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ASPARTAME, METHYLEUGENOL, AND ISOEUGENOL

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IARC MONOGRAPHS ON THE IDENTIFICATION OF CARCINOGENIC HAZARDS TO HUMANS

International Agency for Research on Cancer

World Health Organization

ASPARTAME

1. Exposure Characterization

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 22839-47-0 (Chemical Abstracts Service, 2022a)

EC/List No.: 245-261-3 (ECHA, 2022)

Chem. Abstr. Serv. name: aspartame (Chemical Abstracts Service, 2022a)

IUPAC systematic name: (3S)-3-amino-4-[[(2S)-1-methoxy-1-oxo-3-phenylpropan-2-yl]amino]-4-oxobutanoic acid (NCBI, 2022)

Synonyms: L-phenylalanine, L-α-aspartyl-, 2-methyl ester; succinamic acid, 3-amino-*N*-(α-carboxyphenethyl)-, *N*-methyl ester, stereoisomer; L-phenylalanine, *N*-L-α-aspartyl-, 1-methyl ester; L-aspartyl-L-phenylalanine methyl ester; aspartylphenylalanine methyl ester; methyl aspartylphenylalanine; methyl ester, aspartylphenylalanine; SC-18862; Asp-Phe-OMe (Chemical Abstracts Service, 2022a; ECHA, 2022; NCBI, 2022) [The Working Group noted that aspartame has been historically sold under several brand names (NCBI, 2022). Some of these brands have discontinued the use of aspartame in their products.]

1.1.2 Structural and molecular information

Relative molecular mass: 294.30
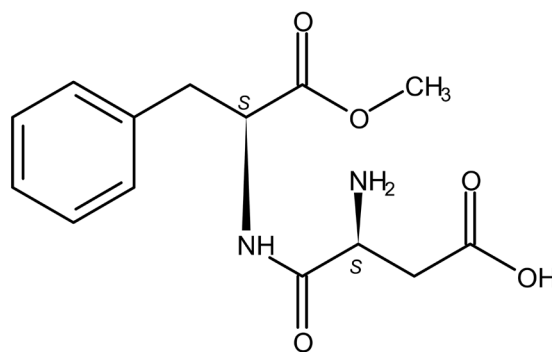
Molecular formula: $C_{14}H_{18}N_2O_5$ (Chemical Abstracts Service, 2022a).

1.1.3 Chemical and physical properties

Description: white, crystalline powder; aspartame has no odour, but an intense sweet taste (about 160–200 times as sweet as sucrose) (Burdock, 2010)

Taste threshold: 6.59 mg/L in water (Dietrich et al., 2021)

Boiling-point: not available

Melting-point: not well defined, often described as having a double melting-point

at 190 °C and 246–247 °C ([Prankerd, 2002](#); [von Rymon Lipinski, 2015](#))

Density: 1.46–1.49 g/cm³ ([Prankerd, 2002](#))

Solubility: sparingly soluble in water; slightly soluble in alcohol ([Burdock, 2010](#))

Dissociation constants: pK_{a1} = 3.19; pK_{a2} = 7.87 ([NCBI, 2022](#))

Stability and reactivity: The dry stability of aspartame is good, with < 5% conversion to its diketopiperazine at 105 °C during 100-hour storage ([Homler, 1984](#)). The stability of aspartame is affected by moisture, pH, and temperature ([Magnuson et al., 2007](#); [Burdock, 2010](#)). Under moist conditions, the dipeptide aspartylphenylalanine and methanol may be formed by hydrolysis. Alternatively, methanol can be eliminated by the cyclization of aspartame to form its diketopiperazine, 5-benzyl-3,6-dioxo-2-piperazineacetic acid, which in turn can be hydrolysed to its individual amino acids, aspartic acid and phenylalanine. When aspartame is decomposed to these compounds, a loss of sweetness is perceived ([Homler, 1984](#)). In solution, the half-life at pH 4.3 (maximum stability) is about 260 days at 25 °C ([Prankerd, 2002](#)). Temperature and pH-dependent racemization of aspartame, or formation of its breakdown products such as the diketopiperazine, aspartic acid, and phenylalanine may occur ([Boehm and Bada, 1984](#)). In addition to these reactions, aspartame can undergo rearrangement at acidic pH to β-aspartame ([Magnuson et al., 2007](#)). The various pathways for the decomposition of aspartame are summarized in [Fig. 1.1](#). Aspartame in aqueous solutions can be stabilized to some extent by the addition of cyclodextrin, modified cyclodextrins, or polyethylene glycol 400 ([Prankerd, 2002](#)).

### 1.1.4 Commercial products and impurities

Commercial qualities with purities in the range of 90–94%, 95–98%, and ≥ 99% are available ([Chemical Abstracts Service, 2022b](#)). Typical specifications for food use include a purity of not less than 98% and maximum limits for 5-benzyl-3,6-dioxo-2-piperazineacetic acid (not more than 1.5% expressed on dry weight basis), other related substances (not more than 2%), residue on ignition (not more than 0.2%), loss on drying (not more than 4.5% at 105 °C, 4 hours); sulfated ash (not more than 0.2% expressed on dry weight basis), transmittance (not less than 0.95, equivalent to an absorbance of not more than approximately 0.022); heavy metals (not more than 10 mg/kg), arsenic (not more than 3 mg/kg expressed on dry weight basis), and lead (not more than 1 mg/kg expressed on dry weight basis) ([Burdock, 2010](#); [European Commission, 2012](#)).

1.2 Production and use

1.2.1 Production process

Although the compound was previously known, the sweet taste of aspartame was accidentally discovered in 1965 by James M. Schlatter during the synthesis of oligopeptides related to the hormone gastrin ([Mazur et al., 1969](#); [Mazur and Ripper, 1979](#); [von Rymon Lipinski, 2015](#)). Aspartame can be synthesized by the coupling of the amino acids L-phenylalanine and L-aspartic acid, and the esterification of the carboxyl group of the phenylalanine moiety to produce the methyl ester. This esterification can occur before or after coupling ([Burdock, 2010](#)). Numerous enzymatic methods are available to couple aspartic acid with phenylalanine methyl ester ([Prankerd, 2002](#)). Some proteinases, such as thermolysin, can catalyse peptide bond formation ([von Rymon Lipinski, 2015](#)). Enzymatic methods applying fermentation were preferred

Fig. 1.1 Degradation pathways for aspartame
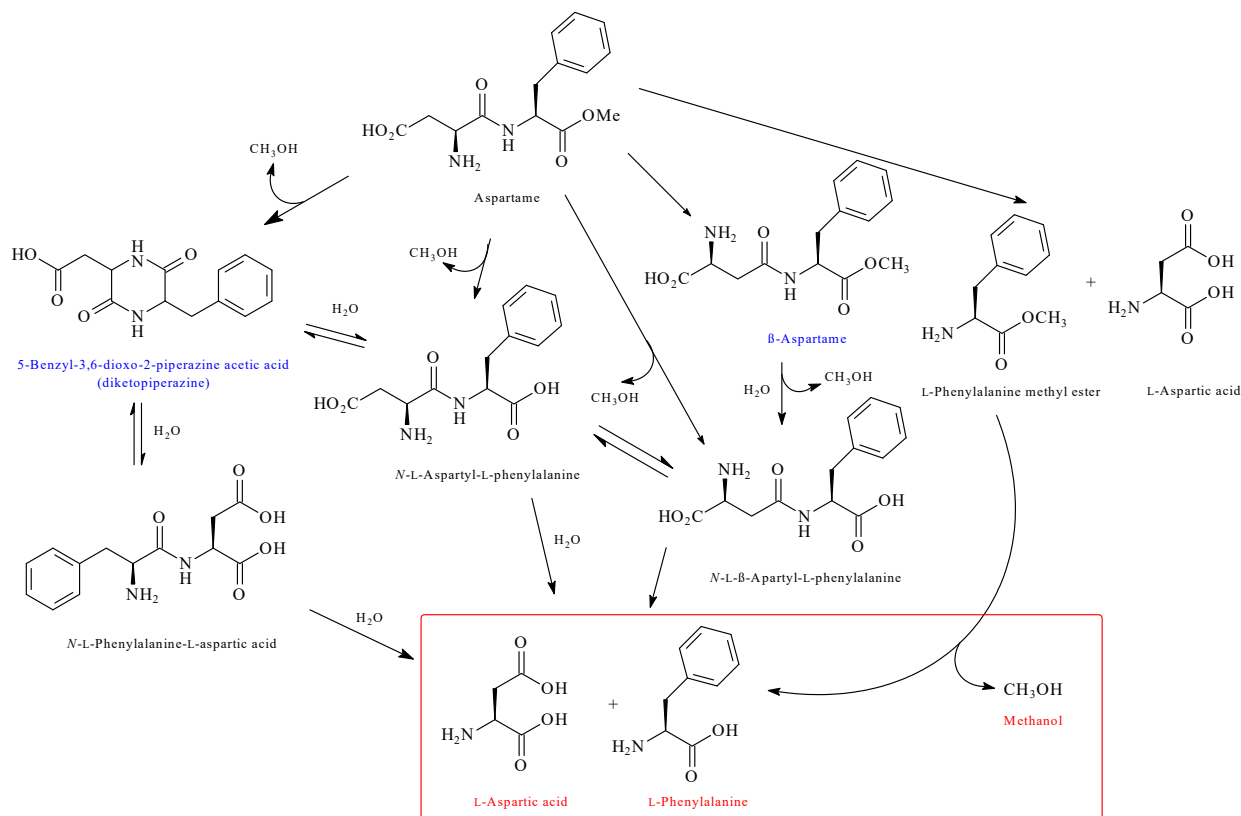
Blue text indicates impurities and red text indicates the three major metabolites.
[The Working Group noted that the diketopiperazine of aspartame is 5-benzyl-3,6-dioxo-2-piperazine acetic acid. The reaction between the diketopiperazine and *N*-ʟ-aspartyl-ʟ-phenylalanine might not be reversible under storage conditions.]
From EFSA (2013), with permission from John Wiley & Sons, from: Scientific opinion on the re-evaluation of aspartame (E 951) as a food additive, EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), *EFSA Journal*, Volume 11, issue 12, 2013; permission conveyed through Copyright Clearance Center, Inc.

for economic commercial production (Prankerd, 2002; Lee, 2006). A combination of an enzymatic process using α-amino acid ester acyltransferase followed by a chemical transformation reaction was recently put into industrial practice (Yokozeki and Abe, 2021).

1.2.2 Production volume

(a) Worldwide

Aspartame's large-scale production began in 1981 (Hollie, 1983). The consumption of aspartame by world regions in tonnes of sugar equivalents is presented in Fig. 1.2. Consumption increased from approximately 5000 tonnes in 1985 to 13 000 tonnes in 2000.

According to encyclopaedia entries published in 2000 and 2006, aspartame was the most widely used non-nutritive sweetener worldwide at that time (Lee, 2000, 2006). In 2001, the consumption of aspartame in the world was 13 185 tonnes (2.64 million tonnes sugar equivalent), of which 1000 tonnes were consumed in Asia, 8500 tonnes in the Americas (North, South, Central, and Caribbean), 735 tonnes in Africa and Oceania, and 2950 tonnes in Europe (IASR, 2004). [More recent data including annual consumption per world region were not available to the Working

Fig. 1.2 Consumption of aspartame in 1980–2020, by world region
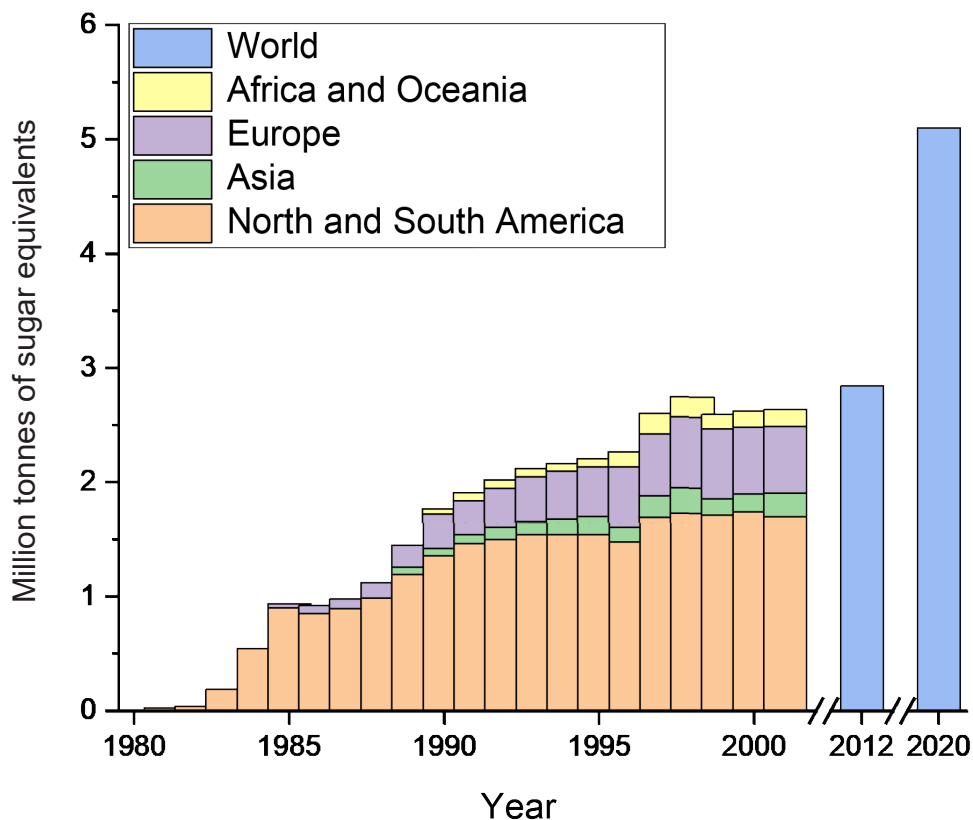
Created by the Working Group with data extracted from figures using WebPlotDigitizer (version 4.6, September, 2022; author, Ankit Rohatgi, Pacifica, California, USA; available from https://automeris.io/WebPlotDigitizer). Sources were Fry (1999) for data for 1980–1983, IASR (2004) for data for 1984–2001; and numerical data from Lewis and Tzilivakis (2021) for 2012 and ECHEMI (2020) for 2020 (sugar equivalents were calculated with a factor of 200 for aspartame). No data for the years 2002–2011 or 2013–2019 were available. No regional data were available for 2012 and 2020. [The Working Group noted that original data on consumption of aspartame are not publicly available and verifiable. The figure was compiled from various secondary sources, and this information may not be consistent between sources. The data for 1980–2012 were considered plausible, showing an increase leading to a plateau. The difference with the data for 2020, suggesting a considerable increase, was not consistent with other sources (see Section 1.4), which indicated a decreasing prevalence of aspartame use over the last decade, and probably cannot be explained by above-average growth rates that had been expected for Central and South America, China, Asia, Africa, and the Middle East (Lewis and Tzilivakis, 2021).]

Group.] The initial increase in production was followed by a period of saturation or even decline beginning in the mid-1990s (Fig. 1.2) (Fry, 1999; IASR, 2004). At that time, drinks that had formerly been sweetened entirely with aspartame began to be sweetened in some countries by blending aspartame with various other sweeteners, particularly acesulfame potassium (acesulfame-K); and the loss of aspartame sales was compounded by the potency gains and synergies from blending (Fry, 1999; IASR, 2004).

By increasing competitiveness, the lower prices of the post-patent era (the patent expired in 1992; Reisch, 2014) were helping stimulate aspartame sales around the world (IASR, 2004). In 2016, aspartame was produced in the European Union, China, Japan, and the Republic of Korea (European Commission, 2016). In 2020, the global production of aspartame was about 25 500 tonnes, and the annual output of aspartame from China was about 19 200 tonnes (ECHEMI, 2020). Present Chinese production capacity was estimated to

be in the range of 30 000 to 35 000 tonnes. The total exports from China amounted to about 16 000 tonnes in 1 year (2020–2021) (European Commission, 2022b).

(b) USA

According to a marketing report, annual sales of aspartame in the USA increased steadily from 3300 tonnes in 1985 to 10 100 tonnes in 2002 (Bizarre et al., 2006; as cited in Magnuson et al., 2007). In 1992, about 8040 tonnes of aspartame were used in the USA (approximately 83% of world consumption) (Lee, 2000). Of this amount, approximately 85% was used for beverages (Lee, 2000). According to data for 1992 from the United States Department of Agriculture, aspartame accounted for 67.4% of the low-calorie sweeteners consumed in the country (Best and Nelson, 1993). Demand in the USA also dominated the increasing global sales of aspartame (IASR, 2004). It was estimated that about half of the global production in 2006 was consumed in the USA (Magnuson et al., 2007). The sole producer of aspartame in the USA withdrew from the market in 2014 (European Commission, 2016).

(c) Europe

In the European Union, the surge in aspartame sales emerged later and more steadily than in the USA. In the mid-1980s, aspartame accounted for a little more than 2% of the European market for intense sweeteners but had risen to 20% in volume terms by 1996 (IASR, 2004). The United Kingdom (UK) was the biggest consumer of intense sweetener in the European Union; in 1987, 31% of the general population used aspartame regularly (i.e. weekly) (IASR, 2004). In 2018–2020, the only aspartame manufacturer in the European Union (France) produced 1181–2921 tonnes of aspartame annually (European Commission, 2022b). In 2018, production volume in the European Union was 1181–1750 tonnes, in 2019 it was 1379–2043 tonnes, and in 2020 it

was 1971–2921 tonnes. Total production in the European Union during 1 year (2020–2021) was in the range of 1963–2909 tonnes (European Commission, 2022b). In Sweden in 2000–2020, annual use of aspartame varied between 3 and 33 tonnes (SPIN, 2023).

1.2.3 Uses

Aspartame is used as a food additive (sweetener) to provide a sugar-like sweetness, both as an ingredient in various foods and beverages and as a tabletop sweetener (Homler, 1984). It is also used for sweetening purposes in various consumer products, such as medicines, cosmetics, tobacco products, and edible cannabis products (see Section 1.4). Soon after its introduction to the market (see Section 1.5), a major use of aspartame in the USA was in artificially sweetened beverages (ASBs), and by the mid-1980s most producers had switched to using aspartame as the only sweetener in these beverages (USDA, 1986). According to industry data supplied to the government of the UK in 1990, [84%] of aspartame use was for soft drinks, followed by tabletop sweeteners [9%], and dry mixed products [4%] (Food Advisory Committee, 1991). Less than 1% was used in pharmaceuticals. A United States Department of Agriculture report published in 2012 stated that between 85% and 90% of aspartame was used as a sweetening agent in beverages, mainly diet carbonated soft drinks (USDA, 2012). Chewing gums and tabletop sweeteners each accounted for 5% of aspartame use.

Aspartame exhibits synergy with carbohydrates and other high-potency sweeteners and may be used in combination (Homler, 1984). Aspartame also has flavour-enhancing and flavour-extending (by up to four times) properties, especially for acid fruit flavours (Abegaz et al., 2012). According to estimates by Abegaz et al. (2012), aspartame is used in approximately 6000 different products worldwide (see Section 1.4).

In biochemical research, aspartame has been used as a model compound to study structure–activity relationships of the sweet taste receptor (Morini et al., 2005).

1.3 Detection and quantification

Aspartame has been analysed in a variety of sample types, but most methods have focused on the application for foods and beverages and usually involve separation by liquid chromatography (LC) followed by different detection methods. Table 1.1 shows a selection of methods used for the detection of aspartame in various matrices.

1.3.1 Environmental samples

Most studies reporting quantification of aspartame in environmental samples have analysed surface water (Baena-Nogueras et al., 2018), wastewater (Kokotou and Thomaidis, 2013; Watanabe et al., 2016), or sewage sludge (Subedi et al., 2014) (see Table 1.1). Analysis of air (NIOSH, 1994; Gan et al., 2013, 2014), dust, and soil (Gan et al., 2014) samples has also been reported. Most methods include filtration before solid-phase extraction and separation by LC. National Institute for Occupational Safety and Health (NIOSH) method 5031 (NIOSH, 1994) describes the measurement of aspartame in air using LC coupled to an ultraviolet (UV) detector with a detection limit of 2 µg per sample of 70–1200 L of air sampled. Detection and quantification in samples with low concentrations of aspartame, e.g. environmental samples (see Section 1.4.1), are usually carried out using mass spectrometry (MS). Methods report varying limits of detection in aqueous samples, ranging from micrograms per litre (e.g. Kokotou and Thomaidis, 2013, 1.6 µg/L) to less than nanograms per litre (e.g. Baena-Nogueras et al., 2018, 0.34 ng/L).

1.3.2 Food

Many methods have described the analysis of aspartame in food and beverages. The analysis of cloudy liquids and semi-solid and solid foods usually involves homogenization and filtration and/or precipitation of particles, which might be followed by solid-phase extraction, whereas clear beverages require little sample preparation (Table 1.1). Separation is usually performed by LC. Aspartame concentrations in foods and beverages are usually higher than in environmental samples, and less sensitive detectors, such as UV detectors or evaporative light-scattering detectors, are sufficient for the analysis of these sample types in a regulatory or food safety context (reviewed in Oktavirina et al., 2021) considering the maximum limits (see Section 1.5). Recently, more sensitive MS-based approaches that allow simultaneous detection of several sweeteners have been reported (Shah and de Jager, 2017). Of the three standardized validated methods in the European Union, two use high-performance liquid chromatography-ultraviolet radiation (HPLC-UV) methodology (CEN, 1996, 1999, 2009) and one uses liquid chromatography-evaporative light-scattering detection (LC-ELSD) (CEN, 2010). One method determines aspartame content in tabletop sweeteners (CEN, 1996), whereas the other two methods allow the measurement of aspartame in a broader range of foods and beverages. Wasik et al. reported an in-house validated procedure to measure aspartame content in soft drinks, canned foods, and yogurts (Wasik et al., 2007). Maes et al. reported a nuclear magnetic resonance (NMR)-based method for the measurement of aspartame in soft drinks that involves only minimal sample preparation, including degassing and pH adjustment (Maes et al., 2012).

Table 1.1 Selected analytical methods for the measurement of aspartame

Sample matrix	Sample preparation	Instrument (LOD)	Comments	Reference
Environmental samples				
Coastal water	Filtration, acidification, SPE	UPLC-MS/MS (0.34 ng/L)		Baena-Nogueras et al. (2018)
Sewage sludge	Freeze-drying, MeOH/water extraction, SPE	HPLC-MS/MS (LOQ, 5 ng/g dw)		Subedi et al. (2014)
Wastewater	Filtration, acidification, SPE	LC-MS/MS (1.6 µg/L)		Kokotou and Thomaidis (2013)
Wastewater	Filtration, acidification, SPE	LC-MS (≤ 23 ng/L*)	*LOD for aspartame was not reported. LODs for five artificial sweeteners including aspartame and five iodinated contrast media were 0.015–23 ng/L.	Watanabe et al. (2016)
Dust and soil	Water extraction, SPE	LC/MS (0.3 ng/L)		Gan et al. (2014)
Air	Quartz fibre filter, extraction with MeOH	LC-MS/MS (0.003 pg/m³)		Gan et al. (2013)
Air	Polytetrafluoroethylene filter	HPLC-UV (2 µg per sample)	NIOSH method 5031, 70–1200 L per sample.	NIOSH (1994)
Foods				
Food and beverages	Clear liquids and beverages: dilution with water, filtration. Cloudy liquids, powders, semi-solid and solid foods: dilution, precipitation with Carrez solution or dilution in NaCl/HCl, filtration	HPLC-UV (NA)	CEN/TS 15606:2009. Validated from 97 mg/kg to 610 mg/L in water-based drink, fruit-based drink, cheesecake with biscuit base, canned soup, and instant chocolate drink.	CEN (2009)
Tabletop sweeteners	Grinding, dissolution, filtration	HPLC-UV (NA)	EN 1378:1996.	CEN (1996)
Beverages, canned fruit	Degassing by ultrasound; homogenization (fruit only), SPE	HPLC-ELSD (NR)	EN 15911:2010. Validated from 38.1 mg/L to 702 mg/L in beverages and from 37.2 mg/kg to 1120.2 mg/kg) in canned fruits.	CEN (2010)
Soft drinks, canned food, yogurt	Homogenization, SPE	HPLC-ELSD (energy drinks, 14 µg/g; yogurt, canned fruit, 13 µg/g)	In-house validated procedure.	Wasik et al. (2007)
Soft drinks	Ultrasound degassing, acidification	NMR (zero cola sample, 2.9 mg/L; light cola sample, 4.6 mg/L)	In-house validated procedure.	Maes et al. (2012)

Table 1.1 (continued)

Sample matrix	Sample preparation	Instrument (LOD)	Comments	Reference
Consumer products				
Alternative tobacco products Lozenges	Acetonitrile/water extraction, filtration	LC-MS (NR)		Miao et al. (2016)

dw, dry weight; HCl, hydrochloric acid; HPLC, high-performance liquid chromatography; HPLC-ELSD, high-performance liquid chromatography-evaporative light scattering detection; LC-MS, liquid chromatography-mass spectrometry; LC-UV, liquid chromatography with ultraviolet detection; LOD, limit of detection; LOQ, limit of quantification; MeOH, methanol; MS/MS, tandem mass spectrometry; NA, not available to the Working Group; NaCl, sodium chloride; NMR, nuclear magnetic resonance; NR, not reported; SPE, solid-phase extraction; UPLC, ultra-performance liquid chromatography.

1.3.3 Drugs and consumer products

An LC-MS based method (Miao et al., 2016) for the analysis of aspartame in lozenges and alternative tobacco products (snus) including solvent extraction has been reported.

1.3.4 Biological specimens

Details on the metabolism of aspartame in humans are described in Section 4.1. Because of the hydrolysis of aspartame to its major constituents – aspartic acid, phenylalanine, and methanol – which are also endogenous metabolites, no validated biomarkers are available for the biomonitoring of aspartame exposure. One study reported the measurement of aspartame in serum using an ultraperformance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) based method (Liu et al., 2022a). [The Working Group noted that the study by Liu et al. (2022a) lacked some detail in the description of the analytical method, since the analytical conditions seemed to be based on a method for the detection of aspartame in beverages (Kubica et al., 2016), but no data on the performance of the method validation for the analysis of serum were provided. The quality of this work was uncertain, since the method was also described to be based on a method that did not aim to detect aspartame (Logue et al., 2017).]

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

Aspartame is not found naturally in the environment, but it can be produced and released into the environment through various industrial processes, such as the manufacture of food and beverage products that contain aspartame. Aspartame can also enter the environment through wastewater emissions from households and industries that use products containing

aspartame. See Table 1.2 for measurements of aspartame in different environmental samples.

(a) Air

Volatilization from dry and moist soil and water surfaces is not expected to be substantial (Lambert et al., 2010). Gan et al. (2014) examined air samples in China for the presence of artificial sweeteners, including aspartame. Aspartame was not detected in any air samples during the summer, but in winter it was detected at concentrations up to 5.39 pg/m^3 in 5 out of the 21 particulate-phase samples (Gan et al., 2014). This was in line with the assumption, based on physicochemical data, that aspartame would be expected to exist solely in the particulate phase (EFSA, 2021). Aspartame does not contain chromophores that absorb at wavelengths > 290 nm and therefore is not expected to be susceptible to direct photolysis by sunlight (EFSA, 2021).

(b) Water

Aspartame appears to be more persistent in freshwater than in saltwater systems. Abiotic degradation half-life (DT$_{50}$) in water at pH 3 was estimated as being approximately 300 days, and degradation results mainly from hydrolysis of the ester and amide bonds (Lambert et al., 2010; EFSA, 2021). Cyclization to the diketopiperazine (see Section 1.1) increases at higher pHs, and the DT$_{50}$ at pH 5, 6, and 7 has been estimated as 245, 120, and 1 day(s), respectively (Crosby and Furia, 1980). The normal pH range for surface water and groundwater systems is usually pH 6.0–8.5; should aspartame reach surface or groundwaters, it may therefore be considered persistent under some conditions (EFSA, 2021).

Table 1.2 lists examples of studies that have aimed to measure aspartame in drinking-water, groundwater, surface water, or wastewater. In drinking-water and tap water in a study from Mexico, aspartame concentrations ranged from below the limit of detection (120 µg/L) to 760 µg/L (Medrano et al., 2019). In a study

Table 1.2 Occurrence of aspartame in environmental samples

Sample type	Location and collection date	No. of samples	Mean (range)	Median (IQR)	Analytical method (LOD)	Comments	Reference
Surface water Urban wastewater	São Paulo state, Brazil, 2019	21 24	ND ND	ND ND	Online SPE-UPLC-MS/MS (0.2 µg/L)	Surface water was sampled from a river at 7 locations, 25 km apart. Wastewater was sampled at 5 WWTPs.	Alves et al. (2021)
Surface water, wastewater, sludge	Shenzhen city, China, 2019	Wastewater, 16 SPM, 8 Dewatered sludge, 4 Surface water, 12 Offshore SPM, 12			UPLC-MS/MS (LOQ, 5 ng/g dw)	Wastewater was sampled at 4 WWTPs. Aspartame was mainly distributed in SPM and sludge, and the fractions in offshore water exceeded 45%.	Guo et al. (2021)
	West coast	4 4 2 12	Influent, 0.7 µg/L Effluent, 0.2 µg/L Sludge, 0.2 µg/g Offshore water, 0.2 µg/L; offshore SPM, (0.1–2.3 µg/g)	NR NR NR NR			
	East coast	22	ND for influent, effluent, sludge, offshore water	NR			
Wastewater	Australia, 2016	Influent, 124 Effluent, 35	16 g ± 38 (SD) g/day Mean population-weighted per capita loads, 0.12 ± 0.14 mg/day ND in effluent	NR NR	HPLC-QTrap MS/MS with ESI (0.08 µg/L)	Nationwide study of 69 WWTPs. Aspartame was detected in 91% of influent samples; 100% removal of aspartame was observed during wastewater treatment.	Li et al. (2020)
Wastewater	Lake Ontario, Canada, 2014	55	Raw sewage, 1200 (61–8007) ng/L	Raw sewage: 676 ng/L	Online SPE-LC-APCI-MS/MS (75 ng/L)	The concentration of aspartame was below the LOD in 4 (out of 55) raw sewage samples and in 18 (out of 18) treated effluents.	Tolouei et al. (2019)

Table 1.2 (continued)

Sample type	Location and collection date	No. of samples	Mean (range)	Median (IQR)	Analytical method (LOD)	Comments	Reference
Wastewater, surface water	Cadiz Bay, Spain, February to July 2015	24	WWTP, 1–4 Influent, NR (< 0.01–0.1) µg/L Effluent, NR (< 0.01–0.09) µg/L	NR	SPE-UPLC-QqQ-MS/MS (0.34 ng/L)	4 WWTPs, 13 coastal water sampling sites. Values are ranges during the sampling period. Aspartame detection frequency, depending on the sampling site, varied between 33% and 100% and it was always detected at < 0.1 µg/L.	Baena-Nogueras et al. (2018)
		24	Surface waters, 1–4 Receiving waters: NR (< 0.01–0.04) µg/L	NR NR			
		28	Coastal waters, [18.2] (7–37) ng/L	[19.5] ng/L			
Wastewater, surface water, groundwater	Hanoi, Viet Nam, 2013	7	*WWTP* Influent, NR (570–3100 ng/L)	2100 ng/L NR	LC-MS with ESI (NR)		Watanabe et al. (2016)
		7	Effluent, ND	ND			
			Surface water				
		5	Hong River, ND	ND			
		1	Canal, ND	ND			
		4	Lu River, NR (1700–2600 ng/L)	2100 ng/L			
		2	Pond, ND	ND			
			Groundwater				
		4	Urban, ND	ND			
		2	Suburb, ND	ND			
		3	Rural, ND	ND			
	Haiphong Viet Nam, 2013	6	*Surface water* Two rivers, ND	ND			
	Halong, Viet Nam, 2013	3	*Groundwater*: ND	ND			
	Manila, Philippines, 2015	5	*Surface water*: ND	ND			
	Pathein, Myanmar, 2014	4	*Surface water*: ND	ND			
	Yangon, Myanmar, 2014	4	*Surface water*: ND	ND			

Table 1.2 (continued)

Sample type	Location and collection date	No. of samples	Mean (range)	Median (IQR)	Analytical method (LOD)	Comments	Reference
Wastewater, sludge	Albany, New York, USA, 2013	11	Influent, 0.13 (0.01–0.44) µg/L Primary effluent, 0.49 (0.03–1.30) µg/L Effluent, 0.11 (0.02–0.22) µg/L Sludge, 0.08 (ND–695) µg/g dw	NR	HPLC-MS/MS (NR)	Wastewater and sludge were sampled in 2 WWTPs. Aspartame detection frequency in influent, primary effluent, and effluent was 100%; in sludge, it was [91%].	Subedi and Kannan (2014)
Sewage sludge	Republic of Korea, 2011	WWTP, 40 Industrial, 15 Mixed, 9 Domestic, 16	129 (36.3–266) ng/g dw 1010 (28.4–2400) ng/g dw 746 (55.9–5220) ng/g dw	140 ng/g dw 321 ng/g dw 311 ng/g dw	HPLC-ESI-MS/MS (LOQ, 5.0 ng/g)	Nationwide study.	Subedi et al. (2014)
Wastewater, surface water, soil	Eggenstein-Leopoldshafen and Karlsruhe, Germany, 2009	NR	ND	ND	HPLC-ESI-MS/MS) (LOQ, 5 ng/L)		Scheurer et al. (2009)
Wastewater	Hanoi, Viet Nam, 2018 to 2020	184	0.17 ± 0.18 (SD) µg/L (ND–2.2 µg/L)	0.12 µg/L	LC-QTrap MS (LOQ, 0.05 µg/L)	Samples collected in urban sewer canal with domestic wastewater.	Li et al. (2023)
Wastewater, surface water, tap water, coastal water, tap water, precipitation, air	Tianjin, China, 2011	Air gas phase, 4 Air particulate phase, 4 Air TSP, 4 Wastewater influent, 6 Surface water, 54 Wetlands, 21 Drainage canal, 24 Sea coast, 30 Groundwater, 15 Tap water, 15	[1.0] (0.5–1.6) pg/m³ [0.7] (0.2–1.4) pg/m³ [0.10] (0.07–0.12) pg/m³ [48.5] (NR) ng/L [33.9] (29–36) ng/L [22.9] (20–28) ng/L [92.5] (53–210) ng/L [16.4] (7.7–37) ng/L ND ND	[1.0] pg/m³ [0.6] pg/m³ [0.11] pg/m³ NR [33.5] ng/L [22.0] ng/L [58.5] ng/L [15.0] ng/L NR NR	LC-MS/MS (tap water and seawater, 0.1 ng/L; river water, 0.3 ng/L)	Water samples collected from 2 WWTPs; 18 sites in Haihe river; 8 sites in Dagu drainage canal; 10 sites in Bohai Sea coast; 7 sites in Dahuangpu wetlands; tap water in city centre; groundwater (well water) in suburbs.	Gan et al. (2013)

Table 1.2 (continued)

Sample type	Location and collection date	No. of samples	Mean (range)	Median (IQR)	Analytical method (LOD)	Comments	Reference
Air, surface water, outdoor dust, soil	Tianjin, China December 2012 (winter); September 2013 (summer)	Air gas phase, 42	NR (ND–0.63) pg/m³	NR	LC-MS/MS Air (0.003 pg/m³); Precipitation (rain or snow) (0.3 ng/L); River water (0.4 ng/L); Wastewater (0.5 ng/L); Dust (dry precipitate) (0.3 ng/g); Soil (0.3 ng/g)	Air and water samples collected once per 2 days, and 7 samples were collected at each of the 3 sampling sites in Tianjin. Precipitation samples were collected in Tianjin. Outdoor dust and soil samples were collected nationwide.	Gan et al. (2014)
		Air particulate phase, 42	NR (ND–5.39) pg/m³	NR			
		Water (river water and wastewater), 42	[0.15] (ND–6.21) ng/L	NR			
		Outdoor dust, 98	[0.28] (ND–23.0) ng/g	NR			
		Soil, 98	ND	NR			
		Dry precipitate, 3	ND	NR			
		Rainfall, 6	NR (ND to < 1.1) ng/L	NR			
		Snow, 3	[9.4] (< 1.1–14.7) ng/L	NR			
Domestic sewage	Two cities in Zhejiang province, China, 2017–2018	NR	NR (ND–7.57) µg/L	NR		Detection rate of aspartame in sewage water was 33.3%.	Ma et al. (2020)
Source water		NR	NR (ND–0.73) µg/L	NR		In source water it was 18.2%.	
Drinking-water, tap water	Pachuca City, Mexico, NR	Bottled water, 7	[0.25] (ND–0.75) mg/L	NR	SPE-LVSS-CE (0.12 mg/L)	Aspartame was found in 3 bottled water samples, 2 tap water samples, 2 distilled water samples, and 1 spring water sample.	Medrano et al. (2019)
		Tap water, 7	[0.14] (ND–0.76) mg/L				
		Drinking-water, 4	[0.13] (ND–0.33) mg/L				
		Spring water, 2	[0.25] (ND–0.49) mg/L				

Table 1.2 (continued)

Sample type	Location and collection date	No. of samples	Mean (range)	Median (IQR)	Analytical method (LOD)	Comments	Reference
River water, groundwater (drinking-water)	Ganges river basin, India, 2014	River water, 14 Groundwater, 14	River water, < LOD Groundwater, < LOD	NR	UPLC-MS (0.2 ng/mL)	Groundwater was collected from handpumps – from which it was used as drinking-water.	Sharma et al. (2019)
Biosolids	Australia, 2016	71 WWTP	GM, 2.8 ng/g (< 1.6–73 ng/g)	0.81 ng/g	LC-MS/MS (LOQ, 1.6 ng/g)	Nationwide study.	Li et al. (2021b)

APCI, atmospheric pressure chemical ionization; dw, dry weight; effluent, treated wastewater; ESI, electrospray ionization; GM, geometric mean; HPLC, high-performance liquid chromatography; influent, raw wastewater; IQR, interquartile range; LC, liquid chromatography; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LOD, limit of detection; LOQ, limit of quantification; MS, mass spectrometry; MS/MS, tandem mass spectrometry ND, not detected; NR, not reported; QTrap, quadrupole ion trap; SD, standard deviation; SPE-LVSS-CE, solid-phase extraction-large-volume sample stacking-capillary electrophoresis; SPE-QqQ-MS/MS, solid-phase extraction triple quadrupole-tandem mass spectrometry; SPE-UPLC-MS/MS, solid-phase extraction-ultra-high-performance liquid chromatography-tandem mass spectrometry; SPM, suspended particulate matter; TSP, total suspended particulates; UPLC-MS, ultra-performance liquid chromatography-mass spectrometry; wt, weight; WWTP, wastewater treatment plant.

from Tianjin, China, aspartame was found in all samples of surface waters at concentrations up to 0.21 µg/L but was not found in groundwater or tap water ([Gan et al., 2013](#)). In India, water from the Ganges River and groundwater in the river basin were sampled in several locations and aspartame was not detected (limit of detection, 0.2 µg/L) ([Sharma et al., 2019](#)).

The occurrence of aspartame in wastewater has been monitored mainly in wastewater treatment plants (WWTPs) since the late 2000s in various locations, namely Australia, Brazil, Canada, China, Germany, the Republic of Korea, Spain, the USA, and Viet Nam ([Table 1.2](#)). Most of the studies either did not detect aspartame or detected it only at low concentrations. In four WWTPs in the Bay of Cadiz, Spain, the highest measured influent aspartame concentrations varied between 0.07 and 0.10 µg/L between February and July 2015 ([Baena-Nogueras et al., 2018](#)), and a median concentration of 2.1 µg/L was measured in a WWTP in Hanoi, Viet Nam, between October and December 2013 ([Watanabe et al., 2016](#)). In another study of wastewater in Hanoi, Viet Nam, in 2018–2020, aspartame was found in 97% of 184 samples; the median concentration was 0.12 µg/L and the maximum was 2.2 µg/L ([Li et al., 2023](#)). Aspartame was detected in 33% of wastewater samples collected in Zhejiang Province, China; the highest concentration measured was 7.57 µg/L ([Ma et al., 2020](#)). In 2014, the median value in the influent of a WWTP located in Lake Ontario, Canada, was 0.68 µg/L and the aspartame detection rate was [93%] ([Tolouei et al., 2019](#)).

In addition to WWTP effluent discharges, the sludge generated can also contain aspartame. In a nationwide survey in 40 WWTPs in the Republic of Korea, aspartame concentrations in sludge ranged from 0.03 to 5.22 µg/g dry weight (dw) ([Subedi et al., 2014](#)). In the Albany area of New York state, USA, sludge samples collected from two WWTPs contained aspartame at a mean concentration of 0.08 µg/g dw ([Subedi and Kannan, 2014](#)).

WWTPs are considered to be a major source of environmental emissions of artificial sweeteners ([Subedi et al., 2014](#)). When aspartame reaches a WWTP, whether the sweetener is then emitted into the aquatic environment will depend upon the efficiency of treatment facilities to remove it from wastewater ([EFSA, 2021](#)). The average removal efficiency was about 70% in one study ([Subedi and Kannan, 2014](#)) and up to 100% in two other studies ([Tolouei et al., 2019](#); [Li et al., 2020](#)).

[The Working Group noted that, because of the complete metabolism of aspartame in mammals, aspartame in wastewater most probably derives from food and beverage residues discarded into the sewage system rather than from human or animal excretions (see Section 4.1 for details on aspartame metabolism).]

(c) Soil

Aspartame is expected to rapidly degrade in soil because of hydrolysis by microbial activity ([EFSA, 2021](#)). Aspartame may degrade in moist soil at pH 7 or higher, with a hydrolysis half-life of about 1 day ([Lambert et al., 2010](#)). This suggests that aspartame would not be persistent in soil systems ([EFSA, 2021](#)). Aspartame would be expected to be highly mobile and not readily absorb to soils, sewage sludge or sediments ([EFSA, 2021](#)). The partition coefficient (log P) for aspartame suggests an affinity for the aqueous phase rather than for lipids and, considering that the predicted bioconcentration factor for aspartame is 3.2, this would imply that the bioaccumulation risk is low ([EFSA, 2021](#)). [The Working Group noted that there were limited data available to support or refute theoretical assumptions about the fate of the compound in soil.]

(d)   Consumer products

Aspartame occurs commonly as an excipient in oral pharmaceutical preparations, such as chewable tablets and liquid formulations, to improve the flavour of medicines and mask unpleasant taste characteristics (Al Humaid, 2018; NCBI, 2022). For example, the taste of pioglitazone can be effectively masked using aspartame, making orally disintegrating tablets palatable to patients (Nakano et al., 2013). The aspartame content of medicinal products is usually < 100 mg per dosage unit. Examples of these products include effervescent tablets containing amoxicillin or ibuprofen, orodispersible tablets containing paracetamol, oral solutions containing penicillin, or bacterial suspensions to treat diarrhoea in infants. Aspartame concentrations of 1–100 mg per dosage unit may be found in medicinal products indicated for short-term use, such as paracetamol or laxatives in adults or children aged > 12 years (Duodecim Drug Database, 2023). The use of some medicinal products may result in daily aspartame doses of > 2000 mg (EMA, 2017).

According to the European Commission database for information on cosmetic substances and ingredients, CosIng (European Commission, 2022a), aspartame can occur as a flavouring agent in cosmetics, e.g. to improve the taste of oral hygiene products such as toothpaste (Welss, 2015). The INCI Beauty database (INCI Beauty, 2022) suggested that aspartame is present in 0.01% of cosmetics, examples including lip products, whitening tooth powders, intimacy moisturizing fluids, and anti-cellulite gels. The Consumer Product Information Database (CPID) lists the use of aspartame in one personal care product (DeLima Associates, 2022). [The Working Group noted that these databases might have different scopes and might not be exhaustive.]

Aspartame can also occur as a sweetener in alternative tobacco products such as snus (for example, 0.008–0.013% in 3 out of 4 analysed samples in one study; Miao et al., 2016) and in edible cannabis products (Health Canada, 2022).

(e)   Food and beverages

This section contains available information on the presence of aspartame in foods and beverages that was obtained from composition and labelling databases, audits, and consumer panels, in addition to published use (concentration) levels of aspartame in retail products.

[The Working Group noted that most data on the occurrence of aspartame were from the USA, Europe, and Oceania, and that there was a lack of studies from Africa and some regions in Asia.]

(i)   Presence of aspartame in food

Occurrence data from composition and labelling databases, audits, and consumer panels were available from published studies in Australia, Brazil, France, New Zealand, Slovenia, and the USA (see Table S1.1, Annex 1, Supplementary material for Section 1, Exposure Characterization, online only, available from: https://publications.iarc.who.int/627).

Recent tracking information from Australia and New Zealand suggested a reduction in occurrence over time. For example, audit data from four supermarket stores in New Zealand in 2013 and in 2019 indicated that by 2019 aspartame was no longer among the top three most commonly available non-nutritive sweeteners (Nunn et al., 2021). Aspartame was present in only 14% of products in 2019 versus 21% in 2015 in a similar Australian audit (Dunford et al., 2022). Beverages, confectionery, snack foods, and sugars and related products were the main food sources of aspartame in these countries (Nunn et al., 2021; Dunford et al., 2022). Data for France indicated that tabletop sweeteners were a key contributing source of aspartame, in addition to ASBs (Chazelas et al., 2021; Debras et al., 2022a). In addition, tracking data for more than 1000 food products during 2017–2020 in Slovenia showed that of 333 beverages that contained

an artificial sweetener, aspartame was present in 102 (31%) (Hafner and Pravst, 2021). It was present only in combination with other sweeteners, primarily acesulfame-K (42 products) (Hafner and Pravst, 2021). Supermarket audit data from Brazil (to support an exposure assessment) revealed that aspartame was one of the top three most commonly used sweeteners, often found in combination with acesulfame-K and in food groups such as "diet/light concentrates and industrialized juices" and "other sweets" (Takehara et al., 2022).

[The Working Group noted that trends in occurrence have been poorly documented in the publicly available literature. A combination of scientific references and other literature sources (e.g. government reports, marketing and industry data and news articles) (see Section 1.2.3, and Annex 2, Scientific and other publicly available data on aspartame use in artificially sweetened beverages, also available from: https://publications.iarc.who.int/627) suggested that although major diet soft drink brands in the USA from the mid-1980s until the late 1990s were sweetened entirely with aspartame, this was probably no longer the case by the mid-2000s. For example, the share of aspartame among high-intensity sweeteners in beverages was more than 80% in 2002, but dropped to about 70% in 2009 and 2014. In the same time frame, the share of aspartame decreased from about 30% to < 15% in tabletop sweeteners, and from 55% to < 20% in other foods (USDA, 2012). Trends for beverages in Europe were likely to be similar, but with probable differences in the dates of introduction of different sweeteners across European countries, generally with an earlier switch from aspartame only to mixed sweetener preparations than in the USA.] It was reported that many diet soft drink bottlers in the European Union, Canada, and the USA switched from 100% aspartame to blends of aspartame and acesulfame-K (IASR, 2004). It has also been suggested that aspartame has become a less popular low-calorie sweetener because sucralose is listed as being more commonly used (Sylvetsky and Rother, 2016).

Databases such as Open Food Facts (Open Food Facts, 2023), Mintel Global New Products Database (Mintel Group Ltd, 2023), and OQALI (data from France; INRAE, ANSES, 2023) cover many countries and also provide information on the occurrence of aspartame in food and beverages. [The Working Group noted the value of such databases, which can also provide information on the presence of aspartame in foods. However, the degree of completeness of the database may vary by country.]

(ii)   Use levels of aspartame in food

Table 1.3 provides data on use levels (concentrations) of aspartame in foods as reported in published scientific papers and by the European Food Safety Authority (EFSA) (EFSA, 2013). All included studies were from European regions except for three studies from Asia (Republic of Korea; Lee et al., 2017; and China; Chang and Yeh, 2014; Li et al., 2021a). [The Working Group noted that data from the USA were not publicly available, although this has been the largest market for aspartame.] The most comprehensive assessment of food types was included in the EFSA opinion (EFSA, 2013), with seven other studies focusing on concentrations present in beverages only (alcoholic: Lachenmeier et al., 2009; Li et al., 2021a; non-alcoholic: Leth et al., 2008; Lino et al., 2008; Basílio et al., 2020; van Vliet et al., 2020; or both: Kubica et al., 2015). This reflected the greater presence of aspartame in non-alcoholic beverages (particularly "light" or sugar-free varieties) across all studies and regions globally. Aspartame was also reported in European studies in food groups such as flavoured fermented milk products (e.g. flavoured yogurts and yogurt drinks), tabletop sweeteners, food supplements, sports foods (e.g. protein or amino acid products), chewing gums, some processed fruit and vegetables (e.g. canned, bottled, dried varieties and jellies, jams and marmalades), powdered

Table 1.3 Concentrations of aspartame in food

Sample type	Location and collection date	No. of samples (no. of detects)	Mean (SD or range, as available) mg/kg or mg/L	Median (IQR) mg/kg or mg/L	Analytical technique (LOD or LOQ)	Comments	Reference
Non-alcoholic beverages Total detects Of which: Light soft drinks with carbon dioxide Light soft drinks without carbon dioxide	Denmark, 2005	Total: 177 76 (67) Of which: 56 (52) 20 (15)	212 (31–560) 85 (11–310)		HPLC (NR)	Study also quantified acesulfame-K, cyclamate, and saccharin (intense sweeteners detected in 76 samples).	Leth et al. (2008)
Non-alcoholic beverages Light (diet, or sugar-free) soft drinks Soft drinks based on mineral waters Light (sugar-free) nectars	Portugal, 2006–2007	Total: 48 (39) 25 (23) 13 (8) 10 (8)	89 (8.8–339) 82 (19–154) 73 (39–129)		RP-HPLC, (LOQ, 3.5 mg/L)	Drinks sampled based on responses by teenagers to a questionnaire on intake of sugar-free soft drinks and nectar; LOD, NR.	Lino et al. (2008)
Alcoholic beverages: fruit wines	Poland	19 (3)	[38] (35–42)		DART-MS/MS (LOD, 30 µg/L)	Aspartame present in combination with acesulfame-K. The mean value was calculated from the three positive samples.	Lachenmeier et al. (2009)
Carbonated cola beverages:	Germany and France, 2010–2011	129			^{1}H NMR (LOD, 3.5 mg/L)	All cola samples. Often co-occurrence of other sweeteners.	Maes et al. (2012)
Branded cola	Germany France	19 (0) 8 (0)	ND ND				
Branded cola light	Germany France	13 (5) 6 (6)	[149] (143–165) [250] (243–255)	[146] [249]			
Branded cola zero	Germany	6 (6)	Overlap (overlapping spectra, no quantification possible)			Overlap, 6/6	

Table 1.3 (continued)

Sample type	Location and collection date	No. of samples (no. of detects)	Mean (SD or range, as available) mg/kg or mg/L	Median (IQR) mg/kg or mg/L	Analytical technique (LOD or LOQ)	Comments	Reference
Branded cola zero	France	8 (7)	[450] (416–601)	[427]		Overlap, 1/8	Maes et al. (2012) (cont.)
Branded cola light no caffeine	France	5 (5)	[237] (224–255)	[237]			
Branded cola vanilla	Germany	2 (0)	ND				
Cola discount	Germany	18 (0)	ND				
	France	13 (0)	ND				
Cola light discount	Germany	10 (10)	Overlap			Overlap, 10/10	
	France	0	0				
Cola zero discount	Germany	10 (10)	[138] (115–168)	[137]		Overlap, 5/10	
	France	6 (6)	[367] (330–388)	[374]			
Cola strong	Germany	3 (0)	ND				
Flavoured fermented milk products included heat-treated products	Europe (use levels from industry, analytical data from Austria, Netherlands, Slovakia, Italy), 2000–2012	Industry: NA Austria: 0 Netherlands: 12 (NR) Slovakia: 1 (1) Italy: 0	Industry: 50–1000 Austria: NA Netherlands: 54–132 Slovakia: 93.5 Italy: NA	NR	NR	Industry typical maximum values listed.	EFSA (2013)
Edible ices		Industry: NA Austria: 0 Netherlands: 0 Slovakia: 0 Italy: 0	Industry: 40–50 Austria: NA Netherlands: NA Slovakia: NA Italy: NA	NR		"Typical-max" values.	
Processed fruit and vegetables		Industry: NA Austria: 137 (NR) Netherlands: 0 Slovakia: 1 (1) Italy: 0	Industry: 350–1000 Austria: 107–695 Netherlands: NA Slovakia: 74.5 Italy: NA	NR		"Typical-max" values.	
Cocoa and chocolate products as covered by Directive 2000/36/EC (European Parliament and Council, 2000)		Industry: NA Austria: 0 Netherlands: 0 Slovakia: 0 Italy: 0	Industry: 500–1000 Austria: NA Netherlands: NA Slovakia: NA Italy: NA	NR		Industry reports for cocoa probably refer to Spain only.	

Table 1.3 (continued)

Sample type	Location and collection date	No. of samples (no. of detects)	Mean (SD or range, as available) mg/kg or mg/L	Median (IQR) mg/kg or mg/L	Analytical technique (LOD or LOQ)	Comments	Reference
Other confectionery without added sugar	Industry: NA Austria: 7 (NR) Netherlands: 4 (NR) Slovakia: 6 (NR) Italy: 0	Industry: 100–1000 Austria: 68.3 Netherlands: 12 Slovakia: 151–912 Italy: NA	NR				EFSA (2013) (cont.)
Chewing gum with added sugar	Industry: NA Austria: 0 Netherlands: 0 Slovakia: 0 Italy: 0	Industry: 600–1450 Austria: NA Netherlands: NA Slovakia: NA Italy: NA	NR			"Typical-max" concentrations.	
Chewing gum without added sugar	Industry: NA Austria: 5 (NR) Netherlands: 0 Slovakia: 1 (1) Italy: 0	Industry: 3650–5420 Austria: 40–1747 Netherlands: NA Slovakia: 561 Italy: NA	NR			"Typical-max" concentrations.	
Breakfast cereals	Industry: NA Austria: 0 Netherlands: 0 Slovakia: 0 Italy: 0	Industry: NA Austria: NA Netherlands: NA Slovakia: NA Italy: NA	NR				
Processed fish and fishery products including molluscs and crustaceans	Industry: NA Austria: 0 Netherlands: 0 Slovakia: 0 Italy: 0	Industry: NA Austria: NA Netherlands: NA Slovakia: NA Italy: NA	NR				

Table 1.3 (continued)

Sample type	Location and collection date	No. of samples (no. of detects)	Mean (SD or range, as available) mg/kg or mg/L	Median (IQR) mg/kg or mg/L	Analytical technique (LOD or LOQ)	Comments	Reference
Tabletop sweeteners	Industry: NA Austria: 19 (NR) Netherlands: 1 (1) Slovakia: 0 Italy:10 (NR)	Industry: 8700 –36 000 (powder); 100 000–360 000 tablet form Austria: < 0.25 to 238 600 (tablet) Netherlands: 230 647 (tablet) Slovakia: NA Italy: 0–500 000 (tablet)	NR			"Typical-max" concentrations.	EFSA (2013) (cont.)
Mustard	Industry: NA Austria: 0 Netherlands: 0 Slovakia: 0 Italy: 0	Industry: NA Austria: NA Netherlands: NA Slovakia: NA Italy: NA	NA				
Soups and broths	Industry: NA Austria: 0 Netherlands: 0 Slovakia: 0 Italy: 0	Industry: NA Austria: NA Netherlands: NA Slovakia: NA Italy: NA	NA				
Sauces	Industry: NA Austria: 2 (NR) Netherlands: 0 Slovakia: 0 Italy: 0	Industry: 200 Austria: < 4 Netherlands: NA Slovakia: NA Italy: NA	NR			Limited representation of the European market. Levels refer to ketchup.	
Salads and savoury-based sandwich spreads	Industry: NA Austria: 6 Netherlands: 0 Slovakia: 0 Italy: 0	Industry: NA Austria: < 20 Netherlands: NA Slovakia: NA Italy: NA	NA			Austria: only Feinkostsalat.	

Table 1.3   (continued)

Sample type	Location and collection date	No. of samples (no. of detects)	Mean (SD or range, as available) mg/kg or mg/L	Median (IQR) mg/kg or mg/L	Analytical technique (LOD or LOQ)	Comments	Reference
Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding 13/1/5) (European Commission, 1999)		Industry: NA Austria: 0 Netherlands: 0 Slovakia: 0 Italy: 0	Industry: 180 Austria: NA Netherlands: NA Slovakia: NA Italy: NA	NA			EFSA (2013) (cont.)
Dietary foods for weight control diets intended to replace total daily food intake or an individual meal		Industry: NA Austria: 0 Netherlands: 0 Slovakia: 0 Italy: 0	Industry: NA Austria: NA Netherlands: NA Slovakia: NA Italy: NA	NA			
Fruit nectars as defined by Council Directive 2001/112/EC and vegetable nectars and similar products (Council of the European Union, 2001)		Industry: NA Austria: 19 (NR) Netherlands: 0 Slovakia: 0 Italy: 0	Industry: NA Austria: < 20 to 71 Netherlands: NA Slovakia: NA Italy: NA	NR			
Flavoured drinks with sweeteners		Industry: NA Austria: 272 (NR) Netherlands: 40 (NR) Slovakia: 60 (NR) Italy: 0	Industry: 100–600 Austria: < 1 to 496 Netherlands: < 3 to 470 Slovakia: 3.5–25.6 Italy: NA	NR			
Alcoholic beverages, including alcohol-free and low-alcohol counterparts		Industry: NA Austria: 47 (NR) Netherlands: 0 Slovakia: 4 (NR) Italy: 0	Industry: 100–600 Austria: < 1 to 104 Netherlands: NA Slovakia: 7.4–25.6 Italy: NA	NR			
Potato-, cereal-, flour-, or starch-based snacks		Industry: NA Austria: 0 Netherlands: 0 Slovakia: 0 Italy: 0	Industry: NA Austria: NA Netherlands: NA Slovakia: NA Italy: NA	NA			

Table 1.3   (continued)

Sample type	Location and collection date	No. of samples (no. of detects)	Mean (SD or range, as available) mg/kg or mg/L	Median (IQR) mg/kg or mg/L	Analytical technique (LOD or LOQ)	Comments	Reference
Processed nuts		Industry: NA Austria: 0 Netherlands: 0 Slovakia: 0 Italy: 0	Industry: NA Austria: NA Netherlands: NA Slovakia: NA Italy: NA	NA			EFSA (2013) (cont.)
Desserts excluding products covered in category 1, 3, or 4 (flavoured fermented milk products, edible ices, and processed fruits and vegetables)		Industry: NA Austria: 0 Netherlands: 0 Slovakia: 0 Italy: 0	Industry: NA Austria: NA Netherlands: NA Slovakia: NA Italy: NA	NA			
Food supplements as defined in Directive 2002/46/EC excluding food supplements for infants and young children		Industry: NA Austria: 3 (NR) Netherlands: 17 (NR) Slovakia: 0 Italy: 0	Industry: NA Austria: < 20–1845 Netherlands: < 20–2245 Slovakia: NA Italy: NA	NR			European Parliament and Council (2002)
Foods and beverages	Pingtung, China, 2012	42		NR	LC-MS/MS (LOQ, 0.1 mg/kg)	The article has a greater focus on method development rather than being a comprehensive survey. Aspartame was detected in only two cola drinks and one sample of marinated olives.	Chang and Yeh (2014)
Non-alcoholic beverages Of which: Lemon tea Sports drinks Soft drinks Juices		11 (2) Of which: 1 (0) 2 (0) 2 (2) 6 (0)	[76.8] (55.1–98.4)				
Alcoholic beverages Of which: Cocktail Plum flavoured wine Grape wines Flavoured beer		16 (0) Of which: 1 (0) 3 (0) 8 (0) 4 (0)		NR			

Table 1.3   (continued)

Sample type	Location and collection date	No. of samples (no. of detects)	Mean (SD or range, as available) mg/kg or mg/L	Median (IQR) mg/kg or mg/L	Analytical technique (LOD or LOQ)	Comments	Reference
Preserved fruit and vegetables Of which: Pickled pepper Preserved fruits Candies		15 (1) Of which: 1 (0) 11 (1) 3 (0)	87.1	NR			Chang and Yeh (2014) (cont.)
Food and food supplements Beverages: Of which: Flavoured drinks Fruit nectars Syrups Jams Ketchups Confectionery Of which: Hard candy Chewing gum Ice-creams Yogurts Tabletop sweeteners Food supplements	Rome, Italy, 2014	Total: 290 78 Of which: 57 (20) 18 (3) 3 (0) 14 (0) 1 (0) 84 Of which: 42 (16) 42 (42) 3 (3) 42 (5) 14 (2) 54 (11)	162 (120) 39 (49) 0 0 0 773 (320) 1922 (1245) 89 (16) 187 (24) 388 (32) 5797 (3814)	NR	UPLC-MS/MS (LOQ, 10 µg/L; LOD, 3 µg/L)	Sampling informed by national food consumption survey. All food contained aspartame at less than maximum levels established by the European Commission. Within the flavoured beverage category, heterogeneity in occurrence was identified between categories – aspartame was more likely to be present in cola-type beverages than in iced tea or sports drinks.	Janvier et al. (2015)
Alcoholic and non-alcoholic drinks Carbonated non-alcoholic Non-carbonated non-alcoholic Carbonated alcoholic Instant drink powders	Gdansk, Poland, NR	24 (2) 5 (0) 11 (0) 5 (2) 3 (0)	[38] (9.88–66) NA NA [38] (9.88–66) NA	NR	RP LC/MS (LOD, 1.63 ng/mL; LOQ, 4.90 ng/mL)	Paper more focused on method development.	Kubica et al. (2015)

Table 1.3 (continued)

Sample type	Location and collection date	No. of samples (no. of detects)	Mean (SD or range, as available) mg/kg or mg/L	Median (IQR) mg/kg or mg/L	Analytical technique (LOD or LOQ)	Comments	Reference
Foods and beverages (no supplements)	Republic of Korea, domestic and imported products, NR	Total: 908		NR	HPLC-ELSD and LC-high-resolution MS (LOD, 3.4 mg/kg; LOQ, 10.3 mg/kg)	Mean of all samples including non-detects. Number of detects was calculated by the Working Group but presented as detection rate (%) in the publication. Means of positive samples were 1.3–23 times as high as means of all samples. *Takju* is a grain-based, non-distilled alcoholic beverage.	Lee et al. (2017)
Snacks		51 [7]	28.4 (ND–310)				
Candy		41 [8]	74.5 (ND–2251)				
Chewing gum		43 [9]	59.3 (ND–1057)				
Chocolate		13 [5]	11.2 (ND–48)				
Processed cocoa products		33 [5]	269 (ND–5649)				
Pickled food		46 [4]	6 (ND–185)				
Yogurt		33 [3]	17 (ND–250)				
Carbonated beverages		47 [3]	10 (ND–202)				
Yogurt flavoured beverages		14 [6]	66 (ND–352)				
Beverages (other)		46 [2]	96 (ND–2474)				
Takju		42 [32]	47 (ND–144)				
Food and food supplements	Dublin, Ireland, NR	Total, 375 (210)		NR	UPLC-MS/MS (NR)	Numbers of sampled foods (occurrence) do not match up; not clear from text how many detects for some foods. Aspartame alone was detected in 35 samples. In addition, it was present in combinations with acesulfame-K, saccharin, sucralose, and/or cyclamate in 175 samples.	Buffini et al. (2018)
ER or NAS dairy products		75 (44)	225.7 (88.7)				
ER or NAS carbonated flavoured drinks		98 (26)	277 (237.7)				
ER or NAS flavoured drinks		98 (60)	179.4 (86.4)				
Solid food supplements		21 (11)	9800 (1.9)				
Tabletop sweeteners		18 (5)	306.3 (396.6)				
Sauces		15 (0)	ND				
Other		50 (20)					
ER or NAS jams, jellies, marmalades		8 (0)	414.7 (127.7)				

Table 1.3 (continued)

Sample type	Location and collection date	No. of samples (no. of detects)	Mean (SD or range, as available) mg/kg or mg/L	Median (IQR) mg/kg or mg/L	Analytical technique (LOD or LOQ)	Comments	Reference
ER or NAS cocoa and chocolate products		13 (NR)	255.4 (98.9)				Buffini et al. (2018) (cont.)
ER or NAS non-chocolate confectionery		4 (NR)	1294.5 (33.47)				
ER or NAS ice-cream cornet and wafers		5 (0)	ND				
ER soups and broths		1 (0)	ND				
Cider and perry		1 (1)	126				
Mixtures of alcoholic and non-alcoholic drinks		2 (0)	ND				
Potato-, cereal-, or flour-based snacks		6 (1)	45				
ER or NAS desserts		4 (1)	135				
Syrup-type or chewable food supplements		3 (NR)	18.9 (3.0)				
Non-alcoholic beverages	Portugal, NR	56 (21)		NR	LC-UV (LOD, 59.8 mg/L)	Small convenience sample. Mean and range values were calculated for positive samples.	Basílio et al. (2020)
Traditional soft drinks		27 [10]	139 (< LOQ to 419.3)				
Tea-based soft drinks		10 [4]	90.7 (< LOQ)				
Soft drinks based on mineral waters		4 [3]	< LOQ (< LOQ)				
Sport drinks		4 (0)	ND				
Energy drinks		2 (2)	479 (278–680)				
Nectars		9 [2]	203 (< LOQ to 315)				

Table 1.3 (continued)

Sample type	Location and collection date	No. of samples (no. of detects)	Mean (SD or range, as available) mg/kg or mg/L	Median (IQR) mg/kg or mg/L	Analytical technique (LOD or LOQ)	Comments	Reference
Non-alcoholic beverages	10 European area countries (Belgium, Denmark, Finland, France, Germany, Spain, Sweden, Netherlands, Türkiye, UK). NR	Total: 111 (95)	30–527 103–1790 µmol/L	NR	HPLC-MS/MS LOD, [0.6 µg/L] 0.002 µmol/L LOQ, [2.1 µg/L] 0.007 µmol/L	Total Phe also available in this study. Groupings not always self-evident, e.g. some cola drinks in the "other category". Numbers sampled per category were unclear, with some overlap between categories. Value used for conversions calculated by the Working Group: 1 mol = 294.30 g aspartame. Numbers per country ranged from Denmark (2), France (4), Türkiye (4), Sweden (4), Germany (7), Spain (7), UK (9), Belgium (15), Finland (21), to Netherlands (38).	van Vliet et al. (2020)
Orange drinks with sugar		8 (4)	[41] [0–108] 0–368 µmol/L	[29]			
Orange drinks without sugar		5 (5)	[230] [75–306] 255–1040 µmol/L	[275]			
Lemon drinks with sugar		12 (8)	[105] [0–232] 0–790 µmol/L	[105]			
Lemon drinks without sugar		7 (7)	[303] [104–462] 594–1570 µmol/L	[302]			
Cola drinks with sugar		2 (0)	ND				
Cola drinks without sugar		36 (35)	[0–527] 0–1790 µmol/L	NR			
Other drinks including "energy drinks"		40 (35)	[0–480] 0–1630 µmol/L	NR			
Alcoholic drinks White liquor Beer Whiskey Red wine Yellow wine	China, NR	17 6 (0) 2 (0) 1 (1) 4 (0) 3 (0)	LOD ND ND < LOQ ND ND		RT QTRAP MS (LOD, 0.03 mg/L)	LOD presented as 30 µg/L in the paper.	Li et al. (2021a)

Table 1.3   (continued)

Sample type	Location and collection date	No. of samples (no. of detects)	Mean (SD or range, as available) mg/kg or mg/L	Median (IQR) mg/kg or mg/L	Analytical technique (LOD or LOQ)	Comments	Reference
Foods and beverages	Germany, 2000–2022	5703 [3720]			Various methods, most commonly HPLC (EN 12856) (LOD, NR)	Most important sample groups out of a data set of 53 116 food monitoring results, of which 14% were positive for aspartame. In column 3, the number of quantifiable samples is shown, not the number of detects.	Schorb et al. (2023)
Chewing gum		312 (241)	1543 (1042)	1369			
Sports foods		297 (125)	1453 (1461)	1030			
Fibre supplements		20 (11)	1248 (175)	1276			
Powdered drink bases		195 (162)	1068 (672)	1133			
Candies		603 (339)	473 (332)	440			
Diet soft drinks		2783 (2021)	91 (101)	60			
Soft drinks		1167 (574)	59 (74)	34			
Flavoured milk drinks		268 (207)	48 (17)	47			
Mixed beer drinks		58 (40)	24 (15)	26			

DART-MS/MS, direct analysis in real time with tandem mass spectrometry; ELSD, evaporative light scattering detector; ER, energy reduced; ^{1}H NMR, ^{1}H nuclear magnetic resonance spectroscopy; HPLC, high-performance liquid chromatography; IQR, interquartile range; LOD, limit of detection; LOQ, limit of quantification; MS, mass spectrometry; NA, not applicable; NAS, no added sugar; ND, not detected; NNS, non-nutritive sweetener; NR, not reported; Phe, phenylalanine; QTRAP, Q Trap mass spectrometry; RP, reverse phase; RT, real time; SD, standard deviation; UPLC, ultra-performance liquid chromatography; UV, ultraviolet.

drink bases, and confectionery without added sugar (EFSA, 2013; Janvier et al., 2015; Buffini et al., 2018; Schorb et al., 2023). This was broadly similar for the most comprehensive study in Asia (Republic of Korea), but the presence in processed cocoa products and *takju* (a type of rice alcohol) was also noted (Lee et al., 2017). Although aspartame was approved for use in many food categories under European Union law, at the time of the analyses submitted to inform the EFSA opinion (EFSA, 2013) it appeared that aspartame was often not present. For example, no detection data were reported in 14 out of the 23 food categories studied, e.g. for mustards, edible ices, cocoa and chocolates, breakfast cereals, processed fish, soups and sauces, sandwich spreads, foods for special medical purposes and weight control diets, processed nuts, or starch-based snacks (EFSA, 2013). [Analytical data were reported from four countries – Austria, the Kingdom of the Netherlands (hereafter "the Netherlands"), Slovakia, and Italy – with variations in the number of samples analysed. The Working Group noted that it was unclear whether detection was not reported in the 14 food categories because the foods were not analysed or because aspartame was not detected.] Aspartame was detected in similar food groups in a large survey (n = 53 116) in Germany in 2000–2022 in which only 14% of samples were positive for aspartame (Schorb et al., 2023).

Aspartame concentrations were typically highest in tabletop sweeteners, chewing gums, and beverages. The highest concentrations were reported in tabletop sweeteners (solid form): industry use levels were reported as 100 000–360 000 mg/kg and analytical values from Italy (tablet form) as 0–500 000 mg/kg (EFSA, 2013); later studies revealed mean concentrations of 388 000 mg/kg in Italy (Janvier et al., 2015) and 306 300 mg/kg (tablet/powder form) in Ireland (Buffini et al., 2018). [The Working Group noted that in the European Union, there was no maximum permitted level concentration outlined for tabletop sweeteners, unlike for other food categories; addition is quantum satis (no numerical maximum is defined; see Section 1.5), and this may explain the variation in concentrations present.]

There was scant information on chewing gum, with recent data suggesting that, when present, aspartame concentrations ranged from 0–1057 mg/kg in the Republic of Korea (Lee et al., 2017) to a mean value of 1922 mg/kg in Italy (Janvier et al., 2015). EFSA reported industry use levels of up to 5420 mg/kg, yet the cited analytical data suggested a range of 40–1747 mg/kg in five samples from a contributing Austrian study (EFSA, 2013). An average of 1543 mg/kg (median, 1369 mg/kg; maximum, 4617 mg/kg) was found in chewing gum (n = 312) on the German market during 2000–2022 (Schorb et al., 2023).

Information on concentrations present in food supplements and foods targeted at individuals participating in sport was scant. Available information indicated concentrations in food supplements of < 20–2245 mg/kg in the Netherlands (EFSA, 2013), a mean of 5797 mg/kg in Italy (Janvier et al., 2015), mean concentrations of 9.8 and 18.9 mg/kg in Ireland (reflecting solid and syrup/chewable food supplements, respectively (Buffini et al., 2018), and mean concentrations of 1248 and 1453 mg/kg for fibre supplements and sports foods (e.g. protein and amino acid powder to prepare drinks), respectively, in Germany (Schorb et al., 2023).

Of note, although studied less frequently in the publications identified, lower concentrations of aspartame were also measured in other food categories e.g. dairy products (including fermented milks and yogurts), ice creams, processed fruits and vegetables, chocolates and processed cocoa products, pickled foods, and confectioneries and candies (EFSA, 2013; Janvier et al., 2015; Lee et al., 2017; Buffini et al., 2018; Schorb et al., 2023).

(iii)   Beverages

Beverages were the most frequently studied food category. Concentrations in beverages depended on categorization; the categories of "light" or "reduced sugar" or "no added sugar" were not always transparent and thus comparison was difficult. Studies published before 2015 indicated mean concentrations in any beverage type of up to 212 mg/L (Leth et al., 2008), 89 mg/L (Lino et al., 2008), 35–42 mg/L (range) (Lachenmeier et al., 2009), [279] mg/L (Maes et al., 2012), and 496 mg/L (upper limit of range) (EFSA, 2013). Studies published after 2015 indicated mean concentrations in beverages of 162 mg/L (Italy; Janvier et al., 2015), 277 mg/L (Ireland; Buffini et al., 2018), 139 mg/L (Portugal; Basílio et al., 2020), 92 mg/L (Republic of Korea; Lee et al., 2017), and 91 mg/L (Germany; Schorb et al., 2023). However, a recent study spanning 10 countries in the European area (Belgium, Denmark, Finland, France, Germany, the Netherlands, Spain, Sweden, Türkiye, and the UK) indicated the presence of aspartame in both sugar-containing and non-sugar-containing beverages (van Vliet et al., 2020). Van Vliet et al. (2020) reported consistently higher aspartame levels in orange, lemon, and cola drinks without sugar [0–527 mg/kg] than in sugar-containing versions [0–232 mg/L]. Other beverages containing aspartame included energy drinks, with levels of [80–160 mg/L] (*n* = 10) (van Vliet et al., 2020) and 278–680 mg/L (*n* = 2) (Basílio et al., 2020) in European studies. Of note, older studies (Leth et al., 2008; Lino et al., 2008; Maes et al., 2012) reported consistently high levels of detection (75–100% of beverages tested). More recent studies revealed greater variability. Van Vliet and colleagues recorded [86%] detection across beverage categories of 111 drinks with and without sugar (van Vliet et al., 2020). In contrast, detection levels in beverages were 38% in a Portuguese study targeting non-alcoholic beverages (Basílio et al., 2020), 8% in a Polish study on

24 soft and alcoholic drinks (Kubica et al., 2015), [66%] in an Irish study on non-alcoholic beverages (*n* = 196) (Buffini et al., 2018), [10%] and 76% in a comprehensive analysis of non-alcoholic and alcoholic (*takju*) beverages in the Republic of Korea, respectively (Lee et al., 2017), and 5% in a small study (*n* = 17) focusing on alcoholic beverages in China (Li et al., 2021a). Data for 2008–2016 from the Danish Veterinary and Food Administration Laboratory, which was responsible for monitoring food additives including intense sweeteners in non-alcohol beverages, suggested that, from 2010, aspartame was not present singly in any beverage, but was used in combination with other sweeteners (Nielsen and Zederkopff Ballin, 2009; Villadsen and Jakobsen, 2012; Jensen and Christiansen, 2014, 2016). [The Working Group noted that although the total samples analysed in these reports were not directly comparable year on year and that across all years there remained considerable variability in the concentrations of aspartame added to beverages, these reports may be suggestive of reduced use levels in recent years.] One study reported aspartame levels in beverages in one urban area of Nigeria (Shinggu and Bekab, 2018). [The Working Group noted that only five samples were analysed, which limits informativeness and comparability to the other studies.]

[For all studies on beverages, the Working Group also noted that the difference in detection rates and concentrations present may be explained by non-representative sampling, often relying on small numbers of samples of beverages. Further, the Working Group noted a lack of information relating to the use of aspartame as part of a mix of sugar and artificial sweeteners in beverages.]

(iv)   General considerations

[The Working Group noted considerable limitations across all data (i.e. for foods and beverages). Differences in the categorization of foods and sampling protocols made strict comparisons

difficult. Four studies used consistent categorization of foods according to European Union legislation (EFSA, 2013; Janvier et al., 2015; Buffini et al., 2018; Schorb et al., 2023), but most other studies had their own categorization process. A variety of analytical methods were also employed with associated differences in detection sensitivity and with some papers focusing on method optimization rather than a comprehensive assessment of retail products. Where provided, sample sizes were in many instances low, except for beverages. The Working Group also noted the variability in concentrations present for a single food category, particularly for tabletop sweeteners, and between countries where specific formulations (e.g. for beverages) may differ.]

1.4.2 Occupational exposure

There was one report from NIOSH on occupational exposure to aspartame for workers in a plant that produced dry dessert and drink mixes in Ohio, USA (NIOSH, 1992). Workplace air measurements on a total of 148 personal breathing zone and general area air samples, across three shifts on four consecutive days were performed. Aspartame concentrations in personal breathing zone samples and area air samples ranged from not detected (ND) to 545 µg/m^3 and from ND to 83 µg/m^3, respectively. Four operations were identified as being of major interest because of higher exposure potential, namely, weigh-out, blending and packing operator, and packing helper. For these operations, full-shift breathing zone personal samples were collected, and the highest values obtained for each operation were 151 µg/m^3, 102 µg/m^3, 100 µg/m^3, and 20 µg/m^3, respectively. The short-term personal sampling performed in the same operations supported the findings; the highest peak concentrations obtained during blending and weigh-out were 213 and 432 µg/m^3, respectively. Thus, tasks for which aspartame

powder was manually handled involved higher exposures.

In 2018–2020, the only aspartame manufacturer in the European Union employed 63–112 persons, some of whom may have been occupationally exposed to aspartame if the risk management measures in place did not provide the effectiveness needed to control exposure by inhalation (European Commission, 2022).

[The Working Group noted the lack of comprehensive exposure data in an occupational context. The Working Group also noted that, despite the lack of exposure data, occupational exposure to aspartame may occur during synthesis of the compound and production of aspartame-containing products.]

1.4.3 Exposure of the general population

Data on estimated aspartame exposure in the general population are presented in Table 1.4 and Fig. 1.3. Levels of exposure of the general population have been assessed in several studies, mostly in the last two decades. Foods, including tabletop sweeteners, and beverages are the main sources of exposure to aspartame (see Sections 1.2 and 1.4.1(e)). The target population of these reports included different sex and age groups (toddlers, children, adolescents, adults, and the elderly), as well as selected populations such as low-income groups and pregnant or lactating women. Although many of the early reports were based mainly on selected volunteer studies (convenience samples), later reports included nationally representative studies or large cohort studies. Most of the studies assessed dietary exposure using dietary assessment data such as multiple-day 24-hour recalls or records, and food frequency questionnaires (FFQs), whereas others were market basket methods and surveys such as food label surveys (market share data) and household surveys. In most reports, estimates were made from the analysis of total diet studies or using a tiered (or scenario) approach

Table 1.4 Measurement of aspartame exposure in the general population

Metabolite and sample type	Location, population group, and collection date	No. of samples	Mean (range)	Median (IQR)	Relevant percentiles	Method of dietary assessment	Comments	Reference
Dietary exposure	Portuguese National Survey, 2015–2016 Overall	5005	Tier 2.1: 3.09 Tier 2.2: 0.31 Tier 3: 0.14		Tier 2.1: 8.92 Tier 2.2: 1.39 Tier 3: 0.59	Adults: 1-day food diaries Children: two 24-hour recalls	Tier 2.1: actual national food consumption data combined with the maximum permitted usage levels for the additive for all foods reported. Tier 2.2: actual national food consumption data combined with the maximum permitted usage levels for the additive for the foods identified as having non-nutritive intense sweetener according to the brand information. Tier 3: actual national food consumption data combined with the actual usage levels of the additive, given by analytical values of aspartame occurrence in foods	Carvalho et al. (2022)
	Children	521:	Tier 1: 8.78 Tier 2: 0.54 Tier 3: 0.30		Tier 1: 17.72 Tier 2: 2.34 Tier 3: 1.31			
	Adolescents	632	Tier 1: 5.68 Tier 2: 0.59 Tier 3: 0.22		Tier 1: 12.02 Tier 2: 2.32 Tier 3: 0.90			
	Adults	3102	Tier 1: 2.74 Tier 2: 0.30 Tier 3: 0.13		Tier 1: 6.95 Tier 2: 1.33 Tier 3: 0.55			
	Elderly	750	Tier 1: 1.36 Tier 2: 0.14 Tier 3: 0.08 (units: mg/kg bw per day)		Tier 1: 3.75 Tier 2: 0.65 Tier 3: 0.37 (95th percentile, units: mg/kg bw per day)			
Dietary exposure	Pubertal and paediatric endocrine outpatient clinics in multiple centres in Taiwan, China, dates not available	102 children and adults	1.58 mg/day			FFQ	Validation study for FFQ	Chu et al. (2022)

Table 1.4 (continued)

Metabolite and sample type	Location, population group, and collection date	No. of samples	Mean (range)	Median (IQR)	Relevant percentiles	Method of dietary assessment	Comments	Reference
Dietary exposure	Beirut and Mount Lebanon, February to August 2020	384 adult Lebanese individuals	98.9 mg/day (1.38 mg/kg bw per day)				FFQ	77.13 mg/day of aspartame from beverages, 21.11 mg/day from food, and 0.66 mg/day from pills and powders.	Daher et al. (2022)
Dietary exposure	French population-based cohort NutriNet-Santé, 2009–2021	102 865 adults	9.35 mg/day				Three non-consecutive web-based 24-hour-dietary records		Debras et al. (2022b)
Dietary exposure	Pregnant Brazilian women, cross-sectional study that used data from the Multicenter Study of Iodine Deficiency (EMDI-Brazil), February 2019 to May 2020	243 pregnant women	Scenario 1: 0.57 Scenario 2: 0.67 Scenario 3: 2.9 (units: mg/kg bw per day)	Scenario 1: 0.00 Scenario 2: 0.00 Scenario 3: 1.82 (units: mg/kg bw per day)	Scenario 1: 2.22 Scenario 2: 2.94 Scenario 3: 7.42 (95th percentile, units: mg/kg bw per day)	24-hour dietary recall	Scenario 1: foods and beverages with confirmed use of aspartame in the list of ingredients. Tabletop sweeteners were also included. Scenario 2: use of aspartame in the food and beverages was defined on the basis of high probability of containing aspartame. Foods and beverages that indicated probability of use such as "diet", "light" etc., were added to those in Scenario 1.	Duarte et al. (2022)

Table 1.4 (continued)

Metabolite and sample type	Location, population group, and collection date	No. of samples	Mean (range)	Median (IQR)	Relevant percentiles	Method of dietary assessment	Comments	Reference
Dietary exposure (cont.)							Scenario 3: all foods and beverages identified in scenarios 1 and 2, added to those in which there was uncertainty of the use of the sweetener, but whose food group indicated the possibility to contain it	Duarte et al. (2022) (cont.)
Dietary exposure	South-eastern Santiago, Chile, 2016–2017	961 low-and middle-income pre-schoolers	2016: 24.6 mg/day 2017: 39.6 mg/day			24-hour dietary recalls		Rebolledo et al. (2022)
Dietary exposure	Brazil, nationwide survey in 2008–2009	34 003 participants	0.08 mg/kg bw per day			1-day dietary records	Intake in general consumers from processed food and beverages and tabletop usage in beverages	Barraj et al. (2021)
Dietary exposure	France, NutriNet-Santé cohort, 2009–2020	106 489 adults	8.63 mg/day 0.13 mg/kg bw per day	0.0 mg/day	95th percentile: 49.92 mg/day, 0.72 mg/kg bw per day	Three non-consecutive web-based 24-hour dietary records		Chazelas et al. (2021)
Dietary exposure	NHANES, USA, 2013–2016 Toddlers aged 12–35 months	126	Scenario 1: 7.4 Scenario 2: 3.4 Scenario 3: 1.7		23.7 10.8 4.3	2 complete days of dietary recalls	Scenario 1: brand loyal-deterministic. Reported maximum global use level applied to all beverages.	Tran et al. (2021)

Table 1.4 (continued)

Metabolite and sample type	Location, population group, and collection date	No. of samples	Mean (range)	Median (IQR)	Relevant percentiles	Method of dietary assessment	Comments	Reference
Dietary exposure (cont.)	Children aged 3–9 yr	579	Scenario 1: 4.4 Scenario 2: 2.2 Scenario 3: 1.4		11.6 6.1 4.3		Scenario 2: brand loyal-deterministic. Reported maximum global use level applied to LNCS CSD; average use level applied to all other beverages. Scenario 3: market share deterministic. Reported maximum global use level applied to LNCS CSD; average use level applied to all other beverages; corresponding fraction of specific LNCS-containing market share data applied to each beverage type.	Tran et al. (2021) (cont.)
	Adolescents aged 10–17 yr	665	Scenario 1: 2.8 Scenario 2: 1.4 Scenario 3: 1.1		7.0 3.9 3.4			
	Adults aged 18–64 yr	2331	Scenario 1: 3.7 Scenario 2: 2.6 Scenario 3: 2.3		10.6 8.5 8.1			
	Elderly aged 65–74 yr	436	Scenario 1: 3.7 Scenario 2: 2.5 Scenario 3: 2.3		13.2 9.0 8.4			
	Very elderly aged 75+ yr	279	Scenario 1: 2.7 Scenario 2: 1.9 Scenario 3: 1.8 (units: mg/kg bw per day)		6.5 5.5 5.4 (95th percentile, units: mg/kg bw per day)			
	UK, NDNS RP, 2008/2009–2016/2017					A 4-day estimated diet diary		
	Toddlers 18–35 months	423	Scenario 1: 12.5 Scenario 2: 5.9 Scenario 3: 4.8		34.4 15.6 13.3			
	Children 3–9 yr	1921	Scenario 1: 7.9 Scenario 2: 4.0 Scenario 3: 3.4		23.3 11.5 10.3			
	Adolescents 10–17 yr	2225	Scenario 1: 4.0 Scenario 2: 2.4 Scenario 3: 2.1		11.5 6.8 6.3			
	Adults 18–64 yr	5019	Scenario 1: 4.7 Scenario 2: 2.4 Scenario 3: 2.3		11.6 6.0 5.6			

Table 1.4   (continued)

Metabolite and sample type	Location, population group, and collection date	No. of samples	Mean (range)	Median (IQR)	Relevant percentiles	Method of dietary assessment	Comments	Reference
Dietary exposure (cont.)	Elderly aged 65–74 yr	818	Scenario 1: 5.5 Scenario 2: 2.6 Scenario 3: 2.4		13.0 6.2 5.8			Tran et al. (2021) (cont.)
	Very elderly aged 75+ yr	574	Scenario 1: 5.5 Scenario 2: 2.6 Scenario 3: 2.4 (units: mg/kg bw per day)		11.0 5.0 4.7 (95th percentile, units: mg/kg bw per day)			
Dietary exposure	Children aged 6–12 yr residing in Santiago, 2018	250		0.88 (0.25–2.10) mg/kg bw per day		FFQ		Martínez et al. (2020)
Dietary exposure	USA, 2002 and 2018 (Nielsen Homescan Consumer panels)	Number not available				Household purchase data	Drop in prevalence of households purchasing products containing aspartame from 60% to 49%. Aspartame had highest volume per capita purchased of all NNS both years (94.7 g/day and 80 g/day)	Dunford et al. (2020)
Dietary exposure	Pregnant women from the two main cities in Chile, 2016	601	0.91 mg/kg bw per day			Survey		Fuentealba Arévalo et al. (2019)

Table 1.4 (continued)

Metabolite and sample type	Location, population group, and collection date	No. of samples	Mean (range)	Median (IQR)	Relevant percentiles	Method of dietary assessment	Comments	Reference
Dietary exposure	A representative sample of Irish adults aged 18–90 yr, the National Adult Nutrition Survey (NANS 2011)	1500	Total population Tier 1: 1.05 Tier 2: 0.62 Tier 3: 0.28 Consumers only Tier 1: 1.04 Tier 2: 0.66 Tier 3: 0.80 (units: mg/kg bw per day)		Total population Tier 1: 5.18 Tier 2: 4.63 Tier 3: 3.57 Consumers only Tier 1: 8.64 Tier 2: 8.65 Tier 3: 7.11 (99th percentile, units, mg/kg bw per day)	4-day, semi-weighed food diaries	Tier 1: a crude assessment that assumed that all foods permitted to contain the additives of interest always did contain them, and at their maximum permitted level. Tier 2: refined assessments estimated intakes of the six sweeteners using food consumption data up to brand level with additive occurrence data from a survey of products currently available on the Irish market. Tier 3: sweetener concentration data (including data from all brands analysed) with sweetener occurrence data from a food label survey.	Buffini et al. (2018)

Table 1.4   (continued)

Metabolite and sample type	Location, population group, and collection date	No. of samples	Mean (range)	Median (IQR)	Relevant percentiles	Method of dietary assessment	Comments	Reference
Dietary exposure	Children and adolescents aged 2–18 yr, First Food and Nutritional/Nutrition Survey of Buenos Aires City, Argentina, 2011	2664				1 day 24-hour recall interview		Garavaglia et al. (2018)
	Age 2–4 yr		6.8	5.5 (2.9–9.3)				
	Age 5–12 yr		4.2	3.2 (1.7–5.3)				
	Age 13–18 yr		2.9 (units: mg/kg bw per day)	2.2 (1.3–4.1) (units: mg/kg bw per day)				
Dietary exposure	Adult participants residing in south-western Virginia, USA, 2015	125	FFQ: 38.7 (0.0–694.5) Dietary recall: 36.5 (0.0–526.4) (units: mg/day)	FFQ: 7.1 Dietary recall: 1.2 (units: mg/day)		FFQ, dietary recall		Myers et al. (2018)
Dietary exposure	Spain, ANIBES study (anthropometric data, macronutrients and micronutrients intake, practice of physical activity, socioeconomic data and lifestyles), a cross-sectional study of a representative sample of the Spanish population (aged 9–75 yr), 2013	2009			Prevalence: 10.7%	3-day dietary records		Samaniego-Vaesken et al. (2018)

Table 1.4 (continued)

Metabolite and sample type	Location, population group, and collection date	No. of samples	Mean (range)	Median (IQR)	Relevant percentiles	Method of dietary assessment	Comments	Reference
Dietary exposure	Republic of Korea, Korea National Health and Nutrition Survey (KNHNES), 2010–2013	20 788				Food consumption data (total diet study)		Kim et al. (2017)
	Whole population Age < 2 yr Age 3–6 yr Age 7–12 yr Age 13–19 yr Age 20–64 yr Age > 65 yr		0.33 1.28 0.97 0.71 0.54 0.23 0.14 (units: mg/kg bw per day)		95th percentile 1.49 3.98 3.40 2.60 2.15 1.09 0.69 (units: mg/kg bw per day)			
Dietary exposure	INRAN-SCAI, 2005–2006	3323	Step 1: 0.97 Step 2: 0.78 (units: mg/kg bw per day)		95th percentile: 3.85 3.46 (units: mg/kg bw per day)	Food label survey (market share data)	Step 1: all food categories and sweeteners according to the legislation and maximum levels. Step 2: only food categories and sweeteners according to the label survey and maximum levels.	Le Donne et al. (2017)
Dietary exposure	University students Chile, male Chile, female Peru, male Peru, female Guatemala, male Guatemala, female Panama, male Panama, female	1229 155 317 64 140 36 217 84 216	0.7 (0.07–1.8) 1.0 (0.9–2.3) 0.6 (0.0–3.7) 0.3 (0.01–3.3) 0.4 (0.1–1.7) 2.1 (0.1–4.7) 0.9 (0.3–2.3) 0.9 (0.3–2.8) (units: mg/kg bw per day)			Survey		Durán Agüero et al. (2017)

Table 1.4 (continued)

Metabolite and sample type	Location, population group, and collection date	No. of samples	Mean (range)	Median (IQR)	Relevant percentiles	Method of dietary assessment	Comments	Reference
Dietary exposure	USA, Nurses' Health Study (NHS), female registered nurses, aged 30–55 yr, 1994	77 218	All: 102 mg/day			FFQ		Schernhammer et al. (2012)
	USA, Health Professionals Follow-Up Study (HPFS), male health professionals, aged 40–75 yr, 1994	47 810	All: 114 mg/day					
Dietary exposure	Belgium, children with diabetes mellitus, 2014				95th percentile for all, consumers only	FFQ		Dewinter et al. (2016)
	Age 1–6 yr Age 7–12 yr Age 13–18 yr	242	0.91 1.07 0.68 (units: mg/kg bw per day)		2.89, 3.89 2.07, 3.46 1.41, 2.72 (units: mg/kg bw per day)			
Dietary exposure	Ireland, pre-schoolchildren (the National Pre-School Nutrition Survey, NPNS, 2010–2011)	500				4-day weighed food diary	Scenario 1: the relevant MPL. Scenario 2: the relevant MPL, taking into account the occurrence of the sweetener in food. Scenario 3: sweetener concentration data; in cases where no analytical data were available for a specific food category, the MPL was applied.	Martyn et al. (2016)
	Total population		Scenario 1: 4.23 Scenario 2: 3.50 Scenario 3: 0.93 Scenario 4: 0.66 (units: mg/kg bw per day)		95th percentile: Scenario 1: 16.42 Scenario 2: 16.07 Scenario 3: 3.17 Scenario 4: 2.72 (units: mg/kg bw per day)			

Table 1.4   (continued)

Metabolite and sample type	Location, population group, and collection date	No. of samples	Mean (range)	Median (IQR)	Relevant percentiles	Method of dietary assessment	Comments	Reference
Dietary exposure (cont.)	Sweetener consumers only		Scenario 1: 4.63 Scenario 2: 5.06 Scenario 3: 1.02 Scenario 4: 0.76 (units: mg/kg bw per day)		95th percentile: Scenario 1: 18.21 Scenario 2: 18.20 Scenario 3: 3.30 Scenario 4: 2.82 (units: mg/kg bw per day)		Scenario 4: sweetener concentration data, taking into account the occurrence of the sweetener in food; in cases where no analytical data were available for a specific food category, the MPL was applied.	Martyn et al. (2016) (cont.)
Biological level (urine and blood)	Tianjin, China, May 2010 to June 2010 Urine and blood in the general population	54	Not detected			NA	Fasting (> 8 hours) samples	Zhang et al. (2016)
Dietary exposure	French survey on individual dietary consumption (BEBE-SFAE dietary survey), randomly sampled children under age 3 yr, 2005					3 consecutive days, food diary		Mancini et al. (2015)
	1–4 months 5–6 months 7–12 months 13–36 months	124 127 195 259	0.19 2.14 7.41 14.11 (units: mg/kg bw per day)	0 0 4.109 12.632 (units: mg/kg bw per day)	0 8.58 19.03 23.93 (90th percentile, units: mg/kg bw per day)			
Biological level (breast milk)	USA, lactating volunteer women, year not indicated	20	Not detected			NA		Sylvetsky et al. (2015)

Table 1.4 (continued)

Metabolite and sample type	Location, population group, and collection date	No. of samples	Mean (range)	Median (IQR)	Relevant percentiles	Method of dietary assessment	Comments	Reference
Dietary exposure	USA, men and women aged 47–95 yr, the Cancer Prevention Study II (CPS-II) nutrition cohort, 1999–2009	100 442 (43 350 men and 57 092 women)	46.6 mg/day	12.6 mg/day (median in quintile 3)	Median in quintile 5 (highest): 145 mg/day	FFQ		McCullough et al. (2014)
Dietary exposure	Norway, dietary surveys, Småbarnskost 2007 and Norkost 3					NR	Scenario 1: Content: average content of aspartame (adjusted for sale). Consumption: actual consumption (the real distribution of consumed beverages added sweeteners from the dietary survey). Scenario 2: Content: average content of aspartame (adjusted for sales). Consumption: it was assumed that all consumed soft drinks, "saft", or nectar contained sweeteners (no sugar).	VKM (2014)
	Age 2 yr	542 1216 542 1216	Scenario 1: 1.50 Scenario 2: 1.73 Scenario 3: 1.79 Scenario 4: 2.03		Scenario 1: 4.32 Scenario 2: 5.29 Scenario 3: 5.11 Scenario 4: 6.26			

Table 1.4 (continued)

Metabolite and sample type	Location, population group, and collection date	No. of samples	Mean (range)	Median (IQR)	Relevant percentiles	Method of dietary assessment	Comments	Reference
Dietary exposure (cont.)	Women aged 18–29 yr	39 93 39 93	Scenario 1: 2.46 Scenario 2: 2.61 Scenario 3: 2.92 Scenario 4: 3.10		Scenario 1: 9.37 Scenario 2: 9.61 Scenario 3: 11.15 Scenario 4: 11.45		Scenario 3: Content: the highest value for the amount of added aspartame in soft drinks and "saft" was used for the calculation. Consumption: actual consumption (the real distribution of consumption of beverages added sweeteners from the dietary survey). Scenario 4: Content: the highest value for the amount of added aspartame in soft drinks, "saft", and nectar was used for the calculation. Consumption: it was assumed that all consumed soft drinks, "saft", or nectar contained sweeteners (no sugar).	VKM (2014) (cont.)
	Men aged 18–29 yr	31 100 31 100	Scenario 1: 1.94 Scenario 2: 3.24 Scenario 3: 2.31 Scenario 4: 3.85		Scenario 1: 4.33 Scenario 2: 8.92 Scenario 3: 5.15 Scenario 4: 10.61			
	Women aged 30–70 yr	209 350 209 350	Scenario 1: 2.56 Scenario 2: 2.28 Scenario 3: 3.04 Scenario 4: 2.71		Scenario 1: 7.88 Scenario 2: 6.54 Scenario 3: 9.38 Scenario 4: 7.79			
	Men aged 30–70 yr	165 365 165 365	Scenario 1: 2.34 Scenario 2: 2.22 Scenario 3: 2.79 Scenario 4: 2.64 (units: mg/kg bw per day)		Scenario 1: 6.79 Scenario 2: 6.53 Scenario 3: 8.08 Scenario 4: 7.78 (95th percentile, units: mg/kg bw per day)			
Dietary exposure	Italy, INRAN-SCAI, age 18–97 yr, 2005 Aged 1–17 yr, 2005 UK, National Diet and Nutrition Surveys (NDNS)	3323	Tier 2: 0.957 Tier 3: 0.182 Tier 2: 3.098 Tier 3: 0.622		Tier 2: 4.055 Tier 3: 1.176 Tier 2: 12.159 Tier 3: 3.884	3-day estimated food diary 4-day or 7-day weighed food diary	Tier 2: Calculated by combining consumption data with the MPLs Tier 3: Calculated by combining consumption data with concentration data	Vin et al. (2013)

Table 1.4 (continued)

| Metabolite and sample type	Location, population group, and collection date	No. of samples	Mean (range)	Median (IQR)	Relevant percentiles	Method of dietary assessment	Comments	Reference
Dietary exposure (cont.)	Aged > 65 yr, 1994–1995	1687	Tier 2: 1.526 Tier 3: 0.307		Tier 2: 4.376 Tier 3: 1.152			Vin et al. (2013) (cont.)
	Aged 19–64 yr, 2000–2001	1724	Tier 2: 2.931 Tier 3: 0.572		Tier 2: 9.629 Tier 3: 2.004			
	Aged 4–18 yr, 1997	1701	Tier 2: 7.445 Tier 3: 1.622		Tier 2: 18.782 Tier 3: 4.906			
	Age 1.5–4.5 yr, 1992–1993	1675	Tier 2: 12.123 Tier 3: 3.069		Tier 2: 28.042 Tier 3: 8.738			
	France, INRAN-SCAI	4079				7-day estimated food diary		
	Age 18–79 yr, 2005		Tier 2: 1.290 Tier 3: 0.530		Tier 2: 4.620 Tier 3: 2.500			
	Age 3–17 yr, 2005		Tier 2: 2.210 Tier 3: 1.000		Tier 2: 6.610 Tier 3: 3.850			
	Ireland, North/South Ireland Food Consumption Survey (NSIFCS), National Children's Food Survey (NCFS), National Teens Food Survey (NTFS)					7-day estimated or weighed food diary		
	Age 18–64 yr, 1997–1999	1379	Tier 2: 4.928 Tier 3: 1.018		Tier 2: 16.175 Tier 3: 3.653	7-day estimated food record		
	Age 13–17 yr, 2005–2006	441	Tier 2: 5.851 Tier 3: 0.989		Tier 2: 14.041 Tier 3: 2.600	7-day estimated food record		
	Age 5–12 yr, 2003–2004	594	Tier 2: 10.498 Tier 3: 1.962 (units: mg/kg bw per day)		Tier 2: 33.034 Tier 3: 5.137 (97.5th percentile, units: mg/kg bw per day)	7-day weighed food record		

Table 1.4 (continued)

Metabolite and sample type	Location, population group, and collection date	No. of samples	Mean (range)	Median (IQR)	Relevant percentiles	Method of dietary assessment	Comments	Reference
Dietary exposure	Belgian Food Consumption Survey, Belgian inhabitants aged ≥ 15 yr, 2004	3245	1.95 mg/kg bw per day	1.74 mg/kg bw per day	4.11, 4.76 (90th, 99th percentile, units: mg/kg bw per day)	2 non-consecutive 24-hour recalls		Huvaere et al. (2012)
Dietary exposure	Japan, National Nutrition Survey, adults aged > 20 yr, 2001–2003	NA		0.14 mg/day		Market basket method	The daily intake based on the analysis of individual foods was more than twice that of the market basket method; therefore, the analysis of individual foods was used.	Kawasaki et al. (2011)
Dietary exposure	Denmark, random sample of people aged 1–80 yr, 1995 Both sexes, aged 1–80 yr Both sexes, aged 1–3 yr Both sexes, aged 1–6 yr Boys, aged 7–10 yr Girls, aged 7–10 yr	3098	Scenario 1: 0.03 Scenario 2: 0.04 Scenario 1: 0.40 Scenario 2: 0.50 Scenario 1: 0.29 Scenario 2: 0.40 Scenario 1: 0.29 Scenario 2: 0.40 Scenario 1: 0.26 Scenario 2: 0.34 (units: mg/kg bw per day)		0.52, 1.77 0.64, 2.11 1.61, 3.26 2.08, 4.28 1.43, 2.80 1.76, 3.81 1.22, 2.63 1.62, 3.37 1.02, 1.73 1.30, 2.21 (90th, 99th percentile, units: mg/kg bw per day)	A questionnaire with 20 beverages (7 consecutive-day record)	Scenario 1: calculated using analytical determined mean value for all the light samples in the category. Scenario 2: calculated using analytical determined mean value for all the light samples with the content of the specific sweetener.	Leth et al. (2008)

Table 1.4 (continued)

Metabolite and sample type	Location, population group, and collection date	No. of samples	Mean (range)	Median (IQR)	Relevant percentiles	Method of dietary assessment	Comments	Reference
Dietary exposure	USA (California, Florida, Pennsylvania, New Jersey, North Carolina, Louisiana, and metropolitan Atlanta and Detroit), NIH-AARP Diet and Health Study cohort, age 50–71 yr, 1995–1996	473 984	All: 111 Users: 205 (units: mg/day)			FFQ		Lim et al. (2006)
Dietary exposure	Republic of Korea, National Health and Nutrition Survey, 1998 All participants Age 1–2 yr Age 3–6 yr Age 7–12 yr Age 13–19 yr Age 20–29 yr Age 30–49 yr Age 50–64 yr Age > 65 yr	11 525	7.7, 0.14 10.5, 0.86 13.1, 0.71 11.6, 0.34 14.5, 0.26 8.3, 0.14 5.2, 0.084 3.8, 0.060 3.4, 0.058 (units: mg/day, mg/kg bw per day)			24-hour recall		Chung et al. (2005)
Dietary exposure	Rome, Italy, randomly selected teenagers, 2000	3982	All: 0.039 Consumers only: 0.054 (units: mg/kg bw per day)		0.170 (95th percentile, unit: mg/kg bw per day)	12-day (3 days of 4 periods) food records		Arcella et al. (2004)

Table 1.4 (continued)

Metabolite and sample type	Location, population group, and collection date	No. of samples	Mean (range)	Median (IQR)	Relevant percentiles	Method of dietary assessment	Comments	Reference
Dietary exposure	France, children with type I diabetes, aged 2–20 yr, 1997	227 (112 girls and 115 boys)	All: 69.4, 1.9 Consumers only: 82.0, 2.4 (units: mg/day, mg/kg bw per day)	All: 44, 1.1 Consumers only: 55.5, 1.4 (Units: mg/day, mg/kg bw per day)	All: 255.8, 7.8 Consumers only: 263.8, 7.8 (97.5th percentile, units: mg/day, mg/kg bw per day)	5-day diary record		Garnier-Sagne et al. (2001)
Dietary exposure	Rome, Italy, children, aged 13–19 yr, 1996	241	Consumers only: 0.03 mg/kg bw per day	Consumers only: 0.01 mg/kg bw per day	0.13 mg/kg bw per day (95th percentile)	14 consecutive-day food records		Leclercq et al. (1999)
Dietary exposure	Germany, 1988	2291 99	All: 0.05 Users: 1.21 (units: mg/kg bw per day)	All: 0.00 Users: 0.86 (units: mg/kg bw per day)	All: 0.00 Users: 2.75 (90th percentile, units: mg/kg bw per day)	24-hour food diary		Bär and Biermann (1992)

bw, body weight; CPS-II, Cancer Prevention Study II; CSD, carbonated soft drinks; EDMI, Estudo Multicêntrico de Deficiência de Iodo; FFQ, food frequency questionnaire; INRAN-SCAI, Italian National Food Consumption Survey; LNCS, low- and no-calorie sweeteners; MPL, maximum permitted level; NA, not applicable; NCFS, National Children's Food Survey; NDNS RP, National Diet and Nutrition Survey Rolling Programme; NHANES, National Health and Nutrition Examination Survey; NHS, Nurses' Health Study; NIH-AARP, National Institutes of Health-American Association of Retired Persons Diet and Health Study; NNS, non-nutritive sweetener; NR, not reported; NSIFCS, North/South Ireland Food Consumption Survey; NTSF, National Teens Food Survey; yr, year(s).

Fig. 1.3 Aspartame exposure reported in general population-based studies
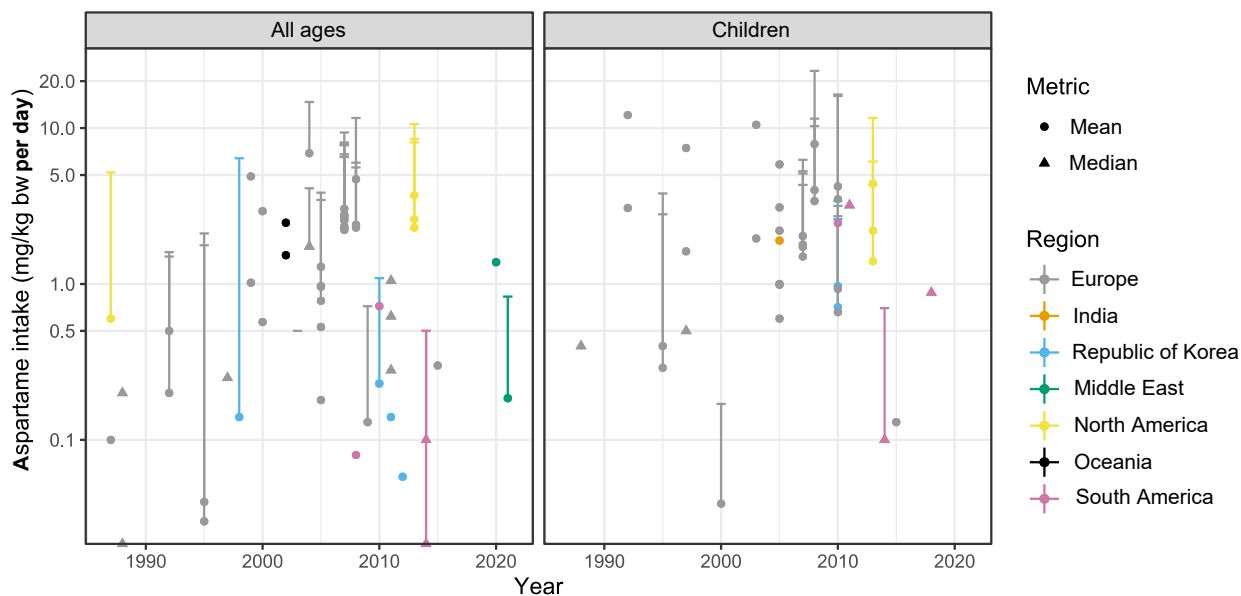
Aspartame intake in the general population (all ages or children only) reported in the scientific literature (see Table 1.4). The scale is logarithmic; circles, triangles, and lines represent mean, median and 95th-percentile values for all participants surveyed (consumers and non-consumers). The dates represent the years for which the aspartame content in foods was calculated, if this information was available, otherwise it represents the year that the dietary intake was assessed.
Created by the Working Group.

in which the assumed levels of aspartame in food varied from the highest permitted level (the most conservative) to the actual levels of use (European Commission, 2001). For example, in a study among Italian adults (Italian National Food Consumption Survey, INRAN-SCAI) in 2005, the mean intake using conservative estimates was higher (0.957 mg/kg body weight (bw) per day) than the mean intake using actual levels (0.182 mg/kg bw per day) (Vin et al., 2013). [The Working Group noted that direct comparisons between studies should be made with caution because of these methodological differences.]

European countries, including Belgium, Denmark, France, Germany, Ireland, Italy, Norway, Portugal, and Spain started conducting assessments in the late 1980s. Early studies based on a small general population in Germany (Bär and Biermann, 1992) and in Italy (Leclercq et al., 1999) indicated rather low estimates (median,

< 1 mg/kg bw per day) among consumers, although a study based on children with type I diabetes in France indicated a slightly higher estimate (1.4 mg/kg bw per day) (Garnier-Sagne et al., 2001). The studies based on larger sample sizes in Italy (Arcella et al., 2004) and Denmark (Leth et al., 2008) also reported the same level of low consumption among the general population. Other studies reported slightly higher estimates of > 1 mg/kg bw per day in some age groups in Belgium (Huvaere et al., 2012), the UK and Ireland (Vin et al., 2013), and Norway (VKM, 2014), even when actual consumption levels were used to estimate realistic intake level. A study in children aged < 3 years in France reported that mean estimated exposure was up to 14 mg/kg bw per day among children aged 13–36 months (Mancini et al., 2015). However, European studies conducted after 2010 reported lower mean estimates (less than or about 1 mg/kg bw per day) in

most tiers (Dewinter et al., 2016; Martyn et al., 2016; Buffini et al., 2018; Carvalho et al., 2022). A study in the USA and UK reported a consumption of up to 4.8 mg/kg bw per day for toddlers (aged 12–35 months in the USA or 18–35 months in the UK) (Tran et al., 2021). EFSA evaluated aspartame as a food additive in 2013 and assessed exposure of the general population in 17 European countries using data from 26 different dietary surveys (EFSA, 2013). The EFSA Scientific Panel on Food Additives and Nutrient Sources added to Food estimated exposure based on the maximum permitted levels and actual levels of aspartame for the general population. Mean levels of exposure for adults (aged 18–64 years) under the conservative approach using the maximum permitted levels were 0.8–8.6 mg/kg bw per day, depending on the dietary survey. High-level exposures, defined as the 95th percentile of consumers, were 2.5–27.5 mg/kg bw per day, depending on the dietary survey. [The Working Group noted that the methodology employed by EFSA for risk assessment was based on the assumption that food groups contained the maximum permitted concentration of aspartame. Therefore, this approach would lead to an overestimation of exposure. Other exposure estimates based on actual levels of use in the general population were highly variable but seemed generally to be much lower (between almost 0 and up to 5 mg/kg bw per day; see Fig. 1.3).]

Data from North America (USA and Canada) have been reported since the late 1990s among large-scale cohort populations (Lim et al., 2006; Schernhammer et al., 2012; McCullough et al., 2014; Myers et al., 2018). Because of the use of FFQs and dietary recalls for estimation, values may not be comparable. Using a total diet study methodology, one study considering data from the National Health and Nutrition Examination Survey (NHANES) in 2013–2016 as the basis for the selection of products indicated that estimated mean levels in a market share deterministic scenario ranged from 1.1 mg/kg bw per day for adolescents to 2.3 mg/kg bw per day for the elderly and adults (Tran et al., 2021). Studies based on a representative large sample in Asian countries were limited to mainly to the Republic of Korea and indicated a mean intake of < 1 mg/kg bw per day for the whole population (Chung et al., 2005; Kim et al., 2017). More recently, data were reported from countries in South America (Brazil, Chile, Argentina) (Durán Agüero et al., 2017; Garavaglia et al., 2018; Fuentealba Arévalo et al., 2019; Martínez et al., 2020; Barraj et al., 2021; Rebolledo et al., 2022) and the Middle East (Lebanon) (Daher et al., 2022). These more recent data appeared to be generally lower (almost 0 to 2.1 mg/kg bw per day, although methods used varied), except for one study from Argentina that reported values of up to 6.8 mg/kg bw per day for children (aged 2–4 years).

Although it was difficult to compare temporal or geographical trends in exposure levels because of methodological differences, some studies have reported a decrease in aspartame use between 2002 and 2018 in the USA (Dunford et al., 2022). [The Working Group noted that mean or median exposure levels for adults in general were consistently at low levels (almost 0 to about 5 mg/kg bw per day, depending on the estimation scenario). Children, adolescents, and other populations such as people with diabetes tended to have elevated levels. Estimated levels will be higher when considering consumers only, but this information was only available in studies that measured intake at the individual level. The proportion of consumers of aspartame-containing products varied between countries and time points, which could explain some of the differences observed between countries, e.g. the very low average consumption levels reported in some studies. The Working Group also noted that there was considerable variability in the data underlying these estimates, and that very few studies used analytical data linked to actual brand consumption scenarios, probably because

of difficulties in collecting data and also in keeping the product database up to date.]

1.5 Regulations and guidelines

Adopted in 1995, the General Standards for Food Additives in the Codex Alimentarius of the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) established the list of food additives, including aspartame, that are suitable for use in food (FAO/WHO, 1995). In addition to the foods in which aspartame could be used, this document also describes the maximum use levels (ranging from 300 mg/kg to 10 000 mg/kg) for each food category (FAO/WHO, 1995).

Within the European Union, several member states have authorized aspartame for use in foods and as a tabletop sweetener since the 1980s. Sweeteners were first regulated in the European Union in the 1990s with the entry into force of Directive 94/35/EC of the European Parliament and Council Directive 94/35/EC on sweeteners in foodstuffs, also known as the "Sweeteners Directive" (European Parliament and Council, 1994). In 2008, the European Parliament and the Council of the European Union adopted a framework regulation, Regulation (EC) No. 1333/2008, to harmonize all current uses of food additives, including sweeteners (European Parliament and Council, 2008). Annex II of this legislation, established by Commission Regulation (EU) No. 1129/2011, provides a Union list of sweeteners approved for use in foods, beverages, and tabletop sweeteners, and their conditions of use (European Commission, 2011). Under the Commission Regulation (EU) No. 1129/2011 on food additives for use in foodstuffs, maximum permitted levels were established, ranging from 25 to 6000 mg/kg in foods, with the exception of the tabletop sweeteners, for which it is authorized quantum satis (no numerical maximum is defined) (European Commission, 2011). The use of aspartame in organically produced foods is not approved (European Commission, 2008). Regarding the use of aspartame in pharmaceuticals, warnings for patients with phenylketonuria with a zero threshold for use were established, and specific information should be mentioned in the package leaflet (EMA/CHMP/302620/2017; European Medicines Agency, 2019). Similarly, in the European Union and USA, foods containing aspartame in the list of ingredients must indicate in the labelling that they include a source of phenylalanine (US FDA, 1974; European Parliament and Council, 1994, 2011).

In the USA, the United States Food and Drug Administration (US FDA) approved aspartame for restricted use in dry foods in 1974 and for uses under certain conditions, as a tabletop sweetener, in chewing gum, cold breakfast cereals, and dry bases for certain foods (i.e. beverages, instant coffee and tea, gelatins, puddings, and fillings, and dairy products and toppings) in 1981 (Office of the Federal Register, 1981). The US FDA approved the use of aspartame in carbonated beverages and carbonated beverage syrup bases in 1983, and its use as a general-purpose sweetener in 1996 (Office of the Federal Register, 1996).

Aspartame has been assessed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) on several occasions (JECFA, 1975, 1976, 1980, 1981), and an acceptable daily intake (ADI) of 40 mg/kg bw per day has been established (JECFA, 1981). EFSA also adopted this ADI of 40 mg/kg bw per day (EFSA, 2013). The US FDA has set the ADI for aspartame at 50 mg/kg bw (Office of the Federal Register, 1984). An ADI of 7.5 mg/kg bw per day was also established for the diketopiperazine of aspartame (JECFA, 1981). The purity of aspartame for use as a food additive is regulated in the European Union (see Section 1.1.4) (European Commission, 2012). Purity regulations are also available for aspartame in medicinal products (European Pharmacopoeia, 2020; United States Pharmacopeia, 2023).

[The Working Group noted that no threshold has been established for occupational exposure to aspartame.]

1.6 Quality of exposure assessment in key epidemiological studies of cancer and mechanistic studies in humans

1.6.1 Quality of exposure assessment in key cancer epidemiology studies

See Table S1.2 (Annex 1, Supplementary material for Section 1, Exposure Characterization, online only, available from: https://publications.iarc.who.int/627).

This section reviews the exposure assessment methods of studies of cancer in humans evaluated in Section 2 of the present monograph and was organized according to the exposure of interest stated in the original publications (aspartame, artificial sweeteners, or ASBs).

No validated biomarker of exposure was available for aspartame (see Section 1.3.4). Aspartame exposure in cancer epidemiology studies was assessed using self-report methods. Few studies specifically considered aspartame as the exposure; most derived aspartame exposure from the consumption of ASBs and the use of tabletop sweetener packets containing aspartame, assigning aspartame concentration values to these products. Other studies considered artificial sweeteners overall or ASBs. Studies were either cohort studies or case–control studies, and most used FFQs and/or 24-hour dietary recalls or records. The studies and their dietary assessment methods are described in detail below.

There are common limitations to the use of ASBs and packets of tabletop artificial sweeteners as sole sources and proxies for aspartame exposure. First, although ASBs, followed by tabletop artificial sweetener packets, can be considered as the main sources of aspartame (Section 1.4.1), other potential dietary sources also contribute to total aspartame exposure (e.g. "sugar-free", "diet", or "light" versions of foods such as yogurt, gelatin/pudding, ice cream, frozen desserts, hot chocolate, or breakfast cereals). These other sources have become more diversified and abundant over time, and their impact on total aspartame exposure has tended to increase. Additionally, most epidemiological studies did not account for aspartame from oral sources other than foods (including tabletop sweeteners) and beverages, i.e. they did not account for aspartame from dietary supplements, medicinal products, or personal care products. Second, even though aspartame was generally the main type of artificial sweetener used in ASBs at the time when the studies were conducted, the actual aspartame content (presence/absence and dose) of ASBs was variable across product types and brands, and over time and between countries (Section 1.2 and Section 1.4.1). After the introduction of aspartame in the early 1980s, individuals may have been exposed to multiple types and doses of artificial sweeteners depending on the evolution of both authorizations and use of artificial sweeteners. This is important to consider when looking at the timing of the baseline assessment and for the interpretation of the follow-up period as an ongoing exposure to aspartame. Finally, the range of ASBs included in the FFQs was variable across studies and may comprise only cola or only carbonated beverages and, less commonly, non-carbonated beverages. Hence there was potential for misclassification of aspartame exposure, with underestimation of exposure and misclassification of consumers as non-consumers because of the partial consideration of dietary sources, but also overestimation and misclassification of non-consumers as consumers because of the assumption that all ASBs contained aspartame, which was not verified in all time periods and regions.

Other limitations concerned the methods of dietary assessment and were shared by studies

using the same tool. FFQs, used by the majority of studies, capture the usual frequency of consumption over a period of time (usually the past 12 months) of a list of food items. In FFQs, the number and type of included items, the range of frequencies available as response options, as well as the possibility of choosing between several portion sizes (versus standard portion sizes applied to all) are important parameters influencing the quality of assessment of aspartame, artificial sweetener, or ASB intakes. A detailed assessment of aspartame exposure from FFQs would be improved by differentiation between sugar-sweetened and artificially sweetened versions for every food item for which these may exist, and by collection of the exact type but also the name and brand of the product usually consumed, since aspartame content varies between product types and between brands for the same type of product. FFQs are generally considered to be efficient tools for ranking individuals according to their dietary intakes, although they may be prone to recall error when participants are asked to provide a description of their diet during the past 1 year, since recall is known to be influenced by more recent intake (Fowke et al., 2004). FFQs are also less precise for the characterization of food and beverages ingested owing to the aggregation of products into food or line items. In contrast, 24-hour dietary records or recalls collect information on all food and beverages consumed during a 24-hour period. Hence the consumption of virtually all types of aspartame-containing product can be captured, depending on the level of detail of the nomenclature used and on the completeness of the underlying food composition databases. Several days of 24-hour dietary records or recalls at different times of the year are nonetheless necessary to consider day-to-day and seasonal variations in dietary intakes and provide a valid assessment of usual dietary intakes, especially when considering foods or beverages that are not consumed daily (including aspartame-containing products).

For both FFQs and 24-hour recalls or records, a major challenge involves the food composition databases available, since the detail on aspartame content (yes/no) and dose of each food and beverage to the level of product brand is ideally required because of the variability in aspartame concentrations in the food supply.

Patterns of consumption of aspartame-containing products may change over time, and this may not be captured in studies that used a single dietary assessment at baseline (especially when considering long follow-up periods). As a result of food innovations and reformulations, new aspartame-containing products may be released onto the market and the aspartame content of products may also vary over time. Beyond assessing changes in dietary intakes over time, food composition tables should also ideally be regularly updated to reflect the exact aspartame content of a given food product or the average aspartame content for a generic food item, considering available products on the market at the time of dietary assessment.

All the above-mentioned limitations are likely to induce measurement errors and some misclassification of participants with regard to consumption of aspartame, artificial sweeteners, or ASBs. Yet in prospective studies such misclassification would be mostly non-differential, i.e. would have a similar effect on the exposure assessment in participants who developed a cancer and in those who did not.

Lastly, the use of a prospective design in cohort studies, in which diet is assessed before cancer diagnosis, limits the risk of differential misclassification. In case–control studies, diet (current or in past years) is assessed after the diagnosis of cancer, and these studies are more at risk of recall bias, biased assessment, and differential misclassification. This may arise if participants with cancer overestimate or underestimate their consumption of artificial sweeteners or

ASBs compared with controls, if early cancer symptoms may induce changes in dietary habits, or if investigators are not blinded to the case or control status.

All studies are reviewed below, classified according to exposure and study design.

(a) Aspartame

(i) Prospective cohort studies

Five cohort studies investigated associations between exposure to aspartame and cancer risk. Four of the five were conducted in the USA, and of these three (Lim et al., 2006; Schernhammer et al., 2012; McCullough et al., 2014) derived aspartame exposure from the consumption of ASBs and the use of aspartame tabletop sweetener packets, assessed through FFQs, whereas the remaining study (Fulgoni and Drewnowski, 2022) assessed exposure through aspartame-containing products reported in 24-hour dietary recalls. More recently, one study in France (Debras et al., 2022b) derived aspartame exposure from all aspartame-containing food and beverages assessed through repeated 24-hour dietary records.

[The Working Group noted that, of all the studies reviewed in Section 1.6.1, these five studies were the only ones that specifically focused on aspartame and provided the most accurate assessment of aspartame exposure. Debras et al. (2022b) performed the most comprehensive and up-to-date assessment of aspartame exposure from all possible sources, whereas the other studies (Lim et al., 2006; Schernhammer et al., 2012; McCullough et al., 2014; Fulgoni and Drewnowski, 2022) only considered the two main sources of aspartame (ASBs and tabletop packets). More details, including the strengths and limitations of each of these studies, are provided below.]

The study by Debras et al. (2022b) was conducted in the NutriNet-Santé prospective cohort study and focused on breast and prostate cancers, obesity-related cancers combined, and all cancers combined. Aspartame intake (in mg/day) was assessed with repeated validated web-based 24-hour dietary records. Three 24-hour dietary records were sent to the participants every 6 months, covering 3 days (2 weekdays, 1 weekend day) randomly assigned over a 2-week period. The main model focused on aspartame estimated from the mean exposure over the first 2 years of follow-up, including a minimum of two dietary records (mean ± standard deviation (SD), 5.6 ± 3.0). In sensitivity analyses, a model accounting for all available dietary records throughout the follow-up was also presented. Measurement of aspartame was calculated from food composition data for all products as follows: presence/absence of additives in food products was retrieved from three large-scale databases: OQALI (a national database the management of which has been entrusted to the National Research Institute for Agriculture, Food and Environment and the French Agency for Food, Environmental and Occupational Health & Safety; INRAE, ANSES, 2023), Open Food Facts (Open Food Facts, 2023), and the Mintel Global New Products Database (Mintel Group Ltd, 2023); quantitative doses of additives were retrieved from ad hoc laboratory assays and completed with information for generic food categories provided by EFSA and JECFA. Changes over time and potential reformulations were accounted for by matching the date of consumption of each food or beverage to the closest date for composition data. The NutriNet-Santé study started in 2009 and is ongoing, with a continuous inclusion strategy. The end of the most recent follow-up for this study was in 2021, hence it had a maximum of 12 years of follow-up. [The Working Group noted that this study provided the most accurate assessment of aspartame exposure, considering all possible dietary sources and including a high level of detail through the use of repeated 24-hour dietary records that collected the brand of industrial product and a detailed food

composition database on variations in aspartame content over time. The baseline assessment between 2009 and 2021 reflected up-to-date consumption patterns of aspartame in France and allowed a sufficient delay between aspartame exposure and the development of cancer. Although this strategy did not allow the consideration of potential changes in dietary behaviours over time, potentially resulting in misclassification of participants, sensitivity analyses were conducted incorporating all 24-hour dietary records available during follow-up. Likewise, there was no information on lifetime exposure to aspartame before study entry, occurring between 2009 and 2021, which could lead to misclassification of participants who had changed or stopped their consumption of aspartame-containing products, although this was considered difficult to quantify.]

The study by Fulgoni and Drewnowski (2022) was conducted using data from the NHANES cycles and focused on cancer mortality. Aspartame exposure was assessed from one or two 24-hour dietary recalls performed by a trained interviewer during NHANES 1988–1994. For other NHANES cycles (1999–2018), only low-calorie sweeteners were considered without distinguishing aspartame, and therefore the analysis of aspartame relied on NHANES 1988–1994 only. Follow-up for cancer mortality was performed through 2019 using public-use mortality files from the National Center for Health Statistics. [The Working Group noted that the study methodology was not reported in sufficient detail. For example, it was unclear whether 2 days of records were used. In addition, although the authors reported identifying a list of products that potentially contained low-calorie sweeteners, for the aspartame assessment the NHANES 1988–1994 study documentation appeared to differentiate aspartame only for diet sodas, hot beverages, or added sweeteners, which was appropriate considering the authorization of aspartame in food products in the USA at that time. However, the assessment relied on a low number of dietary recalls (one or two) and the assessment was performed at baseline only, with up to 31 years of follow-up, during which the use of aspartame in food products changed. There was therefore high potential for non-differential misclassification.]

The study by McCullough et al. (2014) was conducted in the Cancer Prevention Study II (CPS-II) nutrition cohort and focused on lymphoid neoplasms (all types). The usual diet over the past year was assessed using a 152-item self-administered modified Willett FFQ, first at baseline in 1999 and then in 2003, and participants were followed until 2009. There were specific validated questions about the consumption of several types of artificially sweetened carbonated beverage (cola with caffeine, other carbonated beverages with or without caffeine) with frequency categories ranging from "never" to "≥ 4 per day" and a standard serving size (1 glass, bottle, or can, 355 mL). Additional questions related to the use of aspartame tabletop packets, with frequencies ranging from "never" to "≥ 6 per day". Long-term soda consumption patterns were available from data collected in 1982 (CPS-II mortality cohort) but aspartame exposure or intake was not calculated, since aspartame was not included in sodas in the USA before 1983. The mean daily exposure over the past year (in mg/day) was estimated in 1999 and 2003, assigning values for aspartame content of 180 mg/355 mL for low-calorie cola with caffeine, 90 mg/355 mL for other low-calorie sodas with caffeine, 70 mg/355 mL for other low-calorie sodas without caffeine, and 20 mg/packet of one of two branded commercial sweeteners containing aspartame. Estimates from 1999 were used to predict the risk for the 1999–2003 period and the mean of estimates for 1999 and 2003 was used to predict the risk for the 2003–2009 period. [The Working Group noted that the strengths of this study included the updated assessment of usual diet halfway through the maximum

10-year follow-up, allowing the consideration of potential variations in the consumption of ASBs or aspartame tabletop packets over time, thus limiting potential misclassification. Several types of soda were considered with corresponding assigned aspartame values, thus providing a more refined assessment of aspartame intake from different types of beverage. No information was available regarding lifetime aspartame exposure before study entry in 1999, which could misclassify participants who stopped their consumption of ASBs or aspartame tabletop packets. However, some information on patterns of sugar-sweetened and artificially sweetened soda consumption in the early 1980s was available, although artificially sweetened soda did not contain aspartame at that time; hence, this study provided a good assessment of aspartame exposure from artificially sweetened carbonated beverages and tabletop packets. However, other potential dietary sources of aspartame were not considered (although these were more limited), which may cause some non-differential misclassification of participants.]

The study by Schernhammer et al. (2012) was conducted in the Nurses' Health Study (NHS) and the Health Professionals Follow-up Study (HPFS) and focused on lymphoma and leukaemia. A validated self-administered semi-quantitative FFQ of about 130 items was used every 4 years to assess the usual diet of participants, including the frequency of consumption (nine frequencies from never to ≥ 6 times per day) of three types of diet soda (diet cola with caffeine, diet cola without caffeine, and other diet soda) with a standard serving size of 355 mL, starting from 1984 in the NHS and 1986 in the HPFS. The use of two branded commercial sweeteners containing aspartame (added at the table) was queried since 1994 and then every 4 years. Participants were followed until 2006. A cumulative average strategy was used to derive daily intakes of aspartame, whereby intakes were updated after every FFQ with the mean of all reported intakes up to that time. Values of aspartame content were assigned to diet sodas as a weighted average of the representative sodas in that category (i.e. 70–180 mg/serving) and to aspartame packets (20 mg). [The Working Group noted that the repeated assessment of aspartame exposure over a long period (up to 22 years of follow-up) and the use of a cumulative average analysis constitute major strengths of this study. In addition, these cohorts started in the 1980s, that is, concomitantly with the introduction of aspartame in the USA, thus potentially capturing lifetime exposure to aspartame from sodas (and exposure to tabletop aspartame since 1994). Finally, three different types of soda were considered, with corresponding assigned values for aspartame content. Hence, this study provided a good assessment of aspartame exposure from artificially sweetened sodas and tabletop packets. However, other potential dietary sources of aspartame, i.e. non-carbonated soft drinks or other types of foods containing aspartame (although they were limited at the time of this study) were not considered, which may cause some non-differential misclassification of exposure among participants.]

The study by Lim et al. (2006) was conducted in the National Institutes of Health-American Association of Retired Persons Diet and Health Study (NIH-AARP) and focused on haematopoietic cancers and glioma. Usual diet in the past 12 months was assessed at study baseline between 1995 and 1996 using a validated 124-item FFQ, and participants were followed until 2000. Three potentially aspartame-containing beverages were included – soda, fruit drinks, and iced tea – and participants were asked about the frequency of consumption of soft drinks overall (10 frequencies ranging from never to ≥ 6 times per day), the frequency of consumption of the sugar-free or diet versus regular versions of these beverages (usually, or more than half the time) and the portion size (three portion size ranges). The addition of tabletop sweetener packets of

aspartame to cups of coffee and hot tea was also queried. A quantitative average daily exposure to aspartame in milligrams per day was derived from the consumption of diet soft drinks using values of aspartame content assigned per 100 g of beverage (50 mg for diet soda, 14.95 mg for diet fruit drink, 25.55 mg for diet iced tea), and per tabletop sweetener packet (35 mg). [The Working Group noted that the sequential assessment of the frequency of firstly consumption of soft drinks and then of diet/sugar-free versions with imprecise frequencies was likely to induce inaccuracies and thus non-differential misclassification of participants. In addition, this study considered both carbonated and non-carbonated soft drinks but was less precise than the other US studies regarding types of carbonated soft drink (cola or not), which would have an impact on the aspartame exposure assigned to participants (non-differential misclassification). There was a maximum of 5 years between assessment of aspartame exposure and cancer diagnosis; over this limited period of time, the diet could be considered relatively stable, but the impact of aspartame exposure at baseline on the actual onset of cancer was questionable, also because no information was available regarding exposure to aspartame before the study baseline, which occurred 10 years after the introduction of aspartame in the USA. Hence the assessment of aspartame exposure in this study may be weaker than in the other studies described above.]

(ii) Case–control studies

Aspartame was also investigated in relation to cancer in three case–control studies.

Palomar-Cros et al. (2023) conducted a case–control study examining artificial sweetener and common cancers (colorectal, breast, prostate, and stomach, and chronic lymphocytic leukaemia) in Spain. A self-administered, semiquantitative FFQ with 140 food items, capturing usual dietary intake during the previous year, was used. Four items contributed to the assessment of artificial sweetener exposure (low- or no-calorie soft drink, "gaseosa" a typical Spanish soft drink, tabletop sweeteners containing saccharin, and other tabletop sweeteners). The classification of products according to the presence of aspartame was derived from food supply data (OpenFoodFacts, 2023). [The Working Group noted that the validity of the assumption that all low- or no-calorie soft drinks and tabletop sweeteners other than saccharin were aspartame was uncertain, given that this may not have been the case at the time of the exposure assessment (2008–2013). Other potential dietary sources of aspartame were not considered. Also, the retrospective assessment of diet from the year preceding cancer diagnosis could reflect changes in dietary habits as a result of pre-diagnosis symptoms. Non-differential and differential misclassification regarding aspartame exposure was therefore likely in this study.]

Cabaniols et al. (2011) conducted a case–control study on brain cancer in which consumption data were collected in 2005 in France. Aspartame consumption frequency was assessed using a self-administered questionnaire, followed by an in-person interview if information was missing. Participants were asked to recall intakes for the 5 years preceding diagnosis and were categorized as non-consumers (< 1 intake/week) and regular consumers (≥ 1 intake per week). [The Working Group noted that the strength of this assessment was its apparent focus on aspartame, but it was unclear whether this was limited to tabletop use or more broadly included consumption of products containing aspartame. Limitations of the exposure assessment included the retrospective recall of frequency of consumption and the lack of a quantitative assessment (intake was categorized into binary exposure (consumers/non-consumers) only and there was partial consideration of dietary sources). Hence, there was a high potential for misclassification of participants according to their exposure to aspartame in this study.]

Gurney et al. (1997) reported a population-based case–control study of childhood brain tumours in children in the USA. Case patients were born or were in utero in 1981 or later and diagnosed with a primary brain tumour between 1984 and 1991. Biological mothers were queried through in-person interview about their children's consumption of aspartame before the date of diagnosis as well as their own consumption during pregnancy and breastfeeding. Questions related to the age at first consumption of any food, chewing gum, or diet drink containing aspartame, or aspartame tabletop packets, time period of consumption, and frequency of consumption for children, as well as trimesters of consumption, time period of consumption, and frequency of consumption for mothers during pregnancy or while breastfeeding. [The Working Group noted that the period of exposure considered in this study covered lifetime exposure (starting in utero) of the children, which constitutes a major strength. However, only semiquantitative assessment of aspartame exposure could be derived from the questionnaire and, although the sources of aspartame were broadly covered, the lack of specificity regarding the food sources examined may have led to measurement errors. In addition, there was a strong potential for memory bias owing to the retrospective assessment of aspartame consumption several years in the past, and thus a high potential for misclassification of participants according to their exposure to aspartame in this study.]

(b)   Artificial sweeteners

Associations between the consumption of artificial sweeteners overall (without assessment of aspartame exposure in particular) and several cancer sites were assessed in case–control studies conducted in various countries.

Singh et al. (2020) conducted a case–control study on thyroid cancer with cases diagnosed between 2004 and 2014 in the USA. Consumption of artificial sweeteners was assessed using a telephone-based, self-report questionnaire on the use of artificial sweeteners, and the total amount of artificial sweetener consumed and duration of consumption were calculated on the basis of consumption of tabletop sweeteners and ASBs. [The Working Group noted that consumption of artificial sweetener from other sources (e.g. snacks/ice creams) was not considered, and no analysis was provided on the individual types of artificial sweetener, including aspartame. However, the exposure period covered the time when ASBs contained mainly aspartame. For tabletop sweeteners, this was not the case, which may have led to overestimation of aspartame exposure (see Section 1.4.1). Misclassification and/or recall bias was likely to result from the retrospective assessment of diet, the measurement of total artificial sweeteners, and the lack of consideration of other sources of aspartame.]

Mahfouz et al. (2014) conducted a population-based case–control study in Egypt, focusing on colorectal cancers diagnosed between 2010 and 2011. Diet in the 2 years preceding cancer diagnosis was reported to be assessed using an FFQ from Melbourne University consisting of eight food groups from which the frequency of intake (daily, weekly, or monthly) and the number of servings were recorded. The study considered qualitatively the use of artificial sweeteners (yes/no) and the consumption of soft drinks (yes/no) without distinction between artificially sweetened and sugar-sweetened soft drinks. [The Working Group noted that overall this study was not very informative regarding aspartame exposure since there was a high potential for misclassification because of the lack of distinction between sugar-sweetened beverages and ASBs, and the lack of precision in the assessment of artificial sweetener use (including the type of artificial sweetener). In addition, there were inconsistencies in the description of the methods (including a misalignment between the reported use of an FFQ from Melbourne University and

the data reported in the study) and in the references cited.]

Kobeissi et al. (2013) conducted a hospital-based case–control study in Lebanon, focusing on urinary bladder cancer diagnosed between 2002 and 2008. A face-to-face interview (not blinded on the participant's status) collected data on the frequency of artificial sweetener consumption (never, rarely, frequently, always) before diagnosis or hospitalization. [The Working Group assumed that, in the absence of details regarding the sources of artificial sweetener, artificial sweetener consumption referred to tabletop artificial sweetener considered as the only source. No quantitative assessment of artificial sweetener exposure was possible and it was not known whether the artificial sweetener consumed actually contained aspartame. This study was therefore not very informative with regard to aspartame exposure because of the partial consideration of sources and especially the absence of consideration of ASBs, resulting in high misclassification potential.]

Andreatta et al. (2008) conducted a hospital-based case–control study in Argentina focusing on urinary tract tumours diagnosed between 1999 and 2006. Participants were interviewed by a nutritionist regarding their habitual use of artificial sweetener in infusions (tea, coffee, mate) in the 5 years preceding diagnosis or hospitalization: ever consumption (yes/no), brand name of artificial sweetener consumed, and duration of consumption (years). Ever use and duration of use were considered in analyses classifying participants into three categories: non-consumers of artificial sweeteners, short-term consumers (1–9 years), and long-term consumers (≥ 10 years). [The Working Group noted that this study considered only artificial sweeteners added to infusions, which, according to the authors, constituted the main source of artificial sweetener in this population. Aspartame was not specifically considered, and the authors noted that aspartame was only

consumed by a small percentage of the population, saccharin and cyclamate being the most frequently used artificial sweeteners. Hence, data related to artificial sweetener consumption in this study were not very informative with regard to aspartame exposure.]

Two hospital-based case–control studies in Italy were conducted using similar methodologies. Gallus et al. (2007) focused on cancers of the oral cavity and pharynx, oesophagus, colon, rectum, larynx, breast, ovary, prostate, and kidney diagnosed between 1991 and 2004. Bosetti et al. (2009) focused on cancers of the stomach, pancreas, and endometrium diagnosed between 1997 and 2007 (stomach), between 1991 and 2007 (pancreas), or between 1992 and 2006 (endometrium). In both studies, the usual diet over the 2 years preceding diagnosis or hospital admission was assessed using a reproducible and validated 78-item FFQ. The FFQ included specific questions on weekly consumption of sachets or tablets of saccharin and/or other sweeteners, described as mainly aspartame by the authors. An average daily consumption of sachets or tablets of saccharin and other sweeteners was derived. [The Working Group noted that these studies included only tabletop sweeteners as potential sources of aspartame, and aspartame was not specifically considered either in the questionnaire or in analyses ("other sweeteners" only). Even though the authors stated that in this population "other sweeteners" were mainly aspartame, data to support this statement appeared to come from two studies in Italian adolescents, so the extrapolation to a middle-aged adult population may not be straightforward. The Working Group was not able to find evidence in adults in support of the claim that "other sweeteners" was mainly aspartame in Italy for that period. The authors also stated that the frequency of consumption of ASBs or products containing artificial sweeteners in the middle-aged and elderly in the studied Italian populations was rather low, but

no data were provided. There was a high potential for non-differential misclassification in this study.]

Ewertz and Gill (1990) conducted a case–control study of breast cancer in Denmark. Dietary intake was assessed retrospectively with a self-administered, semiquantitative FFQ collected from March 1983 to February 1984, that is, 1 year after diagnosis of the cases. Use of artificial sweeteners in coffee and tea was the only assessment of artificial sweeteners (binary assessment, yes/no) covering the 12 months before diagnosis. [The Working Group noted that there was no specific estimate of aspartame exposure; only sweetener use in tea and coffee was assessed as a proxy. Considering that aspartame only started to be used in the 1980s, it was very unlikely that the artificial sweeteners consumed before cancer diagnosis in this study would be aspartame. Hence this study was probably not informative regarding aspartame exposure. In addition, the case–control design meant that intake was assessed retrospectively after cancer diagnosis.]

Norell et al. (1986) conducted a population-based case–control study of pancreatic cancer in Sweden between 1982 and 1984. Exposure was assessed using a self-administered questionnaire and by telephone by a trained interviewer to clarify or complete specific items whenever necessary; there was a binary question on use of artificial sweeteners before diagnosis, and participants who had changed their diet because of recent illness were asked to report dietary habits before illness. Participants were classified as consumers or non-consumers, and there was no information on dose or duration. [The Working Group noted that the weaknesses of the exposure assessment included that there was no specific assessment of aspartame, the assessment relied on retrospective recall of frequency of consumption, and there was no quantitative assessment as participants were categorized as consumers versus non-consumers

only. In addition, considering that aspartame had only started to be used in the 1980s, it was very unlikely that the artificial sweeteners consumed before cancer diagnosis in this study would have been aspartame. Hence this study was probably not informative regarding aspartame exposure.]

(c) Artificially sweetened beverages

The consumption of ASBs in relation to cancer was considered in several studies. In these studies, ASBs generally referred to soft drinks in which sugar had been replaced by artificial sweetener to maintain the sugary taste ("diet" or "sugar-free" version of soft drinks). It should be noted that some sugar-sweetened soft drinks ("regular" version) containing sugar also contain artificial sweetener. Such beverages were most likely not considered to be ASBs in the following studies. [The Working Group noted that ASB consumption can be considered as a proxy for exposure to artificial sweeteners (including aspartame), since these beverages generally constitute the main source of artificial sweeteners (Sections 1.4.1 and 1.4.3). However, this comes with limitations that applied to all the studies detailed below. Firstly, several other food products that also contain artificial sweeteners (including aspartame), such as tabletop sweeteners, artificially sweetened dairy products or desserts, were not considered. Secondly, the presence and dose of aspartame in different types of ASB and the share of ASBs actually containing aspartame have changed over time; this affects the relevance of considering ASB consumption as a direct proxy for aspartame exposure. Hence, the value of ASBs as a proxy measure for aspartame intake is dependent on the context (time period, country, and types of beverage considered). Sections 1.4.1 and 1.4.3 provide insights into patterns of use of aspartame.]

Considering the data available on aspartame use in ASBs in the USA between the introduction of aspartame in 1983 and the introduction of other

artificial sweeteners used in combination with aspartame in the mid-2000s (see Section 1.4.1), and the major contribution of ASBs to aspartame intake compared with other sources, ASB intake in some studies can be presumed to reflect most of the participants' exposure to aspartame because of the time period of dietary assessment and follow-up. This was the case for the following studies: Bao et al. (2008) (NIH-AARP cohort, dietary data collected in 1995–1996, follow-up until 2003); Schernhammer et al. (2005) (NHS and HPFS cohorts, dietary data collected at least every 4 years since 1980 and 1986, respectively, follow-up until 2000); You et al. (2022) (Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial, PLCO, cohort: dietary data collected at baseline between 1993–2001, follow-up until 2009); and Malik et al. (2019) (NHS and HPFS: dietary data collected every 4 years since 1980 and 1986, respectively; follow-up until 2010). The study by Jones et al. (2022) in the NIH-AARP and PLCO cohorts (dietary data collected in 1995–1996 and 1998, respectively) may also be presumed to reflect most of the aspartame exposure. However, part of the sample was followed for much longer after the relevant exposure window for aspartame from ASBs (follow-up through 2011 for NIH-AARP and through 2017 for PLCO; there was uncertainty regarding aspartame content in ASBs after the mid-2000s), and only one baseline dietary assessment was available. However, the authors presented the analysis separately by follow-up period (≤ 12 years and > 12 years of follow-up), making the analysis of the first 12 years of follow-up very relevant for using ASBs as a proxy for aspartame exposure.

(i)   Prospective cohort studies

Several studies were conducted in the USA in the framework of the NHS, NHS-II, and HPFS cohorts (Schernhammer et al., 2005; Malik et al., 2019; Hur et al., 2021; Joh et al., 2021; Romanos-Nanclares et al., 2021; Wang et al., 2021; Yuan et al., 2022). In all three cohorts, diet was assessed

through a validated self-administered, semi-quantitative FFQ repeated at least every 4 years since inclusion in 1980 for the NHS, 1986 for the HPFS, and 1991 for the NHS-II (61 items in 1980, 116 items in 1984 and 1986, and ≥ 130 items thereafter). ASB consumption was assessed using nine frequencies ranging from "never or less than once per month" to "6 or more times per day" and a standard serving of 355 mL (12 fl oz) corresponding to 1 glass, can, or bottle for caffeinated, caffeine-free, and non-carbonated low-calorie or diet beverages. In the NHS-II, a self-administered high school FFQ (HS-FFQ) was used in 1998 to collect data on the usual diet of participants when they were in high school (aged 13–18 years) between 1960 and 1982 (that is, before the use of aspartame in ASBs). The HS-FFQ comprised 124 food items commonly consumed at that time.

The study by Romanos-Nanclares et al. (2021) focused on breast cancer in the NHS and the NHS-II, with follow-up ending in 2016 (NHS) or 2017 (NHS-II), and 2006 when considering molecular subtypes of breast cancer. The main analyses used a cumulative average strategy, and other strategies were also considered in secondary analyses. These included: simple update (consumption reported on the most recent FFQ before each follow-up interval); latency (consumption reported at different latencies, i.e. 4–8, 8–12, 12–16, and 16–20 years, before a cancer diagnosis); and changes in consumption updated every 4 years to estimate the risk in the subsequent 4-year period (no change or relatively stable, increase or decrease from 1.0 serving/ week to 0.5 serving/day, and increase or decrease by > 0.5 serving/day). The study by Hur et al. (2021) focused on early-onset colorectal cancer in the NHS-II, with follow-up ending in 2015. Adult ASB intakes were cumulatively averaged over questionnaire cycles. The study by Malik et al. (2019) focused on cancer mortality in the NHS and the HPFS, with follow-up ending in 2014. Analyses considered frequencies of ASB intake, with an update of intakes

at the beginning of each FFQ cycle as the main analysis. Secondary analyses used a cumulative average strategy whereby intakes from all FFQs up to the beginning of a follow-up interval were averaged, but also analyses with an 8-year lag, whereby exposures were evaluated in relation to outcomes 8 years later. Dietary intakes were not updated if the participant reported incidence of diabetes mellitus, stroke, coronary heart disease, or cancer. The study by Schernhammer et al. (2005) focused on pancreatic cancer in the NHS and the HPFS, with follow-up ending in 2000. The analyses used a cumulative average strategy, stopping updates after a diagnosis of diabetes mellitus was reported.

[The Working Group noted that in all four studies (Schernhammer et al., 2005; Malik et al., 2019; Hur et al., 2021; Romanos-Nanclares et al., 2021), the repeated assessment of ASB intake at least every 4 years since the 1980s and 1990s and the analytical strategy of updating diet data during follow-up constituted major strengths and allowed potential variations in trends in ASB intake over time to be taken into account. In addition, the time frame of these studies was very relevant to aspartame exposure via ASBs, since the studies started in the 1980s (overlapping the introduction of aspartame in ASBs in 1983), potentially capturing lifetime exposure to aspartame from ASB intake, and carried out diet assessment through the 1990s, when aspartame was almost the only sweetener used in ASBs. However, there was more uncertainty regarding aspartame content in ASBs from the mid-2000s, and the period between 1980 and 1983 is not relevant for aspartame exposure. Hence the quality of ASB intake assessment was high in these studies, which had a positive impact on the assessment of aspartame exposure from ASBs.]

The three remaining studies in the NHS, NHS-II, and/or HPFS (Joh et al., 2021; Wang et al., 2021; Yuan et al., 2022) were less informative with regard to aspartame exposure, considering their exposure of interest and/or analytical

strategy. The study by Yuan et al. (2022) focused on colorectal cancer incidence and mortality in the NHS and the HPFS, with follow-up ending in 2014. The analyses considered the substitution of one serving of sugar-sweetened beverage per day with an equivalent amount of ASBs, without estimates for ASBs as such, using cumulatively averaged intakes as the main analyses and secondary analyses that considered average intake during the most recent 10 years (recent period) and > 10 years previously (distant period). [The Working Group noted that ASBs were not considered as the main exposure of interest in the analyses, which limited the relevance of the results for ASB intake and therefore even more so for aspartame exposure.] The study by Joh et al. (2021) focused on colorectal cancer precursors (polyp, adenoma) in the NHS-II in relation to ASB consumption in adolescence (between 1960 and 1982), with follow-up for health events between 1998 and 2015. Adult diet (cumulative average up to 2 years before the most recent endoscopy), including ASB intake, was considered as a covariate in the analyses. [The Working Group noted that soft drinks in the USA did not contain aspartame before 1983, hence no exposure to aspartame could be derived from the diet for adolescents. Besides, the recalled adolescents' diet (likely to suffer from substantial recall bias) only weakly correlated with the adults' diet, so that ASB intake reported in adolescence did not give insights into ASB intake in adulthood when ASBs potentially contained aspartame. This study was therefore not informative regarding aspartame exposure.] The study by Wang et al. (2021) focused on colorectal cancer in the NHS, NHS-II, and HPFS and considered a "sulfur microbial diet score", consisting of a weighted sum of standardized consumption of 10 food groups with weights reflecting positive or negative correlations with the proliferation of sulfur-producing bacteria in the gut. Low-calorie beverages were included in the score with a positive weight. A cumulative average of the sulfur microbial diet score was

derived for analyses. Participants were followed until 2014 (HPFS), 2016 (NHS), and 2017 (NHS-II). [The Working Group noted that ASBs were only included as a component of the score, so the extrapolation to aspartame exposure and therefore the informativeness of this study with regard to aspartame was very limited.]

Ringel et al. (2023) studied urinary tract cancers in the Women's Health Initiative Observational Study. Consumption of ASBs (defined as "diet drinks such as Diet Coke or diet fruit drinks") was assessed 3 years after study baseline, i.e. in 1996–2001. The frequency of consumption during the past 3 months was questioned using a reference serving size (12 fl oz can [355 mL]) and nine frequencies of servings: from never or < 1 serving/month to ≥ 6 servings/day. Analyses used three categories corresponding to rare (never or < 1 serving/week), frequent (1–6 servings/week) and daily (≥ 1 serving/day) consumption. The follow-up ended in 2020. [The Working Group noted that the time period of the assessment of ASB consumption was very relevant for aspartame exposure, since aspartame was almost the sole sweetener used in ASBs at the time of diet assessment. However, there was only one assessment at baseline, with up to 24 years of follow-up, so potential changes in ASB consumption over time could not be captured, and other sources of aspartame exposure were not considered (although these were more limited), which may have led to non-differential misclassification.]

McCullough et al. (2022) studied mortality from all cancers combined, obesity-related cancers combined, and 20 individual cancer types in the CPS-II study. ASB consumption was assessed at study baseline in 1982 via a question about the usual number of cups, glasses, or drinks consumed per day, and consumption duration for "diet soda or diet iced teas" (one pooled item). Previous intake was queried in case there had been a change in consumption during the past 10 years, and former drinkers (no

current intake but non-zero amount for previous intake) were excluded. The consumption of ASBs was considered as categories – never drinkers (no past or current consumption), < 1 drink/day, 1 drink/day, and ≥ 2 drinks/day – and as continuous intake per 1 drink/day. Participants were followed until 2016 (median follow-up, 27.7 years). [The Working Group noted that the assessment of ASB intake was performed only at baseline in 1982, i.e. a time when aspartame was not used in ASBs. The relevance of this study to aspartame exposure depended on the stability of ASB consumption over time, because aspartame began to be included in ASBs in 1983 in the USA. Although no information was available regarding potential variations in ASB intake over time during up to 34 years of follow-up, some data from McCullough et al. (2014) in the CPS-II nutrition cohort suggested a stable ranking of participants with regard to ASB consumption between 1982 and 1999 for the subsample (n = 100 442) providing data at both time points. However, there was uncertainty regarding aspartame content in ASBs in the mid-2000s. Non-differential misclassification was therefore likely.]

Jones et al. (2022) performed a pooled analysis of data for liver cancer from the NIH-AARP and PLCO studies. For both studies, data on ASB intake were only available at baseline, an FFQ in 1995–1996 for the NIH-AARP study and a diet history questionnaire in 1998 for the PLCO study. Participants were followed until 2011 in the NIH-AARP and 2017 in the PLCO study. The consumption of soda and fruit punches as ASBs was considered as a mean daily frequency (from no consumption to ≥ 6 times/day). [The Working Group noted that ASB consumption was assessed during a very relevant period for aspartame exposure (1995–1996 and 1998 in the USA); however, there was more uncertainty regarding aspartame content in ASBs in the mid-2000s. The dietary assessment occurred only at baseline, and there was a follow-up of up to 16 years in the NIH-AARP

study and 19 years in PLCO. This increased the probability of misclassification of participants with regard to ASB intake and therefore aspartame exposure. However, the authors presented the analysis separately by follow-up period (≤ 12 years and > 12 years of follow-up). Hence the analysis on the first 12 years of follow-up was less prone to such misclassification and ASB consumption could be considered as a relevant proxy of aspartame exposure in this analysis. In addition, sodas and fruit punches (and not just carbonated beverages, as was the case in other studies) were also considered to be ASBs, which constituted a strength of this study.]

Zhang et al. (2021) focused on cancer mortality in the NHANES cohort study. The consumption of ASBs was assessed through 24-hour dietary recalls given by trained investigators at inclusion between 1999 and 2014. Participants included before 2003 completed only one 24-hour dietary recall administered in person, whereas participants included after 2003 completed two 24-hour dietary recalls (one administered in person and one administered by telephone 3–10 days after the first). Participants were followed through registries until 2015. The 24-hour dietary recalls were linked to a main food list of more than 2600 food items. A standard 12 fl oz [335 mL] serving was defined. ASBs were defined as "sugar-free soft drinks" and "carbonated water". [The Working Group noted that 24-hour dietary recalls allowed the consideration of different types of ASB. However, mean intakes were derived from a maximum of two 24-hour dietary recalls, which was likely to introduce measurement errors as a result of potential individual day-to-day variations in ASB intake (especially when only one recall was available). In addition, there was uncertainty regarding the aspartame content of ASBs in the USA after the mid-2000s (i.e. for a substantial part of the study assessment period, 1999–2014). Hence there was the potential for misclassification of participants

with regard to ASB intake and therefore aspartame exposure.]

Inoue-Choi et al. (2013) focused on endometrial cancer in the Iowa Women's Health Study (IWHS). A 127-item Harvard semiquantitative FFQ assessing diet over the past 12 months was used at study baseline in 1986. Sugar-free beverages were included as low-calorie caffeinated and caffeine-free cola and other low-calorie carbonated beverages with a standard serving size. Mean intakes as servings per week were considered in the analyses. Participants were followed until 2010. In 2004, a follow-up survey including an FFQ was performed and a weak correlation was found for sugar-sweetened beverage intake (correlation coefficient, 0.23), with no information on ASBs. [The Working Group noted that only the baseline dietary assessment was used and that there was a follow-up of up to 24 years. Because only a weak correlation was reported for sugar-sweetened beverage intake between the baseline in 1986 and a reassessment of diet in 2004 (with no information on ASBs), there was potential for misclassification of participants with regard to ASB intake and therefore aspartame exposure. However, the dietary assessment was conducted at a very relevant period for aspartame exposure from ASBs.]

Bao et al. (2008) focused on pancreatic cancer in the NIH-AARP cohort. In this study, usual diet in the past 12 months was assessed once at study baseline between 1995 and 1996 using a 124-item FFQ calibrated against two non-consecutive 24-hour recalls. Participants were followed until 2003. Three beverage types potentially containing aspartame were included: soda, fruit drinks, and iced tea (in summer), and participants were asked about frequency of consumption of each type (10 frequencies ranging from never to ≥ 6 times/day). Participants were then asked whether they consumed the sugar-free/diet or regular version of these beverages "usually, or more than half the time" and which portion size (three portion size ranges). An average daily

diet soft drink consumption was derived for the analyses. [The Working Group noted that ASB consumption was assessed during a very relevant period for aspartame exposure (1995–2003 in the USA). This study considered both carbonated and non-carbonated diet soft drinks but was less precise regarding the type of carbonated soft drinks (cola or not), which was likely to affect the classification of participants by type of beverage consumed (and thus the potential corresponding aspartame exposure). The sequential assessment of the frequency of consumption of ASBs was likely to induce measurement errors and thus lead to non-differential misclassification of participants. There was a maximum of 8 years between the assessment of ASB intake and cancer diagnosis; over this limited period, the diet could be considered relatively stable, even though no information was available regarding ASB intake before the study baseline, which occurred 10 years after the introduction of aspartame.]

You et al. (2022) conducted a prospective analysis of the PLCO cohort to examine soft drink consumption and lung and total cancers. In 1993–2001, dietary intake was assessed with a 156-item FFQ. Soft drink (regular and diet) intake was calculated from information on frequency and serving size. Quantitative data on soft drink consumption were then categorized into non-consumers, consumers of regular only, consumers of diet only, and consumers of both or mixed consumption. [The Working Group noted that no quantification of ASB intake was used in the analyses. In addition, only baseline intakes were considered, and there was no consideration of intake before baseline; therefore, there was potential for non-differential misclassification. The timing of exposure assessment was consistent with aspartame being the major sweetener in ASBs in the USA.]

Several studies were conducted in the multi-country framework of the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort (Navarrete-Muñoz et al., 2016; Stepien et al., 2016; Mullee et al., 2019; Heath et al., 2021; Zamora-Ros et al., 2022), which gathers participants from 10 countries (Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, UK). Usual diet was assessed at inclusion using country-specific instruments developed and validated within the various source populations and including hundreds of country- and region-specific foods. These extensive self-administered quantitative dietary questionnaires collected data on usual portions and frequency of consumption of up to 260 food and drink items during different seasons of the year (Riboli et al., 2002). Data on artificially sweetened soft drinks were not available in Spain, nor in parts of Italy (Florence, Turin, Ragusa, Varese) and Sweden (Umeå). Artificially sweetened soft drink consumption was assessed between 1992 and 2000 as the consumption of several glasses per month, week, or day (typical glass sizes in each centre, about 250 mL) of "low-calorie or diet fizzy soft drinks". A mean daily intake of ASBs was derived for the analyses.

The study by Zamora-Ros et al. (2022) focused on thyroid cancer, with a mean follow-up of 14 years. The study by Heath et al. (2021) focused on renal cell carcinoma, with a mean follow-up of 15 years for incidence of renal cell carcinoma and 16 years for mortality. The study by Mullee et al. (2019) focused on cancer mortality, with a mean follow-up of 16.4 (range, 11.1–19.2) years. The study by Navarrete-Muñoz et al. (2016) focused on pancreatic cancer, with a median follow-up of 11.6 years. The study by Stepien et al. (2016) focused on cancers of the liver and biliary tract, with a mean follow-up of 11.4 years.

[The Working Group noted that, for all five studies, only one diet assessment was available at baseline, which was during a period that was relevant for aspartame exposure from ASBs, and the mean follow-up ranged from 11 to 16 years; as a result, potential variations in consumption of ASBs over time could not be captured. In addition, EPIC combined data from multiple

European countries, each having its own pattern of ASB consumption, and potentially its own products (with variable content in aspartame), which limited the direct extrapolation from ASB intake to aspartame exposure. The consumption of ASBs at baseline was relatively low (< 25% of total soft drinks) and no information was available on historic exposure before inclusion. Hence there was some potential for non-differential misclassification of participants with regard to ASB intake and aspartame exposure.]

Three other studies were conducted in Europe and considered beverages that potentially contained aspartame.

The study by Liu et al. (2022b) was conducted within the United Kingdom Biobank and focused on cancer mortality in relation to the consumption of artificially sweetened coffee. Coffee consumption was assessed using validated web-based 24-hour dietary recall questionnaires (Oxford WebQ), with a minimum of one questionnaire completed out of five maximum occasions during 1 year between April 2009 and June 2012 (to account for seasonal variations). The number of drinks of coffee in the previous 24 hours was recorded, together with the number of teaspoons of sugar or artificial sweeteners (any brand) added. An indication of portion size (e.g. mug or cup) was provided. Participants were classified as non-consumers, sole consumers (same kind of coffee over the dietary recalls: unsweetened, sugar-sweetened, artificially sweetened), or overlapped consumers, and analyses considered an average number of drinks (volume, about 250 mL) across multiple dietary recalls. Participants were followed until 2017–2018 (median, 7.0 years). [The Working Group noted that this study was not very informative with regard to aspartame exposure: (i) artificially sweetened coffee was the only ASB considered in this study, and other major sources of aspartame such as carbonated soft drinks (caffeinated or not) were not considered; (ii) there was no indication as to whether the artificial sweeteners used in coffee contained aspartame; and (iii) the mean number of dietary recalls used was relatively low (mean ± SD, 2.2 ± 1.2), which limited the consideration of day-to-day variations in coffee consumption.]

The study by Chazelas et al. (2019) was conducted in France within the NutriNet-Santé prospective cohort study (2009–2017) and examined cancers of the breast, colorectum, and prostate, and all cancers combined. Consumption of artificially sweetened soft drinks was assessed using repeated validated web-based 24-hour dietary records (minimum, 2; mean ± SD, 5.6 ± 3.0). Participants were asked at inclusion and then every 6 months to complete three non-consecutive 24-hour dietary records (2 weekdays, 1 weekend day) randomly assigned over a 2-week period. Baseline data were collected from 2009 through 2017, with follow-up to January 2018. The analyses considered only baseline ASB consumption, defined as the mean consumption over the first 2 years of follow-up. Exposure was assessed for 12 ASB items containing non-nutritive sweeteners, such as diet soft drinks, sugar-free syrups, and diet milk-based beverages, and consumption was categorized into sex-specific quartiles. [The Working Group noted that a major strength of this study was the use of repeated detailed 24-hour-dietary records reflecting up-to-date consumption patterns of ASBs in France. Considering the time period of dietary assessment, ASBs included a variety of products with aspartame only, artificial sweeteners other than aspartame only, or a mix of aspartame and other artificial sweeteners. Therefore, the consumption of ASBs in this study may not reflect only aspartame exposure but also exposure to other sweeteners, as confirmed in the later study by Debras et al. (2022b). In addition, only baseline dietary data were used in analyses, which did not allow the consideration of potential changes in dietary behaviours over time. Potential changes were nonetheless likely to be limited over the maximum of 9 years of follow-up. Likewise,

there was no information on lifetime exposure to aspartame before study entry, which occurred between 2009 and 2017. In terms of relevance for the present monograph, this publication was superseded by the publication of Debras et al. (2022b), which provided estimates specific to aspartame intake in the same cohort. The later publication demonstrated that: (i) aspartame represented only 58% of artificial sweetener intakes in this study; and that (ii) ASBs (with or with added sugar) represented only 57% of artificial sweetener intake.]

The study by Larsson et al. (2016) was conducted in Sweden and focused on cancers of the biliary tract and gallbladder in relation to consumption of sweetened beverages within the Swedish Mammography Cohort and the Cohort of Swedish Men. Usual diet was assessed in 1997 using a self-administered semiquantitative 96-item FFQ covering the past year, including one question about the usual consumption of a standard glass (200 mL) of sweetened beverages (not including fruit juices, energy and sports drinks, or sweetened coffee, tea, or milk). A mean daily intake was derived from the questionnaire. Participants were followed until 2012 (mean follow-up, 13.4 years). [The Working Group noted that no distinction was made between sugar-sweetened and artificially sweetened (low-calorie) beverages, rendering the extrapolation to aspartame exposure very limited. According to Swedish national consumption data, low-calorie soft drinks and "juice" drinks accounted for 9.9% (men) to 19.2% (women) of total soft drink and "juice" drink consumption in 1997.]

Finally, two prospective studies (Hodge et al., 2018; Bassett et al., 2020) were conducted in Australia within the Melbourne Collaborative Cohort Study (MCCS). The study by Hodge et al. (2018) examined associations between ASB consumption and obesity-related cancers, whereas the study by Bassett et al. (2020) focused on cancers not related to obesity. All methods of exposure assessment were the same across the two studies. Exposure to artificially sweetened soft drinks was assessed at baseline only (in 1990–1994) using a self-administered 121-item FFQ with separate questions on frequency of consumption of diet (artificially sweetened) soft drinks. The questionnaire asked respondents to recall their intake over the previous 12 months. Intake of ASBs was categorized into five groups based on frequency of reported consumption (never or < 1 time/month, 1–3 times/month, 1–6 times/week, 1 time/day, > 1 time/day). [The Working Group noted that the relative strengths of this assessment included the semiquantitative assessment for frequency of intake, which allowed multiple dose levels to be assessed. Limitations included that the assessment used for this analysis was conducted at baseline only (1990–1994), which did not allow the consideration of variations in ASB consumption over time, bearing in mind that the follow-up was up to 25 years in Bassett et al. (2020) and 23 years in Hodge et al. (2018). Hence, non-differential misclassification with regard to aspartame exposure was likely.]

(ii) Case–control studies

Four case–control studies (Nomura et al., 1991; Mayne et al., 2006; Chan et al., 2009; Davis et al., 2023) investigating ASBs were conducted in the USA.

Davis et al. (2023) conducted a hospital-based case–control study on pancreatic cancer and mortality in Buffalo, New York, USA, in which exposure was assessed between 1982 and 1998 using a 46-item FFQ on general dietary habits (including daily consumption of diet cola) in the few years before diagnosis. Participants were categorized in three groups: never, occasional (< 1 drink/day), and habitual (≥ 1 drink/day) consumers. [The Working Group noted that the retrospective assessment of ASB consumption during the few years before diagnosis may cause misclassification that could be both non-differential and differential, according to case or

control status. In addition, this study included only diet cola as an ASB and as a potential source of aspartame, which was a partial assessment that probably induced some misclassification. Additionally, for cancers diagnosed at the beginning of the study period (the first half of the 1980s), the assessment of ASB consumption was not relevant for aspartame exposure since aspartame was not included in ASBs in the USA before 1983). However, for the remaining study period, ASB consumption was relevant for aspartame exposure.]

Chan et al. (2009) conducted a population-based case–control study of pancreatic cancer in California, USA, in which exposure data were collected between 1995 and 1999. Total ASB consumption during the 12 months before diagnosis was assessed using a 131-item food questionnaire via in-person interviews with questions on low-calorie colas, low-calorie caffeine-free colas, and other low-calorie carbonated beverages). ASB frequency was categorized into three groups (0, < 1, ≥ 1 beverage/day). [The Working Group noted that although there was no specific estimate of aspartame exposure and ASBs were used as a proxy, the timing of exposure assessment was relevant for aspartame being the major artificial sweetener in ASBs. However, the retrospective assessment of diet after diagnosis may have led to differential or non-differential misclassification of participants with regard to ASB intake and therefore aspartame exposure.]

Mayne et al. (2006) conducted a population-based case–control study of oesophageal cancer in Connecticut, USA. Exposure was assessed in 1993–1995 using an in-person structured questionnaire covering the 3–5 years before diagnosis. The usual frequency of consumption of diet soft drinks or soda (per day, week, month, or year) was then categorized into quartiles, the top quartile being compared with non-consumers. [The Working Group noted that the limitations of the assessment included the retrospective

assessment of diet after diagnosis, and that there was no consideration of lifetime consumption of ASBs, which made differential and non-differential misclassification of participants more likely with regard to ASB intake and therefore aspartame exposure. Nonetheless, ASB intake in this study was probably very relevant for aspartame exposure, considering the time frame of dietary assessment.]

Nomura et al. (1991) conducted a case–control study of bladder cancer in Hawaii, USA. Dietary intake was assessed via a diet history interview of 29 food items consumed during a usual week, or usual month for less frequently consumed items, on the basis of the participants' usual diet in the year before diagnosis, with assessment between 1977 and 1986. The consumption of ASBs (such as diet or low-calorie sodas) and frequency of use of saccharin, cyclamates, and other artificial sweeteners (but not aspartame) were calculated. Participants were categorized on the basis of frequency of consumption of ASBs as a non-user or user (1–2 can-years, 3+ can-years; can-years indicate number of servings/day × years). [The Working Group noted that the time frame of this study (i.e. cancers diagnosed between 1977 and 1987) was of questionable relevance for aspartame exposure, considering that the US authorization of aspartame for use in ASBs was in 1983. Other limitations were that diet was assessed retrospectively after diagnosis and that interviewers were not blinded to case or control status, both of which increased the potential for differential exposure misclassification.]

(d) Other related exposures

Exposures to agents previously evaluated by IARC that are potentially associated with the consumption of aspartame, artificial sweeteners, and ASBs include alcoholic beverages and tobacco smoking (both *carcinogenic to humans*, IARC Group 1, Volume 100E, IARC, 2012). These co-exposures were taken into account in almost all the studies described above. The consumption

of tabletop artificial sweeteners may also be associated with the consumption of very hot beverages (*probably carcinogenic to humans*, IARC Group 2A, Volume 116; IARC, 2018a), but this was rarely considered. Other potential co-exposures, as part of overall dietary patterns, include dietary cancer hazards such as red meat (IARC Group 2A, Volume 114; IARC, 2018b) and processed meat (IARC Group 1; IARC, 2018b), or environmental exposures. These factors were seldom considered in analyses. Other probable cancer risk factors that were not considered in analyses include, for instance, night shift work (IARC Group 2A, Volume 124; IARC, 2020). Other factors potentially associated with aspartame exposure include coffee intake, food additives, sugar and sugar-sweetened beverages, body fatness, and diabetes (which could induce some bias from reverse causation, whereby individuals at a higher risk of developing cancer because of higher body fatness or diabetes are more likely to consume ASBs and, more generally, artificial sweeteners).

1.6.2 Quality of exposure assessment in mechanistic studies in exposed humans

See Table S1.3 (Annex 1, Supplementary material for Section 1, Exposure Characterization, online only, available from: https://publications.iarc.who.int/627).

This section reviews the exposure assessment methods of mechanistic studies in exposed humans, evaluated in Section 4 of the present monograph. Studies included in this section were a mixture of those that examined mechanistic responses to: (a) added dose of aspartame (as a beverage or in capsule form and often investigating the impact of increasing doses); (b) added dose mixed with other artificial sweeteners (typically as a beverage); (c) intake of artificial sweeteners either from dietary intake of beverages only or from the total diet assigning intake estimates for aspartame; and (d) intake of aspartame from a commercial beverage but in situations where this acted as the control arm of interventions focusing on sugar-sweetened beverages rather than on aspartame per se.

There was a mix of challenge studies, randomized controlled trials, observational studies, matched control studies and one genome-wide association study (GWAS). There was a high degree of heterogeneity, with study participants including those who were generally healthy, groups with medical conditions (e.g. HIV infection), and those who self-selected for adverse responses to aspartame intake (e.g. headaches or urticaria).

Where present, dietary assessments typically used food diaries or records, FFQs, or 24-hour dietary recalls; associated challenges related to the accuracy and precision of each tool and concomitant food composition data for quantifying aspartame intake (see Section 1.6.1 for a detailed review). Habitual or background dietary intake was not always assessed, often because the study focus was on examining the impact of increasing doses of aspartame. Hence, habitual intakes were generally poorly characterized across all studies; in addition, there was no clear indication of co-exposure to other agents that may be carcinogenic. Differential misclassification of exposure among participants was unlikely in studies in which exposure to aspartame was allocated. However, in studies in which habitual or background diet was not assessed or controlled, there was a potential for diluted treatment effect. All studies are reviewed below and classified according to exposure and study design.

(a)   *Controlled doses of aspartame added to the diet*

(i)   *Crossover challenge studies*

Six studies adopted a crossover approach to investigating the impact of aspartame on health outcomes (Baraniuk et al., 1988; Garriga et al.,

1991; Nguyen et al., 1998; EFSA_UN07, 2011; Ahmad et al., 2020a, b). Two studies were carried out in the USA (Garriga et al., 1991; EFSA_UN07, 2011), one in France (Nguyen et al., 1998), and one was available only as an abstract with no information on location (Baraniuk et al., 1988). [The Working Group assumed that the study was performed in the USA because of the stated affiliation of the authors.] All studies involved adding doses of aspartame to usual daily intakes but over different time periods and different doses. The study populations included those who self-reported headaches related to aspartame ingestion (Baraniuk et al., 1988), those who self-reported urticaria related to aspartame ingestion (EFSA_UN07, 2011), a generally healthy adult population (Nguyen et al., 1998), and participants who had a confirmed positive adverse reaction of any sort to aspartame that was documented by the researchers (Garriga et al., 1991). Each study adopted an approach whereby added doses of aspartame were included as an acute challenge study over a single day (single meal approach), either as a beverage (Nguyen et al., 1998), capsule (Garriga et al., 1991; EFSA_UN07, 2011), or not stated (Baraniuk et al., 1988). Sample sizes ranged from 7 (Nguyen et al., 1998), 12 (Garriga et al., 1991), and 21 (EFSA_UN07, 2011), to 40 individuals (Baraniuk et al., 1988). EFSA_UN07 (2011) included two children in the study population. The doses under study ranged from 30 mg/kg bw (the exact doses administered were not available; Baraniuk et al., 1988), 250 mg per challenge (total amount) (Nguyen et al., 1998), and 950 mg/day for individuals with a body weight of > 40 kg (450 mg where body weight was < 40 kg) (EFSA_UN07, 2011) and 2000 mg per challenge (Garriga et al., 1991). Given the acute nature of these studies, there was no background information on habitual dietary aspartame exposure in any study. It was not possible to characterize usual dietary exposure, if any.

[The Working Group noted that, across the studies, the dose of aspartame was controlled and known, and therefore the certainty of exposure was high, and risk of misclassification (except for background exposure) was low.]

Two publications (Ahmad et al., 2020a, b) reported on primary and secondary outcomes for a crossover randomized controlled trial in which participants received a dose of aspartame of 14% (0.425 g) of the ADI, mixed with water, every day for 2 weeks. Among the inclusion criteria for the study was that participants were not regular users of non-nutritive sweeteners, i.e. artificial sweeteners (i.e. consuming less than one can of diet beverages and/or one spoonful of non-nutritive sweetener or the equivalent per week in food products, which was confirmed via screening with a web-based FFQ (Canadian Diet History Questionnaire II). The FFQ included questions on the type, quantity, and frequency of use of artificial sweeteners in tea, coffee, and other drinks, and on the consumption of diet beverages (including fruit drinks, diet soda, iced tea, and flavoured water). Participants were asked to avoid food or drink products that contained non-nutritive sweeteners for the study period. Compliance with this advice and the treatment was assessed via completion of a 3-day food diary (2 weekdays, 1 weekend day, during the 2-week study period), a daily beverage checklist over the 14-day intervention period, and return of beverage containers each week. Aspartame was the specific exposure tested in both studies.

In the EFSA clinical research report (EFSA_UN01, 2011), 10 participants with type 2 diabetes and 12 participants without type 2 diabetes received a single dose of aspartame-containing beverage (approximately 400 mg of aspartame was added to 300 mL of an unsweetened beverage, cherry-flavoured reconstituted drink mix). No measurement of background or usual diet was conducted in this study, and it was not reported whether participants were consumers or non-consumers of artificial sweeteners before the study. Aspartame was the specific exposure tested in this study.

Kashima et al. (2019) conducted a crossover randomized controlled trial in Japan in which participants (*n* = 9) received either 25 mL of water (control) or a 2.5% solution of *Gymnema sylvestre* (a plant that suppresses sweet taste sensation) as a mouth rinse, followed by 200 g (four doses of 50 g over 80 minutes) of either 0.09% aspartame or 15% glucose solution containing 100 mg of ^{13}C-labelled sodium acetate. There was no information provided on time between administration of each intervention arm. There was no measurement of background or usual diet conducted in this study, and it was not reported whether participants were consumers or non-consumers of artificial sweeteners before the study. Aspartame was the specific exposure tested in this study. There was no information on the purity of aspartame delivered; however, a branded commercial product containing aspartame was used (see Section 1.1.4 for purity requirements of commercial food products).

(ii) Randomized parallel-arm challenge studies

Six studies adopted a parallel-arm approach (Okuno et al., 1986; Leon et al., 1989; EFSA_UN08, 2011; Higgins et al., 2018; Higgins and Mattes, 2019; Suez et al., 2022). Four of these were carried out in the USA (Leon et al., 1989; EFSA_UN08, 2011; Higgins et al., 2018; Higgins and Mattes, 2019), one in Israel (Suez et al., 2022), and one in Japan (Okuno et al., 1986). [The Working Group noted for all studies that the dose of aspartame was controlled over a short period of time during which there was a high certainty regarding short-term exposure to aspartame.]

Higgins et al. (2018) completed a three-parallel-arm study whereby a total of 93 generally healthy participants were randomized to consume one of three doses of aspartame as a beverage and/or capsule – 0 mg/day (dextrose capsule), 350 mg/day (beverage) or 1050 mg/day (350 mg as a beverage plus 700 mg as a capsule) – over 12 weeks. There was no record of dietary intakes of aspartame from any other sources during this time, although participants were selected at screening to be low- or non-consumers of low-calorie sweeteners.

In the trial described in EFSA_UN08 (2011), 108 generally healthy adults were randomized in a double-blind fashion to consume either a placebo or a total of 75 mg/kg per day of aspartame over three doses each day for 24 weeks. This dose was considered by the authors to be approximately equivalent to the amount of aspartame in 10 L of aspartame-sweetened beverage per day for a 70 kg person at that time (around 1985). Aspartame was delivered as capsules (300 mg/capsule) and the placebo was microcrystalline cellulose. No data were provided on the purity of aspartame delivered. Use of aspartame-containing products was recorded at each visit, and participants were encouraged to continue their usual diet but to avoid aspartame-containing products (list provided). There was no detailed record of habitual intakes of aspartame (if any) or of exposure to any other artificial sweetener during the study period (EFSA_UN08, 2011).

Okuno et al. (1986) described two studies. The first examined the effect of a single dose of aspartame (500 mg mixed with water) in 7 individuals without diabetes (controls) and 22 individuals with untreated diabetes, whereas the second involved daily intake of aspartame (125 mg/day) as part of a jelly cake, for 2 weeks, by Japanese adults living with diabetes but with controlled glycaemia. There was no record of usual intakes of aspartame or other low-calorie sweeteners at baseline or throughout the study period. The authors reported that in the second study the administered dose was considered equivalent in sweetness to mean daily sugar consumption for Japanese adults (20–30 g of sugar).

Leon et al. (1989) conducted a randomized controlled trial in the USA in 108 male and female participants aged 18–62 years; aspartame delivered in capsule form at a dose of 75 mg/kg (taken at three times, spread across the day)

for 24 weeks. A sample of the aspartame was analysed and found to be 98% pure aspartame. Compliance was assessed by counting unused capsules at clinic visits every 3 weeks. In addition, blood and urine metabolites including the products of aspartame metabolism, i.e. aspartic acid, phenylalanine, and methanol, were used to assess exposure five or six times over the 24-week intervention. [Measures of compliance were a strength of this work.]

Higgins and Mattes (2019) conducted a randomized controlled trial (n = 154) with aspartame provided as a beverage in one of five intervention arms (the other arms were sucrose, saccharin, sucralose, or rebaudioside A), with daily consumption for 12 weeks (n = 30). Participants consumed between 1.25 L and 1.75 L of a coloured, fruit-flavoured beverage (made from a reconstituted drink mix) per day, according to body weight at baseline (body weight, 60–75 kg, 1.25 L/day; body weight, 76–90 kg, 1.50 L/day; body weight, > 91 kg, 1.75 L/day), with 0.58 g of aspartame. Food and energy intake were measured on 3 days (2 non-consecutive weekdays and 1 weekend day) during baseline and weeks 4, 8, and 12, using the Automated Self-Administered 24-hour Dietary Assessment Tool (ASA24), a standardized dietary assessment tool. There was also a brief questionnaire that measured habitual beverage intake over the past month, completed at baseline, and in weeks 4, 8, and 12, which was used to assess whether participants made other changes to beverage intake. *para*-Aminobenzoic acid was added to the supplied beverages, and urinary *para*-aminobenzoic acid was measured to determine compliance for the intervention beverages. Urinary concentrations of low-calorie sweeteners were not measured. Background diet assessments were not used for aspartame exposure. Blood was sampled to conduct an oral glucose tolerance test at baseline and week 12. A single fasting blood draw was collected at week 6 for measurement of whole-blood glycated haemoglobin (HbA1c) and serum glucose, insulin,

triacylglycerol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) cholesterol. [Measures of compliance were a strength of this work.]

Suez et al. (2022) conducted a randomized controlled trial with aspartame as one intervention arm (2 sachets, three times per day; total amount per day, 0.24 g) for 3 weeks, in Israel (120 participants overall, 20 participants per arm of intervention). Among the inclusion criteria for the study was being a non-consumer of non-nutritive sweeteners, which was assessed through completion of an FFQ based on products containing non-nutritive sweeteners on the Israeli market. Dietary intake during the trial was assessed with a smartphone food diary; however, this was not used to assess aspartame.

(iii) Genome-wide association study

Hwang et al. (2019) completed a genome-wide association study (GWAS) focusing on sugars and sweeteners, using data from the USA, UK, and Australia. The aim was to identify associations with genes involved in the peripheral receptor systems relating to human perception of sweetness, i.e. taste 1 receptor members 2 and 3 (*TAS1R2* and *TAS1R3*), and G protein subunit alpha transducing 3 (*GNAT3*). In the US sample, aspartame (or any other artificial sweetener) was not included in the taste test. In the cohort from the United Kingdom Biobank, only total sugar and sweets (confectionery) was assessed (not aspartame, ASBs, or artificial sweeteners). In the Australian cohort, aspartame was added as an additional dose as part of a taste test in teenagers. The taste battery included duplicated presentations of four sweet solutions (0.60 M glucose, 0.30 M fructose, 8.0×10^{-5} M neohesperidin dihydrochalcone, and 1.4×10^{-3} M aspartame), and five bitter solutions (propylthiouracil, sucrose octaacetate, quinine, caffeine, and denatonium benzoate), plus two water solutions as controls. [The Working Group noted that there was no indication of habitual or background intakes of

the teenagers and no information available for the other cohorts beyond the Australian teenagers.]

(b) Controlled doses of aspartame mixed with other artificial sweeteners added to the diet

Two randomized studies examined the impact of added doses of aspartame mixed with other artificial sweeteners on mechanistic outcomes (Sørensen et al., 2005; Kim et al., 2020). One study with a randomized controlled design was carried out in Denmark (Sørensen et al., 2005) and the second study had a randomized crossover design and was carried out in Australia (Kim et al., 2020).

Randomized controlled trial

The study by Sørensen et al. (2005) involved the secondary analysis of an existing randomized controlled trial in which the aspartame-consuming group acted as a control (Sørensen et al., 2005). For 10 weeks the control group of healthy adults consumed a mix of artificial sweeteners as foods and beverages at three different levels based on energy intakes dependent on body weight (694 kJ/day, 832 kJ/day, or 971 kJ/day). Aspartame was consumed as a mix along with cyclamate, acesulfame-K, and saccharin (54% aspartame). Foods containing artificial sweeteners were listed as soft drinks, fruit juices, yogurt, marmalade, ice creams, and stewed fruits, but exact compositions and intakes were not provided. There was no quantitative estimate of intakes of any artificial sweetener, including aspartame, or of usual dietary exposure. Participants were advised to continue their usual diet ad libitum. [The Working Group noted that there was co-ingestion of other sweeteners (cyclamate, acesulfame-K, saccharin).]

Kim et al. examined the impact of added doses of water or an artificially sweetened soft drink (containing a mix of acesulfame-K and aspartame) on glucose metabolism in healthy adults after consuming each for 2 weeks in a randomized crossover fashion (Kim et al., 2020). The dose of aspartame was 144 mg/L, which translated as a daily intake of 86.4 mg of aspartame per 600 mL of beverage. There was no record of usual dietary intakes of aspartame (or any other artificial sweetener) or of intakes during the study period, although participants were encouraged to keep diets constant and minimize eating out. Study participants were recruited on the basis of lack of use of non-nutritive sweeteners in the previous 2 weeks and, although no results were provided, changes in dietary intakes or consumption of soft drinks were recorded (Kim et al., 2020). [The Working Group noted that there was co-ingestion of acesulfame-K.]

(c) Estimated intake of aspartame from beverage consumption or total diet

Seven studies focused on intakes of aspartame or artificial sweeteners either from the total diet or from beverages only (Auerbach and Garfinkel, 1989; Suez et al., 2014; Frankenfeld et al., 2015; Hall et al., 2017; Hess et al., 2018; Tamez et al., 2018; Ramne et al., 2021). One study was carried out in Mexico (Tamez et al., 2018), one in Israel (Suez et al., 2014), one in Sweden (Ramne et al., 2021), and four in the USA (Auerbach and Garfinkel, 1989; Frankenfeld et al., 2015; Hall et al., 2017; Hess et al., 2018). All were cross-sectional and observational in nature.

[Across the studies, common strengths included the assessment of habitual diet using a mix of FFQs, food diaries, and dietary recalls, and often assessing total dietary intakes of aspartame from a variety of foods rather than relying on intakes of specific food groups (e.g. beverages). However, common limitations also existed, including the potential for recall bias, timing of exposure relative to the introduction of aspartame into the food supply, reliance on food composition databases that may not reflect product reformulations and composition changes for aspartame, and the potential for non-differential exposure misclassification.]

Tamez et al. (2018) completed a cross-sectional analysis of beverage intake and biomarkers of cardiovascular risk among 825 generally healthy Mexican women. Intakes of sugar and ASBs were compared over the previous year using a 138-item FFQ from which three questions were extracted regarding beverage intake (colas, other sodas, and diet sodas). Intake of artificial sweeteners was estimated on the basis of diet soda intake, with a serving size being 355 mL. The total questionnaire was previously validated with dietary histories and dietary recalls for total energy, carbohydrate, protein, and fat, but not specifically for sugar or artificial sweeteners. [There was no specific estimate of aspartame intake available, and so the study was of limited value since the focus was on total beverage intakes (sugar and diet), and it was not possible to determine aspartame exposure.]

Hess et al. (2018) completed a short-term assessment of intakes of non-nutritive sweeteners over a 2-week period and compared intakes with biomarkers of metabolic syndrome among 125 adults. The participants completed three 24-hour dietary recalls (2 weekdays and 1 weekend day). The authors adopted a previously published approach of identifying consumers as those who ingested from their total diet a quantity equivalent to that of non-nutritive sweeteners present in 1 fl oz [29 mL] of dietary beverages (equivalent to 17 mg of aspartame together with other sweeteners, including cyclamate, saccharin and acesulfame-K) (Hedrick et al., 2017). The authors used a nutrition software platform (Nutrition Data System for Research) that had a publication date of 2015, indicating that compositions of aspartame (and other sweeteners) may relate to that time period. The authors aggregated the groups as either consumers (≥ 17 mg/day aspartame) or non-consumers (presumably < 17 mg/day aspartame) to examine the relationship with the metabolic syndrome, but the analysis was limited to 33 aspartame consumers and intake patterns were not characterized further.

Hall et al. (2017) evaluated dietary sweetener consumption among individuals infected with HIV and matched healthy controls, assessing the relationship to immune and inflammatory markers and coronary plaque characteristics. The data set focused on a cohort of 36 HIV-infected participants and 15 non-infected participants (controls) who consumed aspartame. Dietary intakes of aspartame were quantified using a 4-day food diary (3 weekdays, 1 weekend day) and the Nutrition Data System for Research (2012 version). Within the HIV-infected cohort, 29% were aspartame consumers, with a mean daily intake of 164 mg/day, whereas in the control group 27% were consumers, with a mean daily intake of 89 mg/day. No detail was provided on food or beverages contributing to these intakes.

Frankenfeld et al. (2015) examined aspartame intake using a paper-based, estimated (not weighed) 4-day food record, collected over consecutive days and analysed using appropriate food composition databases (Nutrition Data System for Research for nutrient analysis, 2010 version) for the period when the data were collected. All sources of aspartame from the diet were assessed, contingent on the available food composition data. No data were presented on completeness of the food records (i.e. whether participants completed all 4 days). [The Working Group noted that aspartame intakes were calculated in milligrams per day; however, for the analysis, participants were classified as consumer or non-consumers, with no consideration of dose.]

Ramne et al. (2021) examined ASB consumption among 1371 participants via two separate dietary assessment methods that were combined to reflect habitual consumption using data collected at baseline between 2013 and 2017. Participants completed a web-based 4-day food record and a web-based short FFQ covering the past 6 months; consumption frequencies addressed ASB intakes ranging from never or seldom to several times per day on an eight-level

scale. Data from each method were grouped into three levels and cross-tabulated to create a new classification of participants as non-consumers, medium consumers, and high consumers of ASBs. [The Working Group noted that no specific assessment of aspartame was conducted, and intakes of ASBs were used as a proxy for aspartame intake. Other artificial sweeteners were used at the time of intake data collection, which limited the certainty of the exposure estimate. No information or reference was provided to be able to examine the validity of the FFQ, and it was unclear how many items or questions on the short FFQ covered ASBs. Completeness of the food record was not reported.]

Suez et al. (2014) assessed long-term non-caloric artificial sweetener consumption among 381 non-diabetic individuals (males, 44%; females, 56%) from a single question in an FFQ with data collected in 2013 in Israel. Consumption of any non-caloric artificial sweetener was assessed, rather than specifically aspartame. [The Working Group noted that the dietary assessment was poorly described, and no further information on the FFQ was provided (e.g. validity, reference period, or specific details of the questionnaire). Furthermore, there was no reference cited from which these details could be obtained.]

Auerbach and Garfinkel (1989) conducted a retrospective case analysis of 149 non-smokers and smokers who had died from various causes between 1976 and 1984. Frequency of use of artificial sweeteners in soft drinks or added to coffee or tea or other beverages or foods was assessed by retrospective recall by family members of the cases. Frequency information was collected in broad categories (regular use, occasional use, or rarely used). [The Working Group noted that there was no specific assessment of aspartame, only of total artificial sweeteners. Given the dates when the study was conducted, and that aspartame was approved as a tabletop sweetener (relevant for tea and coffee exposure) in 1981 and in

ASBs in 1983, significant exposure to aspartame was unlikely in the majority of this cohort.]

Yu et al. (2018) conducted a pooled analysis from two time points of the NHS and compared intakes of ASBs with several biomarkers of cardiometabolic health. Frequency of use of low-energy beverages or ASBs was assessed as a cumulative average of intakes from at least two FFQs, before blood collection, and ranked into five categories ranging from never/almost never to ≥ 1 time/day. Time points for collection of dietary intake were 1986–1990 and 1994–1998, and for blood biomarkers were 1989–1990 and 2000–2001. [The Working Group noted that there was no specific assessment of aspartame, only total artificial sweeteners. However, given the dates when the study was conducted in the USA, the timing of exposure was consistent with aspartame being the major artificial sweetener in beverages.]

(d) Intake of aspartame from commercial beverages used as the control arm in studies focusing on sugar-sweetened beverages

Randomized controlled trials

Four randomized controlled trials studies used commercially available beverages containing aspartame as a control in studies focused on sugar-sweetened beverages rather than aspartame per se (Hieronimus et al., 2020; Sigala et al., 2020, 2021, 2022). All studies were involving the same US study group and used the same aspartame-containing drink mix (fruit-flavoured, commercial) although the dose of aspartame was not specified. The study populations of healthy adults were all matched for sex, body mass index (BMI), fasting triglyceride, LDL-cholesterol, HDL-cholesterol, and insulin concentrations. In each study, 24-hour recalls were taken, but there was no detail provided on usual intakes of aspartame or of any other artificial sweetener. Participants were advised during the studies

to limit their consumption of sugar-containing beverages to one serving of 100% fruit juice, but no such guidance was provided regarding artificial sweeteners, and it was not possible to determine whether there were any changes in intake of aspartame in any of the groups beyond the beverages provided. [The Working Group noted that exposure was clearly defined in these study protocols and therefore there was a high certainty of aspartame exposure, despite the amount of aspartame exposure and background exposure being unknown.]

Bishop et al. (2002) conducted a randomized controlled trial ($n = 6$) in the UK comparing a carbohydrate solution with an artificially sweetened solution (the placebo); however, there was no information on the type of beverage used for the placebo. Diet was assessed (but not reported) for 2 days before the trial, to standardize intakes before the second trial. There was no assessment of long-term diet reported.

2. Cancer in Humans

Introduction

This section reviews studies of exposure to aspartame and to artificial sweetener formulations containing aspartame as the primary sweetener in relation to cancer incidence or mortality in humans. A systematic search was conducted in the PubMed database (NCBI, 2023) to identify cohort, case–control, and nested case–control studies evaluating exposure to aspartame or artificially sweetened beverages (ASBs) with cancer as an outcome. The search terms used and the resulting literature tree are available online from: https://hawcproject. iarc.who.int/lit/assessment/680/. Case reports, studies using ecological designs, and studies that did not include cancer as an end-point were not considered further.

The Working Group considered cohort and case–control studies with various exposure definitions aimed at capturing aspartame consumption. All identified studies were nutritional epidemiology studies, and no studies were identified in occupationally or environmentally exposed populations. Only a handful of studies assessed aspartame specifically. Many studies measured intake of ASBs, which were considered by the Working Group to be a proxy for aspartame exposure on the basis of the time period and country in which the study was conducted (see Section 1.4 and Annex 2, Scientific and other publicly available data on aspartame use in artificially sweetened beverages, also available from: https://publications.iarc.who.int/627). In addition to ASBs, some studies also measured consumption of tabletop sweeteners containing aspartame. Control for confounding, in particular, body mass index (BMI) and consumption of sugar or sugar-sweetened beverages, was carefully evaluated, as was effect modification by diabetes. Several studies provided information on use of artificial sweeteners that were likely to be a mix of aspartame and other artificial sweeteners. The Working Group also considered meta-analyses of aspartame or ASBs and cancer, but carefully reviewed whether the summary results were pertinent to exposure to aspartame versus exposure to other artificial sweeteners. A recent systematic review and meta-analysis by the World Health Organization (WHO) reported on non-sugar sweeteners and cancer risk (WHO et al., 2022). It is important to note that this systematic review was limited to studies with interventions or exposures within the respective acceptable daily intakes (ADIs) established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), so may not include some studies for which it was concluded that the ADI may have been exceeded. Additionally, this meta-analysis was focused on all types of non-sugar sweetener and therefore was only considered relevant to the present evaluation if

the summary estimates included studies relevant to aspartame exposure only (either directly or by proxy with ASBs).

The studies are presented below in sections by cancer end-point and are ordered by study design and then publication year (oldest to most recent). Cohort studies are described first, followed by case–control studies and meta-analyses of potential relevance.

2.1 Cancer of the liver, colon and rectum, pancreas, and other organs of the digestive tract

2.1.1 Cancer of the liver

See Table 2.1.

Three prospective cohort studies including a total of four cohorts examined the association between aspartame exposure or ASB consumption and risk of liver cancer (Stepien et al., 2016; Jones et al., 2022; McCullough et al., 2022).

A study in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort (Stepien et al., 2016) examined soft drink consumption and liver cancer risk among 477 206 men and women from 10 European countries. Over a mean follow-up of 11.4 years, 191 cases of hepatocellular cancer were diagnosed. Usual diet was assessed at study enrolment between 1992 and 1998 using country-specific diet assessment instruments. Artificially sweetened soft drink intake was assessed by asking participants to recall the number of glasses (approximately 250 mL) of "low-calorie or diet fizzy soft drinks" per month, week, or day (Mullee et al., 2019). This quantity was then converted to 330 mL servings per week for the analysis (Stepien et al., 2016). In the analysis restricted to the centres with available information on artificially sweetened soft drink consumption, each 330 mL serving of artificially sweetened soft drink per week was associated with an increased risk of hepatocellular cancer (hazard ratio, HR, 1.06; 95% CI,

1.03–1.09; 151 cases). [The Working Group noted that a correction to the number of hepatocellular carcinomas reported in the original paper was published on 17 April 2024 and the original paper was updated.] Individuals who were obese or reported a history of diabetes consumed artificially sweetened soft drinks more frequently than sugar-sweetened soft drinks. [Strengths included prospective analysis of a geographically heterogeneous population with detailed and validated dietary instruments specific to each country (which potentially had its own products with variable content in aspartame) during relevant periods of aspartame exposure. However, towards the end of the follow-up period for countries that started recruitment later (i.e. towards 2000), co-exposure to other artificial sweeteners was likely. The study controlled finely for BMI (height and weight were measured by trained investigators), diabetes status, smoking, lifetime alcohol exposure, physical activity, and education. The authors also evaluated potential confounding by total sugar, sugar-sweetened beverages, coffee consumption, and other dietary factors and noted that these did not appreciably change risk estimates. The study did not control for hepatitis B virus/hepatitis C virus (HBV/HCV) status, which is a strong risk factor for liver cancer, but whether it was associated with ASB consumption is unclear. Moreover, the prevalence of HBV/HCV infection in the EPIC cohort is very low (around 3%) (Trichopoulos et al., 2011). A limitation of this study was that baseline-only assessment of ASB intake may contribute to non-differential measurement error and bias risk estimates towards the null.]

Jones et al. (2022) pooled data from two cohorts in the USA, the National Institutes of Health-American Association of Retired Persons Diet and Health Study (NIH-AARP) and the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial, which included 553 874 participants of whom 1060 developed liver cancer (ICD-O-3, C22.0 and C22.1, liver and intrahepatic bile

Table 2.1 Epidemiological studies on consumption of aspartame and cancers of the liver, pancreas, and other digestive organs

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Schernhammer et al. (2005) USA Enrolment, 1976 (NHS), 1986 (HPFS/ follow-up, 1980–2000 (NHS), 1986–2000 (HPFS) Cohort	88 794 women and 49 364 men; female registered nurses aged 30–55 yr in the NHS and male health professionals aged 40–75 yr in the HPFS Exposure assessment method: prospective assessment of ASB consumption through repeated FFQs between 1980–1986 and 2000	Pancreas, incidence	Frequency of diet soft drink consumption (RR): < 1/mo 1–12/mo > 3/wk Trend-test P value: 0.98	155 116 108	1 1.08 (0.85–1.38) 1.02 (0.79–1.32)	Age (years), sex, follow-up cycle, history of diabetes, smoking status, caloric intake, nonvigorous physical activity, other soft drink consumption, BMI	*Exposure assessment critique*: A key strength was the prospective assessment of ASB consumption as a cumulative average from repeated diet assessments (using validated instruments) every 4 yr, the majority at a very relevant period for aspartame exposure from ASBs (the USA between 1980–1986 and 2000) potentially capturing lifetime exposure to aspartame. A key limitation was that other sources of aspartame were not considered (although these were more limited). *Other strengths*: updated smoking and history of diabetes covariates, stopped updating exposure information with new report of diabetes mellitus; control for BMI at baseline.
		Pancreas, incidence	Frequency of diet soft drink consumption, women (RR): < 1/mo 1–12/mo > 3/wk Trend-test P value: 0.64	77 62 66	1 1.10 (0.78–1.55) 1.12 (0.79–1.59)	Age (years), follow-up cycle, history of diabetes, smoking status, caloric intake, nonvigorous physical activity, other soft drink consumption, BMI	
		Pancreas, incidence	Frequency of diet soft drink consumption, men (RR): < 1/mo 1–12/mo > 3/wk Trend-test P value: 0.52	78 54 42	1 1.08 (0.76–1.53) 0.89 (0.60–1.33)		
		Pancreas, incidence	Frequency of diet cola consumption (RR): < 1/mo 1–12/mo > 3/wk Trend-test P value: 0.39	182 105 92	1 0.90 (0.67–1.21) 0.85 (0.59–1.21)	Age (years), sex, follow-up cycle, history of diabetes, smoking status, caloric intake, nonvigorous physical activity, other soft drink consumption, BMI	

Table 2.1   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Schernhammer et al. (2005) (cont.)		Pancreas, incidence	Frequency of diet cola consumption, women (RR):			Age (years), follow-up cycle, history of diabetes, smoking status, caloric intake, nonvigorous physical activity, other soft drink consumption, BMI	*Other limitations*: potential for confounding from unmeasured factors; diet soft drink intake was not high; limited number of cases in some stratified analyses resulted in wide confidence intervals.
			< 1/mo	83	1		
			1–12/mo	63	0.84 (0.49–1.42)		
			> 3/wk	59	0.83 (0.43–1.59)		
			Trend-test *P* value: 0.96				
		Pancreas, incidence	Frequency of diet cola consumption, men (RR):				
			< 1/mo	99	1		
			1–12/mo	42	0.86 (0.58–1.26)		
			> 3/wk	33	0.85 (0.54–1.32)		
			Trend-test *P* value: 0.38				
		Pancreas, incidence	Frequency of other diet soft drink consumption (RR):			Age (years), sex, follow-up cycle, history of diabetes, smoking status, caloric intake, nonvigorous physical activity, other soft drink consumption, BMI	
			< 1/mo	211	1		
			1–12/mo	111	1.14 (0.85–1.53)		
			> 3/wk	57	1.40 (0.93–2.11)		
			Trend-test *P* value: 0.13				

Table 2.1   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Schernhammer et al. (2005) (cont.)		Pancreas, incidence	Frequency of other diet soft drink consumption, women (RR):			Age (years), follow-up cycle, history of diabetes, smoking status, caloric intake, nonvigorous physical activity, other soft drink consumption, BMI	
			< 1/mo	87	1		
			1–12/mo	74	1.44 (0.85–2.44)		
			> 3/wk	44	1.50 (0.76–2.97)		
			Trend-test *P* value: 0.54				
		Pancreas, incidence	Frequency of other diet soft drink consumption, men (RR):				
			< 1/mo	124	1		
			1–12/mo	37	0.89 (0.59–1.34)		
			> 3/wk	13	1.62 (0.88–2.97)		
			Trend-test *P* value: 0.15				
		Pancreas, incidence	Frequency of diet soft drink consumption, BMI < 25 kg/m² (RR):			Age (years), sex, follow-up cycle, history of diabetes, smoking status, caloric intake, nonvigorous physical activity, other soft drink consumption	
			< 1/mo	86	1		
			1–12/mo	59	1.24 (0.88–1.74)		
			> 3/wk	37	0.95 (0.63–1.41)		
			Trend-test *P* value: 0.69				
		Pancreas, incidence	Frequency of diet soft drink consumption, BMI ≥ 25 kg/m² (RR):				
			< 1/mo	66	1		
			1–12/mo	56	0.96 (0.67–1.38)		
			> 3/wk	67	1.03 (0.72–1.47)		
			Trend-test *P* value: 0.88				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Schernhammer et al. (2005) (cont.)		Pancreas, incidence	Frequency of diet soft drink consumption, low physical activity (men, < 11.5 MET/wk; women, < 7.7 MET/wk) (RR):			Age (years), sex, follow-up cycle, history of diabetes, smoking status, caloric intake, nonvigorous physical activity, other soft drink consumption	
			< 1/mo	72	1		
			1–12/mo	53	1.07 (0.74–1.54)		
			> 3/wk	51	0.99 (0.68–1.44)		
			Trend-test *P* value: 0.84				
		Pancreas, incidence	Frequency of diet soft drink consumption, high physical activity (men, ≥ 11.5 MET/wk; women, ≥ 7.7 MET/wk) (RR):				
			< 1/mo	83	1		
			1–12/mo	63	1.07 (0.77–1.50)		
			> 3/wk	57	1.03 (0.72–1.47)		
			Trend-test *P* value: 0.94				
		Pancreas, incidence	Frequency of diet soft drink consumption, women, BMI, < 25 kg/m^2 (RR):			Age (years), follow-up cycle, history of diabetes, smoking status, caloric intake, nonvigorous physical activity, other soft drink consumption	
			< 1/mo	55	1		
			1–12/mo	34	1.05 (0.68–1.62)		
			> 3/wk	30	1.02 (0.64–1.62)		
			Trend-test *P* value: 0.93				
		Pancreas, incidence	Frequency of diet soft drink consumption, women, BMI ≥ 25 kg/m^2 (RR):				
			< 1/mo	21	1		
			1–12/mo	28	1.31 (0.74–2.32)		
			> 3/wk	34	1.32 (0.75–2.33)		
			Trend-test *P* value: 0.62				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Schernhammer et al. (2005) (cont.)		Pancreas, incidence	Frequency of diet soft drink consumption, women, low physical activity (< 7.7 MET/wk) (RR):			Age (years), follow-up cycle, history of diabetes, smoking status, caloric intake, nonvigorous physical activity, other soft drink consumption	
			< 1/mo	24	1		
			1–12/mo	25	0.72 (0.41–1.28)		
			> 3/wk	31	1.05 (0.63–1.76)		
			Trend-test P value: 0.38				
		Pancreas, incidence	Frequency of diet soft drink consumption, women, high physical activity (≥ 7.7 MET/wk) (RR):				
			< 1/m	53	1		
			1–12/mo	37	1.52 (0.98–2.36)		
			> 3/wk	35	1.21 (0.75–1.94)		
			Trend-test P value: 0.72				
		Pancreas, incidence	Frequency of diet soft drink consumption, men, BMI < 25 kg/m^2 (RR):				
			< 1/mo	31	1		
			1–12/mo	25	1.65 (0.96–2.83)		
			> 3/wk	7	0.68 (0.29–1.57)		
			Trend-test P value: 0.34				
		Pancreas, incidence	Frequency of diet soft drink consumption, men, BMI ≥ 25 kg/m^2 (RR):				
			< 1/mo	45	1		
			1–12/mo	28	0.77 (0.48–1.25)		
			> 3/wk	33	0.81 (0.55–1.40)		
			Trend-test P value: 0.74				
		Pancreas, incidence	Frequency of diet soft drink consumption, men, low physical activity (< 11.5 MET/wk) (RR):				
			< 1/mo	48	1		
			1–12/mo	28	0.94 (0.58–1.52)		
			> 3/wk	20	0.71 (0.41–1.22)		
			Trend-test P value: 0.21				

Table 2.1   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Schernhammer et al. (2005) (cont.)		Pancreas, incidence	Frequency of diet soft drink consumption, men, high physical activity (≥ 11.5 MET/wk) (RR):			Age (years), follow-up cycle, history of diabetes, smoking status, caloric intake, nonvigorous physical activity, other soft drink consumption	
			< 1/mo	30	1		
			1–12/mo	26	1.26 (0.74–2.15)		
			> 3/wk	22	1.19 (0.66–2.12)		
			Trend-test P value: 0.67				
Bao et al. (2008) USA Enrolment, 1995–1996/ follow-up through 2003 (mean, 7.2 yr) Cohort	487 922; NIH-AARP Diet and Health Study: AARP members (men and women) aged 50–71 yr residing in 8 study areas, excluding those with prevalent cancer, history of diabetes, proxy respondents, and persons reporting extreme energy intake	Pancreas (exocrine, adenocarcinoma), incidence	Diet soft drink consumption (RR):			Sex, age	*Exposure assessment critique*: Key strengths were that ASB consumption was assessed and studied during a very relevant period for aspartame exposure from ASBs (1995–2003 in the USA); three different types of ASB were considered; and the prospective assessment.
			Never drinkers (0 g/day)	716	1		
			1st quintile (0.1–26.3 g/day)	137	1.14 (0.90–1.43)		
			2nd quintile (26.4–76.9 g/day)	86	0.91 (0.70–1.18)		
			3rd quintile (77.0–258.9 g/day)	99	0.96 (0.75–1.24)		
			4th quintile (259.0–494.4 g/day)	118	1.17 (0.92–1.49)		
			5th quintile (494.5–4897.6 g/day)	102	1.14 (0.89–1.47)		
			Trend-test P value: 0.46				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Bao et al. (2008) (cont.)	Exposure assessment method: FFQ at baseline, average follow-up of 7.2 yr Consumption frequency of 3 types of soft drink (soda, fruit drinks, and iced tea) + frequency of consumption of diet vs regular versions + 3 portion sizes	Pancreas (exocrine, adenocarcinoma), incidence	Diet soft drink consumption (RR):			Sex, age, race, education, BMI, alcohol, smoking, physical activity, energy-adjusted red meat consumption, energy-adjusted folate consumption, total energy, regular soft drinks	Key limitations were the sequential assessment of first the frequency of consumption of soft drinks and then that of diet/sugar-free versions, with imprecise frequencies inducing inaccuracies; other sources of aspartame were not considered (although more limited). *Other strengths*: large study; estimated 90% completeness of case identification via linkage with cancer registries, detailed adjustment for confounders, including BMI; control for diabetes at baseline by exclusion of participants who reported having diabetes at baseline. *Other limitations*: potential for bias from non-differential measurement error.
			Never drinkers	716	1		
			1st quintile (0.1–26.3 g/day)	137	1.23 (0.97–1.55)		
			2nd quintile (26.4–76.9 g/day)	86	0.97 (0.74–1.27)		
			3rd quintile (77.0–258.9 g/day)	99	1.03 (0.80–1.33)		
			4th quintile (259.0–494.4 g/day)	118	1.23 (0.96–1.56)		
			5th quintile (494.5–4897.6 g/day)	102	1.11 (0.86–1.44)		
			Trend-test *P* value: 0.68				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Bao et al. (2008) (cont.)		Pancreas (exocrine, adenocarcinoma), incidence	Diet soft drink consumption, excluding the first 2 yr of follow-up (RR):			Sex, age, race, education, BMI, alcohol, smoking, physical activity, energy-adjusted red meat consumption, energy-adjusted folate consumption, total energy, regular soft drinks	
			Never drinkers	NR	1		
			1st quintile (0.1–26.3 g/day)	NR	1.22 (0.94–1.59)		
			2nd quintile (26.4–76.9 g/day)	NR	1.00 (0.74–1.35)		
			3rd quintile (77.0–258.9 g/day)	NR	1.00 (0.75–1.34)		
			4th quintile (259.0–494.4 g/day)	NR	1.35 (1.03–1.77)		
			5th quintile (494.5–4897.6 g/day)	NR	1.25 (0.94–1.66)		
			Trend-test *P* value: 0.19				
		Pancreas (exocrine, adenocarcinoma), incidence	Diet soft drink consumption, non-obese (BMI, < 30 kg/m²) (RR):			Sex, age, race, education, BMI, alcohol, smoking, physical activity, energy-adjusted red meat consumption, energy-adjusted folate consumption, total energy, regular soft drinks	
			1st quintile (median, 0 g/day)	NR	1		
			2nd quintile (median, 16.2 g/day)	NR	1.31 (1.03–1.68)		
			3rd quintile (median, 74.8 g/day)	NR	0.88 (0.67–1.17)		
			4th quintile (median, 260.6 g/day)	NR	1.20 (0.93–1.56)		
			5th quintile (median, 816.9 g/day)	NR	1.16 (0.88–1.53)		
			Trend-test *P* value: 0.59				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Bao et al. (2008) (cont.)		Pancreas (exocrine, adenocarcinoma), incidence	Diet soft drink consumption, obese (BMI, ≥ 30 kg/m²) (RR):			Sex, age, race, education, BMI, alcohol, smoking, physical activity, energy-adjusted red meat consumption, energy-adjusted folate consumption, total energy intake, regular soft drinks	
			1st quintile (median, 0 g/day)	NR	1		
			2nd quintile (median, 16.2 g/day)	NR	0.91 (0.51–1.63)		
			3rd quintile (median, 74.8 g/day)	NR	0.94 (0.53–1.64)		
			4th quintile (median, 260.6 g/day)	NR	0.93 (0.54–1.61)		
			5th quintile (median, 816.9 g/day)	NR	0.89 (0.51–1.53)		
			Trend-test *P* value: 0.73				
		Pancreas (exocrine, adenocarcinoma), incidence	Diet soft drink consumption, high physical activity (≥ 3 times/wk) (RR):			Sex, age, race, education, BMI, alcohol, smoking, physical activity, energy-adjusted red meat consumption, energy-adjusted folate consumption, total energy, regular soft drinks	
			1st quintile (median, 0 g/day)	NR	1		
			2nd quintile (median, 16.2 g/day)	NR	1.21 (0.89–1.66)		
			3rd quintile (median, 74.8 g/day)	NR	0.87 (0.61–1.23)		
			4th quintile (median, 260.6 g/day)	NR	1.01 (0.72–1.41)		
			5th quintile (median, 816.9 g/day)	NR	1.14 (0.81–1.62)		
			Trend-test *P* value: 0.52				

Table 2.1   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Bao et al. (2008) (cont.)		Pancreas (exocrine, adenocarcinoma), incidence	Diet soft drink consumption, low physical activity (≤ 3 times/week) (RR):			Sex, age, race, education, BMI, alcohol, smoking, physical activity, energy-adjusted red meat consumption, energy-adjusted folate consumption, total energy, regular soft drinks	
			1st quintile (median, 0 g/day)	NR	1		
			2nd quintile (median, 16.9 g/day)	NR	1.27 (0.92–1.75)		
			3rd quintile (median, 74.8 g/day)	NR	1.04 (0.74–1.47)		
			4th quintile (median, 260.6 g/day)	NR	1.27 (0.92–1.76)		
			5th quintile (median, 816.9 g/day)	NR	1.12 (0.79–1.57)		
			Trend-test P value: 0.84				
		Pancreas (exocrine, adenocarcinoma), incidence	Diet soft drink consumption, never-smoker or quit ≥ 10 yr ago (RR):			Sex, age, race, education, BMI, alcohol, smoking, physical activity, energy-adjusted red meat consumption, energy-adjusted folate consumption, total energy, regular soft drinks	
			1st quintile (median, 0 g/day)	NR	1		
			2nd quintile (median, 16.2 g/day)	NR	1.22 (0.92–1.64)		
			3rd quintile (median, 74.8 g/day)	NR	1.03 (0.76–1.40)		
			4th quintile (median, 260.6 g/day)	NR	1.11 (0.82–1.49)		
			5th quintile (median, 816.9 g/day)	NR	1.25 (0.92–1.71)		
			Trend-test P value: 0.32				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
[Bao et al. (2008)](#) (cont.)		Pancreas (exocrine, adenocarcinoma), incidence	Diet soft drink consumption, current smoker or quit < 10 yr ago (RR):			Sex, age, race, education, BMI, alcohol, smoking, physical activity, energy-adjusted red meat consumption, energy-adjusted folate consumption, total energy, regular soft drinks	
			1st quintile (median, 0 g/day)	NR	1		
			2nd quintile (median, 16.2 g/day)	NR	1.21 (0.81–1.79)		
			3rd quintile (median, 74.8 g/day)	NR	0.80 (0.51–1.27)		
			4th quintile (median, 260.6 g/day)	NR	1.19 (0.80–1.79)		
			5th quintile (median, 816.9 g/day)	NR	0.92 (0.60–1.40)		
			Trend-test P value: 0.45				

Table 2.1   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Navarrete-Muñoz et al. (2016) Europe Enrolment, 1992–2000/ follow-up, through 2004 to 2009 (depending on country) Cohort	477 199 (142 202 men and 334 997 women); EPIC cohort study participants from 23 centres in 10 European countries (Denmark, France, Germany, Greece, Italy, Norway, Spain, Sweden, Netherlands, and United Kingdom), excluding participants with prevalent cancer other than nonmelanoma skin cancer at baseline or with extreme energy intake/expenditure; Additionally, data from Italy, Spain, and Umeå were excluded from ASB analyses	Pancreas (exocrine, adenocarcinoma), incidence	Artificially sweetened soft drink consumption (HR): Nondrinker 1st quintile (0.1–2.0 g/day) 2nd quintile (2.1–9.9 g/day) 3rd quintile (9.9–28.6 g/day) 4th quintile (28.7–92.2 g/day) 5th quintile (> 92.2 g/day) Continuous (per 100 g/day) Trend-test *P* value: 0.55	340 54 50 53 42 47 586	0.88 (0.58–1.35) 1 1.13 (0.69–1.86) 1.12 (0.71–1.77) 1.03 (0.64–1.67) 1.07 (0.67–1.73) 1.04 (0.98–1.10)	Centre, sex, age, educational level, physical activity, smoking status, alcohol consumption, sugar-sweetened soft drinks, juice consumption	*Exposure assessment critique*: A key strength was the prospective assessment of ASB consumption (several types of beverage) in several western Europe countries at a period relevant for aspartame exposure (between 1991 and 2000). Key limitations were that no other sources of aspartame were considered; uncertainty regarding aspartame content of ASBs in every country; and there was only one assessment at baseline.

Table 2.1   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
[Navarrete-Muñoz et al. (2016)](#) (cont.)	Exposure assessment method: questionnaire; exposure to ASBs overall assessed once at baseline through country-specific validated tools (mainly FFQ) covering the usual diet over the past year; no specific assessment of aspartame or AS content in ASB	Pancreas (exocrine, adenocarcinoma), incidence	Artificially sweetened soft drink consumption (HR):			Centre, sex, age, educational level, physical activity, smoking status, alcohol consumption, sugar-sweetened soft drinks, juice consumption, energy intake, diabetes, BMI	Other information: context of low ASB consumption in middle-aged adults; continuous analyses were per 100 g increment which spans from nondrinker to the highest quintile; analyses across countries were meta-analysed; no heterogeneity by country; adjustment set for sex-stratified models by 336 g increment were confirmed on a personal communication with authors.
			Nondrinker	340	0.89 (0.58–1.36)		
			1st quintile (0.1–2.0 g/day)	54	1		
			2nd quintile (2.1–9.9 g/day)	50	1.12 (0.68–1.84)		
			3rd quintile (9.9–28.6 g/day)	53	1.09 (0.69–1.73)		
			4th quintile (28.7–92.2 g/day)	42	0.99 (0.61–1.60)		
			5th quintile (> 92.2 g/day)	47	0.99 (0.61–1.60)		
			Continuous (per 100 g/day)	586	1.02 (0.96–1.08)		*Other strengths*: large cohort, geographical diversity, validated questionnaires, cancer subtype available; results adjusted for diabetes and BMI.
			Trend-test *P* value: 0.81				
		Pancreas (exocrine, adenocarcinoma), incidence	Artificially sweetened soft drink consumption, women (HR):			Centre, age, education, smoking, alcohol consumption physical activity, BMI, diabetes, energy intake	
			Continuous (per 336 g/day)	373	0.69 (0.44–1.09)		*Other limitations*: co-exposure to other ASs likely towards the end of follow-up for countries that started recruitment later.
		Pancreas (exocrine, adenocarcinoma), incidence	Artificially sweetened soft drink consumption, men (HR):				
			Continuous (per 336 g/day)	313	1.25 (1.03–1.52)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Navarrete-Muñoz et al. (2016) (cont.)		Pancreas (exocrine, adenocarcinoma), incidence	Artificially sweetened soft drink consumption, age < 62 yr at diagnosis (HR): Continuous (per 100 g/day)	215	1.00 (0.90–1.10)	Centre, sex, age, educational level, physical activity, smoking status, alcohol consumption, sugar-sweetened soft drinks, juice consumption	
		Pancreas (exocrine, adenocarcinoma), incidence	Artificially sweetened soft drink consumption, age ≥ 62 yr at diagnosis (HR): Continuous (per 100 g/day)	471	1.05 (0.98–1.12)		
		Pancreas (exocrine, adenocarcinoma), incidence	Artificially sweetened soft drink consumption, BMI < 25 kg/m^2 (HR): Continuous (per 100 g/day)	297	0.95 (0.81–1.11)		
		Pancreas (exocrine, adenocarcinoma), incidence	Artificially sweetened soft drink consumption, BMI ≥ 25 kg/m^2 (HR): Continuous (per 100 g/day)	389	1.05 (0.99–1.12)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Navarrete-Muñoz et al. (2016) (cont.)		Pancreas (exocrine, adenocarcinoma), incidence	Artificially sweetened soft drink consumption, waist circumference normal/moderate (HR): Continuous (per 100 g/day)	452	1.05 (0.98–1.13)	Centre, sex, age, educational level, physical activity, smoking status, alcohol consumption, sugar-sweetened soft drinks, juice consumption	
		Pancreas (exocrine, adenocarcinoma), incidence	Artificially sweetened soft drink consumption, waist circumference large (HR): Continuous (per 100 g/day)	180	1.02 (0.93–1.11)	Centre, sex, age, education, physical activity, alcohol consumption, sugar-sweetened beverage intake	
		Pancreas (exocrine, adenocarcinoma), incidence	Artificially sweetened soft drink consumption, low physical activity (HR): Continuous (per 100 g/day)	381	1.07 (1.00–1.15)	Centre, sex, age, educational level, physical activity, smoking status, alcohol consumption, sugar-sweetened soft drinks, juice consumption	
		Pancreas (exocrine, adenocarcinoma), incidence	Artificially sweetened soft drink consumption, high physical activity (HR): Continuous (per 100 g/day)	268	1.02 (0.93–1.12)		
		Pancreas (exocrine, adenocarcinoma), incidence	Artificially sweetened soft drink consumption, never-smokers (HR): Continuous (per 100 g/day)	257	1.00 (0.88–1.14)	Centre, sex, age, educational level, physical activity, alcohol consumption, sugar-sweetened soft drinks, juice consumption	
		Pancreas (exocrine, adenocarcinoma), incidence	Artificially sweetened soft drink consumption, former smokers (HR): Continuous (per 100 g/day)	201	1.05 (0.96–1.15)		
		Pancreas (exocrine, adenocarcinoma), incidence	Artificially sweetened soft drink consumption, current smokers (HR): Continuous (per 100 g/day)	213	1.03 (0.93–1.13)		

Table 2.1   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Stepien et al. (2016) Europe Enrolment, 1992–1998/ follow-up, through 2006 to 2010 (depending on country) Cohort	424 123; EPIC study participants from 23 centres in Denmark, France, Greece, Germany, Italy, Netherlands, Norway, Spain, Sweden, and the United Kingdom (for artificially sweetened soft drinks analysis, participants from selected centres in Italy (north and Ragusa) and Sweden (Umeå) were excluded); men and women generally aged 35–70 yr; excluding participants with prevalent cancer other than nonmelanoma skin cancer at baseline or with extreme energy intake/expenditure	Liver (HCC), incidence	Artificially sweetened soft drink consumption (HR): Continuous (per serving of 330 mL)/ wk	151	1.06 (1.03–1.09)	Sex, age, study centre, non-alcoholic energy intake, alcohol at enrolment, lifetime alcohol intake, smoking status and intensity, BMI, physical activity, education, diabetes status	*Exposure assessment critique:* A key strength was the prospective assessment of ASB consumption (several types of beverages) in several western European countries at a period relevant for aspartame exposure (between 1991 and 2000). Key limitations were that no other sources of aspartame were considered; uncertainty regarding aspartame content in ASBs in each country; and there was only one assessment at baseline. Other information: context of low ASB consumption in middle-aged adults; did not present detailed data by ASB, e.g. unknown whether models controlled for biochemical measures of hepatitis and liver enzymes.

Table 2.1   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Stepien et al. (2016) (cont.)	Exposure assessment method: questionnaire; exposure to artificially sweetened soft drinks overall assessed once at baseline through country-specific validated tools (mainly FFQ) covering the usual diet over the past year; no specific assessment of aspartame or AS content of the artificially sweetened soft drinks						[Note: the Working Group noted that a correction to the number of hepatocellular carcinomas reported in the original paper was published on 17 April 2024 and the original paper was updated.] *Other strengths*: prospective design; diverse European population; control for multiple confounders, including BMI. *Other limitations*: small number of cases; co-exposure to other ASs likely towards the end of follow-up for countries that started recruitment later; did not control for HBV/HCV status.
Hodge et al. (2018) Australia Enrolment, 1990–1994/ follow-up through 30 June 2013 Cohort	35 593MCCS – a prospective cohort study of men and women aged 40–69 yr at recruitment and free of cancer, angina, heart attack, or diabetes at baseline; participants with extreme baseline energy intake were excluded	Stomach, (gastric cardia), incidence	Artificially sweetened soft drink consumption (HR): Never or < 1/mo 1–3/mo 1–6/wk ≥ 1/day Continuous (per beverage/ day) Trend-test *P* value: 0.46	123 9 23 10 165	1 0.86 (0.42–1.73) 1.46 (0.92–2.34) 1.03 (0.53–1.98) 1.24 (0.70–2.18)	Age, sex, socioeconomic index, country of birth, alcohol intake, smoking status, physical activity, Mediterranean diet score, sugar-sweetened soft drink consumption, waist circumference	*Exposure assessment critique*: Key strengths were that it was a prospective study; assessment after aspartame introduction in diet soft drinks in Australia (1987), and first half of follow-up largely overlapping with period of aspartame use in Australia.

Table 2.1   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Hodge et al. (2018) (cont.)	Exposure assessment method: self-administered 121-item FFQ with separate questions on frequency of consumption in the past year of diet (artificially sweetened) soft drinks	Colon and rectum, incidence	Artificially sweetened soft drink consumption (HR): Never or < 1/mo 1–3/mo 1–6/wk ≥ 1/day Continuous (per beverage/ day) Trend-test *P* value: 0.46	 802 77 125 51 1055	 1 0.87 (0.68–1.11) 1.15 (0.95–1.40) 0.79 (0.60–1.06) 0.91 (0.71–1.17)	Age, sex, socioeconomic index, country of birth, alcohol intake, smoking status, physical activity, Mediterranean diet score, sugar-sweetened soft drink consumption, waist circumference	Key limitations were the FFQ assessment with no specific estimate of aspartame exposure, ASBs as a proxy, and exposure data at baseline only. *Other information*: other non-colorectal gastrointestinal cancers were included in the "overall obesity-related cancers" outcome but were not examined individually. *Other strengths*: adjustment for key confounders, including a measure of obesity (waist circumference); ability to examine stomach cancer subsite. *Other limitations*: small number of consumers; likely bias from non-differential exposure misclassification given single baseline assessment and long follow-up.

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Chazelas et al. (2019) France Enrolment, 2009–2017/ follow-up, through 11 January 2018 Cohort	101 257; NutriNet-Santé prospective cohort (web-based); men and women aged ≥ 18 yr Exposure assessment method: participants were asked every 6 mo to complete a series of three validated web-based 24 h dietary records randomly assigned over a 2-wk period (2 weekdays, 1 weekend day); at least two 24 h dietary records during the first 2 yr of follow-up were considered in analyses (mean ± SD, 5.6 ± 3.0)	Colon and rectum, incidence	ASB intake (subdistribution HR): 1st quartile (men, < 2.7 mL/ day; or women, < 4.6 mL/day)	119	1	Age, sex, energy intake without alcohol, sugar intake from other dietary sources, alcohol, sodium, lipid, fruit and vegetable intakes, BMI, height, physical activity, smoking status, number of 24 h records, family history of cancer, educational level, prevalent type 2 diabetes, hypertension, major cardiovascular event and dyslipidaemia at baseline	*Exposure assessment critique*: A key strength was that it was a prospective study using repeated dietary records. Key limitations were that there was no specific estimate of aspartame exposure, use of ASBs as a proxy (includes 12 ASB items, all beverages containing non-nutritive sweeteners, such as diet soft drinks, sugar-free syrups, and diet milk-based beverages); and exposure data at baseline only. *Other strengths*: large population-based prospective study; comprehensive adjustment for confounders. *Other limitations*: only 166 cases of colorectal cancer; low level of consumption of ASB, with median of 6.9 mL/day; non-representative population, predominantly women, may limit result generalizability.
			2nd quartile (men, 2.7 to < 4.7 mL/day; or women, 4.6 to < 7.7 mL/day)	29	0.65 (0.37–1.14)		
			3rd quartile (men, 4.7 to < 7.9 mL/day; or women, 7.7 to < 11.6 mL/day)	4	0.84 (0.21–3.42)		
			4th quartile (men, ≥ 7.9 mL/ day; or women, ≥ 11.6 mL/day)	14	0.80 (0.44–1.46)		
			Continuous (per 10 mL/day)	166	1.02 (0.94–1.10)		
			Trend-test *P* value: 0.40				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Malik et al. (2019) USA Enrolment, 1976 (NHS), 1986 (HPFS)/ follow-up, 1980–2014 (NHS), 1986–2014 (HPFS) Cohort	37 716 men and 80 647 women; female registered nurses aged 30–55 yr in the NHS and male health professionals aged 40–75 yr in the HPFS, excluding those with history of diabetes, cardiovascular disease, or cancer at baseline, or with implausible dietary intake Exposure assessment method: prospective assessment of ASB consumption through repeated FFQs between 1980–1986 and 2010	Colon, mortality	ASB intake (HR): < 1 serving/mo 1–4 servings/mo 2–6 servings/wk 1 to < 2 servings/day ≥ 2 servings/day Continuous (per serving/ day) Trend-test P value: 0.69	NR NR NR NR NR NR	1 0.97 (0.81–1.16) 1.01 (0.86–1.18) 1.11 (0.89–1.37) 1.01 (0.77–1.31) 1.00 (0.91–1.07)	Age, race, smoking, alcohol intake, postmenopausal hormone use (NHS), physical activity, family history of diabetes; family history of myocardial infarction, family history of cancer, multivitamin use, aspirin use, baseline history of hypertension and hypercholesterolaemia, intake of whole grains, fruit, vegetables, or red and processed meat, total energy, BMI, SSB intake	*Exposure assessment critique*: A key strength was the prospective assessment of ASB consumption from repeated diet assessments (using validated instrument) every 4 yr, the majority at a very relevant period for aspartame exposure from ASBs (the USA between the 1980s and 2010) potentially capturing lifetime exposure to aspartame. Key limitations were that other sources of aspartame were not considered (although these were more limited); and uncertainty regarding aspartame content in ASBs after the mid-2000s. *Other strengths*: large cohort with long follow-up. *Other limitations*: likely bias from non-differential misclassifications of exposure to aspartame; stratified numbers of deaths not provided for specific cancer sites.

Table 2.1   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Mullee et al. (2019) Europe Enrolment, 1992–2000/ follow-up, through 2009–2013 (depending on study centre, mean 16.4 yr) Cohort	451 743; EPIC cohort study participants from 10 European countries (Denmark, France, Greece, Germany, Italy, Netherlands, Norway, Spain, Sweden, and the United Kingdom); men and women excluding those with prevalent cancer, heart disease, stroke, or diabetes, or with implausible dietary intake; for artificially sweetened soft drinks; participants from Italy, Spain, and Sweden were not included; 2095 colorectal cancer deaths	Colon and rectum, mortality	Glasses (250 mL) of artificially sweetened soft drink consumption (HR): < 1 glass/mo 1 to 4 glasses/mo > 1 to 6 glasses/ wk ≥ 1 glass/day Trend-test *P* value: 0.21	NR NR NR NR	1 1.08 (0.91–1.28) 1.02 (0.85–1.22) 1.22 (0.91–1.64)	Age, centre, sex, BMI, physical activity index, educational status, alcohol consumption, smoking status, smoking intensity, smoking duration, ever use of contraceptive pill, menopausal status, ever use of menopausal hormone therapy, intakes of total energy, red and processed meat, fruits and vegetables, coffee, fruit and vegetable juice, sugar-sweetened soft drinks	*Exposure assessment critique*: A key strength was the prospective assessment of ASB consumption (several types of beverage) in several western European countries at a period relevant for aspartame exposure (between 1991 and 2000). Key limitations were that no other sources of aspartame were considered; uncertainty regarding the aspartame content in ASBs in every country; and there was only one assessment at baseline. Other information: context of low ASB consumption in middle-aged adults. *Other strengths*: large population-based cohort spanning multiple countries with different behaviours; large number of cases; results adjusted for appropriate potential confounders, including BMI.

Table 2.1   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Mullee et al. (2019) (cont.)	Exposure assessment method: questionnaire; exposure to artificially sweetened soft drinks overall assessed once at baseline through country-specific validated tools (mainly FFQ) covering the usual diet over the past year; no specific assessment of aspartame or AS content of the artificially sweetened soft drinks						

Table 2.1   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level		Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Hur et al. (2021) USA Enrolment, 1989/follow-up, 1991 through June 2015 Cohort	95 464; NHS-II cohort; female registered nurses aged 25–42 yr at enrolment; excluded women with colorectal cancer or inflammatory bowel disease before baseline, or with implausible energy intake Exposure assessment method: semiquantitative FFQ every 4 yr since 1991 assessing the frequency of ASB consumption with standard serving size; cumulative average of intakes updated at every FFQ cycle	Colon and rectum, early onset (before age 50 yr), incidence	Low-calorie carbonated beverage intake (RR): < 1 serving/wk 1 serving/wk to < 1 serving/day 1 to < 2 servings/ day ≥ 2 servings/day Continuous (per serving/ day) Trend-test *P* value: 0.11	32 33 19 25 109		1 1.20 (0.73–1.98) 0.86 (0.48–1.54) 0.73 (0.42–1.27) 0.93 (0.83–1.04)	Age, total energy intake, race, height, BMI, menopausal status, menopausal hormone use, family history of colorectal cancer, pack-years of smoking, physical activity, regular aspirin use, regular non-steroidal anti-inflammatory use, current multivitamin use, intake of alcohol, intake of red and processed meat, dietary fibre, total folate, total calcium, Alternative Healthy Eating Index-2010 without alcohol or SSB, lower endoscopy for screening or other indications in past 10 yr	*Exposure assessment critique*: A key strength was the prospective assessment of ASB consumption as a cumulative average from repeated diet assessments every 4 yr (from 1991 to 2015) at a very relevant period for aspartame exposure from ASBs in the USA, potentially capturing lifetime exposure to aspartame. Key limitations were that other sources of aspartame including non-carbonated ASBs were not considered (although these were more limited); there was uncertainty regarding aspartame content in ASBs after the mid-2000s; and the repeated intake data could have been better used by analysing as time-dependent data rather than just averaging. *Other limitations*: relatively small numbers of cases, only 109 early-onset colon or rectal cancers, i.e. diagnosed before age 50 yr.

Table 2.1   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Jones et al. (2022) USA Enrolment, 1995–1996 (NIH-AARP), 1993–2001 (PLCO)/ follow-up, through 2011 (NIH-AARP), 1998–2011 (PLCO) Cohort	553 874; pooled analysis of two cohorts: (i) NIH-AARP Diet and Health Study – men and women aged 50–71 yr residing in six states and two metropolitan areas; (ii) PLCO – men and women aged 55–74 yr randomized to screening trial between 1993–2001 across 10 screening centres in the USA; all participants were without prior cancer diagnoses; proxy respondents were not included; 1060 incident liver cancers. Exposure assessment method: questionnaire; frequency of consumption of ASBs assessed in two cohorts at baseline in 1995–1996 and 1998, no indication of volume consumed	Liver (HCC), incidence	ASB consumption, non-diabetics, ≤ 12 yr follow-up (HR): Continuous (per time/day)	587	1.01 (0.92–1.11)	Age, sex, race/ ethnicity, BMI, smoking, alcohol use, study, total energy intake (kcal/day)	*Exposure assessment critique*: A key strength was the prospective assessment of ASB consumption (soda and fruit punches) at a very relevant period for aspartame exposure from ASBs (the USA in 1995–1996 and 1998). Similar exposure assessment in both cohorts. Key limitations were that no other sources were considered (although these were more limited); there was only one assessment at baseline (possible variations over up to 19 yr of follow-up); and uncertainty regarding aspartame content in ASBs after the mid-2000s. *Other strengths*: prospective, pooled data from two large studies resulted in large sample size, stratification by diabetes status.
		Liver (HCC), incidence	ASB consumption, non-diabetics, 12+ yr follow-up (HR): Continuous (per time/day)	252	0.99 (0.86–1.15)		
		Liver (HCC), incidence	Artificially sweetened soda consumption, non-diabetics, ≤ 12 yr follow-up (HR): Continuous (per time/day)	587	1.00 (0.90–1.11)		
		Liver (HCC), incidence	Artificially sweetened soda consumption, non-diabetics, 12+ yr follow-up (HR): Continuous (per time/day)	252	0.98 (0.83–1.15)		
		Liver (HCC), incidence	Artificially sweetened fruit punch consumption, non-diabetics, ≤ 12 yr follow-up (HR): Continuous (per time/day)	587	1.06 (0.80–1.41)		

Table 2.1   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Jones et al. (2022) (cont.)		Liver (HCC), incidence	Artificially sweetened fruit punch consumption, non-diabetics, 12+ yr follow-up (HR):			Age, sex, race/ ethnicity, BMI, smoking, alcohol use, study, total energy intake (kcal/day)	*Other limitations*: single assessment of covariates; stratification by diabetes status may be misclassified due to potential changes in status during follow-up; the study did not control for coffee consumption or HBV/HCV infection status.
			Continuous (per time/day)	252	1.11 (0.74–1.65)		
		Liver (HCC)	ASB consumption, diabetics, ≤ 12 yr follow-up (HR):				
			Continuous (per time/day)	158	1.13 (1.02–1.25)		
		Liver (HCC), incidence	ASB consumption, diabetics, 12+ yr follow-up (HR):				
			Continuous (per time/day)	63	0.82 (0.64–1.05)		
		Liver (HCC), incidence	Artificially sweetened soda consumption, diabetics, ≤ 12 yr follow-up (HR):				
			Continuous (per time/day)	158	1.13 (1.01–1.27)		
		Liver (HCC)	Artificially sweetened soda consumption, diabetics, 12+ yr follow-up (HR):				
			Continuous (per time/day)	63	0.78 (0.59–1.03)		
		Liver (HCC), incidence	Artificially sweetened fruit punch consumption, diabetics, ≤ 12 yr follow-up (HR):				
			Continuous (per time/day)	158	1.17 (0.92–1.48)		
		Liver (HCC), incidence	Artificially sweetened fruit punch consumption, diabetics, 12+ yr follow-up (HR):				
			Continuous (per time/day)	63	1.01 (0.61–1.69)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level		Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2022) USA Enrolment, 1982/follow-up, through 2016 (median, 27.7 yr) Cohort	934 777 (416 313 men, 518 464 women); CPS-II prospective cohort; adults aged ≥ 28 yr; excluded participants with personal history at baseline of diabetes or cancer other than nonmelanoma skin cancer, men aged > 90 yr or women aged > 95 yr at enrolment, and those reporting only prior but not current consumption of either SSBs or ASBs Exposure assessment method: questionnaire; exposure to ASBs assessed in 1982 through a question about number of drinks per day of diet soda or ice teas (one pooled item) and potential changes over the past 10 yr; no specific assessment of aspartame content in ASBs	Larynx[a], pharynx, and oral cavity combined, mortality	ASB consumption, BMI adjusted (HR):				Age, sex, race/ ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	*Exposure assessment critique*: A key strength was the prospective assessment of ASB consumption. Key limitations were that there was only one dietary assessment at baseline in 1982 that was before the use of aspartame in ASBs, hence the relevance to aspartame exposure depends on the stability of ASB consumption over up to 34 yr of follow-up, but such information was not directly available; no other sources were considered (although these were more limited); and uncertainty regarding aspartame content in ASBs after the mid-2000s. Other information: exclusion of participants who reported only prior but not current consumption of either SSBs or ASBs at baseline.
			Never	NR		1		
			< 1 drink/day	NR		0.95 (0.79–1.13)		
			1 drink/day	NR		1.17 (0.97–1.41)		
			≥ 2 drinks/day	NR		1.13 (0.94–1.37)		
			Continuous (per drink/day)	1852		1.04 (0.99–1.09)		
			Trend-test *P* value: 0.107					
		Larynx[a], pharynx, and oral cavity combined, mortality	ASB consumption, men, BMI adjusted (HR):				Age, race/ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR		1		
			< 1 drink/day	NR		1.23 (0.98–1.53)		
			1 drink/day	NR		1.40 (1.10–1.78)		
			≥ 2 drinks/day	NR		1.13 (0.87–1.48)		
			Continuous (per drink/day)	1215		1.07 (1.00–1.14)		
			Trend-test *P* value: 0.034					

Table 2.1   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2022) (cont.)		Larynx[a], pharynx, and oral cavity combined, mortality	ASB consumption, women, BMI adjusted (HR):			Age, race/ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	*Other strengths*: large, prospective cohort with long follow-up; ability to examine multiple cancer types, stratify by sex or BMI, and limit to never-smokers; comprehensive adjustment for confounders, including SSB consumption.
			Never	NR	1		
			< 1 drink/day	NR	0.68 (0.51–0.89)		
			1 drink/day	NR	0.89 (0.66–1.20)		
			≥ 2 drinks/day	NR	1.06 (0.80–1.40)		
			Continuous (per drink/day)	637	0.99 (0.91–1.07)		
			Trend-test *P* value: 0.845				
		Oesophagus, mortality	ASB consumption, BMI adjusted (HR):			Age, sex, race/ ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	*Other limitations*: potential for residual confounding because unable to distinguish gastric cardia and non-cardia cancers or oesophageal SCC and adenocarcinoma with different risk factors; unable to control liver cancer for HBV/HCV infection status.
			Never	NR	1		
			< 1 drink/day	NR	0.98 (0.85–1.11)		
			1 drink/day	NR	0.89 (0.75–1.05)		
			≥ 2 drinks/day	NR	0.92 (0.78–1.08)		
			Continuous (per drink/day)	2727	0.99 (0.94–1.03)		
			Trend-test *P* value: 0.155				
		Stomach, mortality	ASB consumption, BMI adjusted (HR):				
			Never	NR	1		
			< 1 drink/day	NR	0.92 (0.81–1.05)		
			1 drink/day	NR	0.92 (0.79–1.09)		
			≥ 2 drinks/day	NR	1.06 (0.91–1.24)		
			Continuous (per drink/day)	2798	1.00 (0.96–1.05)		
			Trend-test *P* value: 0.892				

Table 2.1   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2022) (cont.)		Liver (HCC), mortality	ASB consumption, BMI adjusted (HR):			Age, sex, race/ ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR	1		
			< 1 drink/day	NR	1.01 (0.90–1.15)		
			1 drink/day	NR	1.01 (0.87–1.17)		
			≥ 2 drinks/day	NR	0.95 (0.81–1.10)		
			Continuous (per drink/day)	2722	0.98 (0.94–1.02)		
			Trend-test *P* value: 0.578				
		Liver (HCC), mortality	ASB consumption, men, BMI adjusted (HR):			Age, race/ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR	1		
			< 1 drink/day	NR	1.10 (0.92–1.32)		
			1 drink/day	NR	1.07 (0.87–1.32)		
			≥ 2 drinks/day	NR	1.00 (0.80–1.24)		
			Continuous (per drink/day)	1576	0.99 (0.93–1.05)		
			Trend-test *P* value: 0.701				
		Liver (HCC), mortality	ASB consumption, women, BMI adjusted (HR):				
			Never	NR	1		
			< 1 drink/day	NR	0.93 (0.79–1.11)		
			1 drink/day	NR	0.94 (0.76–1.16)		
			≥ 2 drinks/day	NR	0.90 (0.72–1.12)		
			Continuous (per drink/day)	1146	0.97 (0.92–1.04)		
			Trend-test *P* value: 0.270				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2022) (cont.)		Liver (HCC), mortality	ASB consumption, never-smokers, BMI adjusted (HR):			Age, sex, race/ ethnicity, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR	1		
			< 1 drink/day	NR	0.98 (0.81–1.20)		
			1 drink/day	NR	1.01 (0.80–1.28)		
			≥ 2 drinks/day	NR	0.93 (0.72–1.20)		
			Continuous (per drink/day)	996	0.96 (0.89–1.04)		
			Trend-test *P* value: 0.637				
		Liver (HCC), mortality	ASB consumption, men, never-smokers (HR):			Age, race/ethnicity, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption	
			Never	NR	1		
			< 1 drink/day	NR	1.13 (0.80–1.60)		
			1 drink/day	NR	1.17 (0.80–1.73)		
			≥ 2 drinks/day	NR	1.44 (0.99–2.08)		
			Continuous (per drink/day)	413	1.08 (0.98–1.19)		
			Trend-test *P* value: 0.040				
		Liver (HCC), mortality	ASB consumption, men, never-smokers, BMI adjusted (HR):				
			Never	NR	1		
			< 1 drink/day	NR	1.03 (0.73–1.47)		
			1 drink/day	NR	1.04 (0.70–1.53)		
			≥ 2 drinks/day	NR	1.21 (0.83–1.77)		
			Continuous (per drink/day)	413	1.03 (0.93–1.15)		
			Trend-test *P* value: 0.335				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2022) (cont.)		Liver (HCC), mortality	ASB consumption, women, never-smokers, BMI adjusted (HR):			Age, race/ethnicity, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR	1		
			< 1 drink/day	NR	0.96 (0.75–1.21)		
			1 drink/day	NR	0.99 (0.74–1.32)		
			≥ 2 drinks/day	NR	0.76 (0.54–1.08)		
			Continuous (per drink/day)	583	0.91 (0.81–1.01)		
			Trend-test *P* value: 0.169				
		Pancreas, mortality	ASB consumption (HR):			Age, sex, race/ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption	
			Never	NR	1		
			< 1 drink/day	NR	1.03 (0.97–1.11)		
			1 drink/day	NR	1.09 (1.01–1.18)		
			≥ 2 drinks/day	NR	1.16 (1.07–1.26)		
			Continuous (per drink/day)	9196	1.04 (1.02–1.06)		
			Trend-test *P* value: < 0.0001				
		Pancreas, mortality	ASB consumption, BMI adjusted (HR):			Age, sex, race/ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR	1		
			< 1 drink/day	NR	1.00 (0.94–1.07)		
			1 drink/day	NR	1.06 (0.98–1.14)		
			≥ 2 drinks/day	NR	1.11 (1.02–1.20)		
			Continuous (per drink/day)	9196	1.03 (1.01–1.05)		
			Trend-test *P* value: 0.008				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2022) (cont.)		Pancreas, mortality	ASB consumption, men, BMI adjusted (HR):			Age, race/ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR	1		
			< 1 drink/day	NR	0.96 (0.86–1.08)		
			1 drink/day	NR	1.10 (0.97–1.25)		
			≥ 2 drinks/day	NR	1.15 (1.01–1.30)		
			Continuous (per drink/day)	4339	1.04 (1.00–1.08)		
			Trend-test *P* value: 0.023				
		Pancreas, mortality	ASB consumption, women, BMI adjusted (HR):				
			Never	NR	1		
			< 1 drink/day	NR	1.01 (0.93–1.10)		
			1 drink/day	NR	1.02 (0.92–1.13)		
			≥ 2 drinks/day	NR	1.08 (0.97–1.19)		
			Continuous (per drink/day)	4857	1.02 (0.99–1.05)		
			Trend-test *P* value: 0.167				
		Pancreas, mortality	ASB consumption, never-smokers, BMI adjusted (HR):			Age, sex, race/ ethnicity, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR	1		
			< 1 drink/day	NR	0.99 (0.90–1.10)		
			1 drink/day	NR	1.07 (0.95–1.20)		
			≥ 2 drinks/day	NR	1.19 (1.05–1.34)		
			Continuous (per drink/day)	3955	1.05 (1.02–1.09)		
			Trend-test *P* value: 0.005				

Table 2.1   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2022) (cont.)		Pancreas, mortality	ASB consumption, men, never-smokers, BMI adjusted (HR):			Age, race/ethnicity, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR	1		
			< 1 drink/day	NR	0.93 (0.77–1.14)		
			1 drink/day	NR	1.00 (0.81–1.25)		
			≥ 2 drinks/day	NR	1.34 (1.10–1.65)		
			Continuous (per drink/day)	1456	1.08 (1.02–1.14)		
			Trend-test *P* value: 0.018				
		Pancreas, mortality	ASB consumption, women, never-smokers, BMI adjusted (HR):				
			Never	NR	1		
			< 1 drink/day	NR	1.01 (0.90–1.13)		
			1 drink/day	NR	1.09 (0.95–1.25)		
			≥ 2 drinks/day	NR	1.12 (0.96–1.30)		
			Continuous (per drink/day)	2499	1.03 (0.99–1.08)		
			Trend-test *P* value: 0.089				
		Pancreas, mortality	ASB consumption, normal weight (BMI, 18.5 to < 25 kg/m^2) (HR):			Age, sex, race/ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR	1		
			< 1 drink/day	NR	0.96 (0.87–1.06)		
			1 drink/day	NR	1.01 (0.89–1.14)		
			≥ 2 drinks/day	NR	1.07 (0.94–1.22)		
			Continuous (per drink/day)	4234	1.00 (0.97–1.04)		
			Trend-test *P* value: 0.432				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2022) (cont.)		Pancreas, mortality	ASB consumption, overweight (BMI, 25 to < 30 kg/m²) (HR):			Age, sex, race/ ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR	1		
			< 1 drink/day	NR	1.03 (0.93–1.14)		
			1 drink/day	NR	1.08 (0.96–1.22)		
			≥ 2 drinks/day	NR	1.18 (1.04–1.33)		
			Continuous (per drink/day)	3625	1.05 (1.01–1.08)		
			Trend-test P value: 0.006				
		Pancreas, mortality	ASB consumption, obese (BMI, ≥ 30 kg/m²) (HR):				
			Never	NR	1		
			< 1 drink/day	NR	1.10 (0.92–1.31)		
			1 drink/day	NR	1.02 (0.83–1.26)		
			≥ 2 drinks/day	NR	0.99 (0.80–1.21)		
			Continuous (per drink/day)	1022	1.01 (0.95–1.07)		
			Trend-test P value: 0.992				
		Bile duct or gallbladder, mortality	ASB consumption (HR):			Age, sex, race/ ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption	
			Continuous (per drink/day)	1024	1.07 (1.01–1.14)		
			Trend-test P value: 0.011				

Table 2.1   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2022) (cont.)		Bile duct or gallbladder, mortality	ASB consumption, BMI adjusted (HR):			Age, sex, race/ ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR	1		
			< 1 drink/day	NR	1.12 (0.93–1.36)		
			1 drink/day	NR	1.18 (0.94–1.48)		
			≥ 2 drinks/day	NR	1.15 (0.90–1.46)		
			Continuous (per drink/day)	1024	1.05 (0.98–1.11)		
			Trend-test *P* value: 0.105				
		Bile duct or gallbladder, mortality	ASB consumption, never-smokers, BMI adjusted (HR):			Age, sex, race/ ethnicity, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR	1		
			< 1 drink/day	NR	1.05 (0.80–1.39)		
			1 drink/day	NR	1.14 (0.81–1.60)		
			≥ 2 drinks/day	NR	1.25 (0.88–1.77)		
			Continuous (per drink/day)	476	1.05 (0.96–1.16)		
			Trend-test *P* value: 0.170				
		Small intestine, mortality	ASB consumption (HR):			Age, sex, race/ ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption	
			Continuous (per drink/day)	324	1.12 (1.01–1.23)		
			Trend-test *P* value: 0.162				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2022) (cont.)		Small intestine, mortality	ASB consumption, BMI adjusted (HR):			Age, sex, race/ ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR	1		
			< 1 drink/day	NR	0.98 (0.68–1.42)		
			1 drink/day	NR	1.09 (0.72–1.66)		
			≥ 2 drinks/day	NR	1.28 (0.85–1.93)		
			Continuous (per drink/day)	324	1.11 (1.00–1.22)		
			Trend-test *P* value: 0.244				
		Small intestine, mortality	ASB consumption, never-smokers, BMI adjusted (HR):			Age, sex, race/ ethnicity, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR	1		
			< 1 drink/day	NR	1.25 (0.75–2.07)		
			1 drink/day	NR	1.13 (0.60–2.13)		
			≥ 2 drinks/day	NR	1.20 (0.62–2.32)		
			Continuous (per drink/day)	138	1.10 (0.94–1.30)		
			Trend-test *P* value: 0.479				
		Colon and rectum, mortality	ASB consumption (HR):			Age, sex, race/ ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption	
			Never	NR	1		
			< 1 drink/day	NR	0.99 (0.94–1.05)		
			1 drink/day	NR	1.03 (0.97–1.11)		
			≥ 2 drinks/day	NR	0.97 (0.90–1.04)		
			Continuous (per drink/day)	13 752	1.00 (0.98–1.02)		
			Trend-test *P* value: 0.704				

Table 2.1   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2022) (cont.)		Colon and rectum, mortality	ASB consumption, BMI adjusted (HR):			Age, sex, race/ ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR	1		
			< 1 drink/day	NR	0.96 (0.91–1.02)		
			1 drink/day	NR	1.00 (0.93–1.07)		
			≥ 2 drinks/day	NR	0.92 (0.86–0.99)		
			Continuous (per drink/day)	13 752	0.99 (0.97–1.01)		
			Trend-test *P* value: 0.034				
		Colon and rectum, mortality	ASB consumption, men, BMI adjusted (HR):			Age, race/ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR	1		
			< 1 drink/day	NR	1.01 (0.92–1.11)		
			1 drink/day	NR	0.96 (0.86–1.07)		
			≥ 2 drinks/day	NR	0.95 (0.84–1.06)		
			Continuous (per drink/day)	6660	0.99 (0.96–1.02)		
			Trend-test *P* value: 0.276				
		Colon and rectum, mortality	ASB consumption, women, BMI adjusted (HR):				
			Never	NR	1		
			< 1 drink/day	NR	0.94 (0.87–1.01)		
			1 drink/day	NR	1.02 (0.93–1.11)		
			≥ 2 drinks/day	NR	0.90 (0.82–0.99)		
			Continuous (per drink/day)	7092	0.98 (0.96–1.01)		
			Trend-test *P* value: 0.052				

Table 2.1   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2022) (cont.)		Colon and rectum, mortality	ASB consumption, never-smokers, BMI adjusted (HR):			Age, sex, race/ ethnicity, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR	1		
			< 1 drink/day	NR	0.97 (0.89–1.05)		
			1 drink/day	NR	1.04 (0.94–1.15)		
			≥ 2 drinks/day	NR	0.92 (0.82–1.03)		
			Continuous (per drink/day)	5799	0.98 (0.95–1.01)		
			Trend-test P value: 0.294				
		Colon and rectum, mortality	ASB consumption, men, never-smokers, BMI adjusted (HR):			Age, race/ethnicity, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR	1		
			< 1 drink/day	NR	1.03 (0.88–1.20)		
			1 drink/day	NR	0.95 (0.79–1.16)		
			≥ 2 drinks/day	NR	0.90 (0.74–1.11)		
			Continuous (per drink/day)	2107	0.96 (0.90–1.02)		
			Trend-test P value: 0.335				
		Colon and rectum, mortality	ASB consumption, women, never-smokers, BMI adjusted (HR):				
			Never	NR	1		
			< 1 drink/day	NR	0.95 (0.86–1.04)		
			1 drink/day	NR	1.07 (0.95–1.20)		
			≥ 2 drinks/day	NR	0.92 (0.80–1.06)		
			Continuous (per drink/day)	3692	0.99 (0.95–1.02)		
			Trend-test P value: 0.469				

Table 2.1   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Norell et al. (1986) Sweden 1982–1984 Case–control	Cases, 99; aged 40–79 yr diagnosed with cancer of the exocrine pancreas at three surgical departments in Stockholm and Uppsala; diagnosis based on resection or autopsy; of 120 confirmed cases, 21 were lost to the study, leaving 99 (55 men, 44 women) Controls: 301 (163 hospital-based, 138 population-based); population controls selected from parish registries, which list all inhabitants by date of birth; matched on sex, closest date of birth in the same parish; hospital controls were selected as a stratified (age, sex) random sample of patients aged 40–79 yr who had a diagnosis of inguinal hernia during the study period from the same three surgical departments; survey completed by proxy for 16 cases,	Pancreas (exocrine), incidence Pancreas (exocrine), incidence	Use of AS, population controls (RR): No 78 1 Yes 18 1.2 (90% CI, 0.7–2.0) Use of AS, hospital controls (RR): No 78 1 Yes 18 1.1 (90% CI, 0.6–2.0)			Age, sex, parish Age, sex, hospital	*Exposure assessment critique*: Key limitations were the retrospective recall with a single item on use of AS and not specifically aspartame; study participants could not consume aspartame before it was approved in the early 1980s, therefore, exposure of participants to AS does not reflect aspartame. *Other strengths*: population-based controls should reflect exposure experience of the study population (minimize selection bias). *Other limitations*: potential for bias from exposure misclassification due to long induction periods and variable exposure by time; potential for recall bias; limited number of exposed cases; reported results for ASs did not control for confounders other than sex, age, and hospital/parish.

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Norell et al. (1986) (cont.)	2 hospital controls, and 1 population control Exposure assessment method: self-administered questionnaire including use of AS; exposure categorized as consumers vs non-consumers only						
Mayne et al. (2006) USA 1993–1995 Case–control	Cases: 255 gastric cardia adenocarcinoma; 352 noncardia gastric adenocarcinoma; 206 oesophageal SCC; 282 oesophageal adenocarcinoma; aged 30–79 yr with incident cancer in rapid reporting systems in three areas (Connecticut, New Jersey, and western Washington State); medical records and slides reviewed by study pathologists to confirm diagnoses	Stomach (gastric cardia, adenocarcinoma), incidence	Diet carbonated soft drink consumption (OR): No intake NR 1 Top 20% of intake NR 0.50 (0.31–0.81)			Age, sex, study centre, race, proxy interview status, BMI, mean caloric intake, consumption (each) of beer/wine/liquor, consumption of meat, cigarettes per day, education, income, frequency of reflux symptoms	*Exposure assessment critique*: Key limitations were the retrospective recall; the lack of a specific estimate of aspartame exposure with ASBs as a proxy (diet soft drinks or soda), but timing of exposure was consistent for aspartame being used as the major AS in beverages; lack of data on duration of exposure; the authors repeated analyses limiting to those without reflux symptoms.

Table 2.1   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Mayne et al. (2006) (cont.)	Controls: 687; population-based controls were frequency-matched on the expected distribution of target cases by 5-year age group, sex, and geographical area (study site); controls aged 30–64 yr were identified by Waksberg's random-digit dialling method; controls 65–79 yr were identified by Health Care Financing Administration rosters Exposure assessment method: in-person, structured questionnaire of usual frequency of consumption of "diet soft drinks or soda" covering the period 3–5 yr before diagnosis	Stomach (gastric noncardia, adenocarcinoma), incidence	Diet carbonated soft drink consumption (OR):			Age, sex, study centre, race, proxy interview status, BMI, mean caloric intake, consumption (each) of beer/wine/liquor, consumption of meat, cigarettes per day, education, income, frequency of reflux symptoms	*Other strengths*: large sample size; separate cancer histologies and sites; population-based; control for key confounders, including BMI. *Other limitations*: 30% proxy interviews; potential for recall bias; reverse causation (potential to change behaviours based on reflux), and unmeasured confounders; selection bias due to low participation rate of controls (70.2%); use of random-digit dialling to identify controls may result in non-representative sample.
			No intake	NR	1		
			Top 20% of intake	NR	0.58 (0.38–0.90)		
		Oesophagus (SCC), incidence	Diet carbonated soft drink consumption (OR):				
			No intake	NR	1		
			Top 20% of intake	NR	0.43 (0.23–0.82)		
		Oesophagus (adenocarcinoma), incidence	Diet carbonated soft drink consumption (OR):				
			No intake	NR	1		
			Top 20% of intake	NR	0.52 (0.32–0.83)		

Table 2.1   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Gallus et al. (2007) Italy 1991–2004 Case–control	Cases: 598 (oral or pharyngeal), 304 (oesophagus), 1225 (colon), 728 (rectum); histologically confirmed cancers of oral cavity and pharynx (512 men, 86 women; median age, 58 yr), oesophagus (275 men, 29 women; median age, 60 yr), colorectum (1225 colon, 728 rectum; 1125 men, 828 women; median age, 62 yr); > 95% participation Controls: 1491 (oral/pharyngeal), 743 (oesophagus), 4154 (colorectum); controls admitted to same network of general and teaching hospitals as cases for acute, non-neoplastic disorders; total of 7028 patients (3301 men and 3727 women; 4838 included in more than one study); 24% trauma, 31% other nontraumatic orthopaedic	Oral or pharyngeal combined, incidence	Consumption of ASs other than saccharin (OR):			Age, sex, study centre, education, tobacco smoking, alcohol drinking, BMI, total energy intake, consumption of hot beverages	*Exposure assessment critique*: Key limitations were that there was no specific assessment of aspartame, aspartame was considered as "other sweeteners" but with unclear actual contribution; only one source was considered (tabletop sweeteners); and the retrospective assessment in a case–control study (potential for differential misclassification). Other information: according to the authors, limited consumption of sources of sweeteners (including ASBs) in the study population of middle-aged adults in Italy between 1991 and 2004. *Other strengths*: high response rates (< 5% refusals) for cases and controls reduced the potential for selection bias; large sample size with large case numbers for rarer cancers; control for key potential con-founders, including BMI.
			Non-consumers	586	1		
			> 0 sachets or tablets/day	12	0.77 (0.39–1.53)		
		Oesophagus, incidence	Consumption of ASs other than saccharin (OR):				
			Non-consumers	294	1		
			> 0 sachets or tablets/day	10	0.77 (0.34–1.75)		
		Colon, incidence	Consumption of ASs other than saccharin (OR):			Age, sex, study centre, education, tobacco smoking, alcohol drinking, BMI, total energy intake, consumption of hot beverages	
			Non-consumers	1137	1		
			> 0 sachets or tablets/day	88	0.90 (0.70–1.16)		

Table 2.1   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Gallus et al. (2007) (cont.)	conditions, 17% acute surgical disorders, 28% miscellaneous other diseases; > 95% participation Exposure assessment method: questionnaire; assessment of use of tabletop sweeteners containing either saccharin or other sweeteners as sachets or tablets per week in the 2 yr before cancer diagnosis	Rectum, incidence	Consumption of ASs other than saccharin (OR):			Age, sex, study centre, education, tobacco smoking, alcohol drinking, BMI, total energy intake, consumption of hot beverages	*Other limitations*: potential for recall bias and reverse causation; exposure may have been related to disease conditions of controls.
			Non-consumers	689	1		
			> 0 sachets or tablets/day	39	0.71 (0.50–1.02)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Bosetti et al. (2009) Italy 1997–2007 Case–control	Cases: 230 (stomach), 326 (pancreas); hospital-based, greater Milan area; cases had histologically confirmed cancer of the stomach (143 men, 87 women; median age, 63 yr) or pancreas (174 men, 152 women; median age, 63 yr) Controls: 547 (stomach), 652 (pancreas); controls selected from same network of general and teaching hospitals as cases for acute, non-neoplastic disorders, frequency-matched on age, sex, and study centre; 25% traumas, 32% nontraumatic orthopaedic conditions, 15% acute surgical disorders, 27% miscellaneous other diseases; > 95% control participation	Stomach, incidence Pancreas, incidence	Consumption of low-calorie sweeteners other than saccharin (OR): Non-users Users Consumption of low-calorie sweeteners other than saccharin (OR): Non-users Users	213 17 291 35	1 0.86 (0.45–1.67) 1 1.16 (0.66–2.04)	Age, sex, study centre, year of interview, education, BMI, tobacco smoking, history of diabetes, consumption of hot beverages, total energy intake	*Exposure assessment critique*: Key limitations were that there was no specific assessment of aspartame, aspartame was included in "other sweeteners" but with unclear actual contribution; only one source was considered (tabletop sweeteners); exposure assessment limited to users vs non-users, which increases the potential for exposure misclassification; and the retrospective assessment in a case–control study (potential for differential misclassification). Other information: according to the authors, low consumption of sources of sweeteners (including ASBs) in the study population of middle-aged adults in Italy between 1991 and 2007). *Other strengths*: > 95% participation rate among controls; adjustment for several key confounders, including BMI and diabetes status.

Table 2.1   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Bosetti et al. (2009) (cont.)	Exposure assessment method: questionnaire; assessment of use of tabletop sweeteners containing either saccharin or other sweeteners as several sachets or tablets per week in the 2 yr before cancer diagnosis; exposure to "other sweeteners" considered as ever users vs non-users only						*Other limitations*: potential for reverse causation, recall bias; exposure may have been related to disease conditions of controls; no distinction by stomach cancer subtype.
Chan et al. (2009) San Francisco Bay area, California, USA 1995–1999 Case–control	Cases: 532; men and women with incident adenocarcinoma of the exocrine pancreas were identified in 6 counties of the San Francisco Bay Area using rapid case ascertainment by the Northern California Cancer Center (in-area); cases verified by contacting participants' physicians and through SEER abstracts; eligible cases were aged 21–85 yr, residents of one of six counties,	Pancreas (exocrine, adenocarcinoma), incidence	Daily servings of sugar-free carbonated beverages (OR): 0 — 310 — 1 < 1/day — 124 — 0.8 (0.7–1.1) ≥ 1/day — 92 — 1.5 (1.1–2.1) Trend-test *P* value: 0.2			Age, sex, energy intake, BMI, race, education, smoking, history of diabetes, physical activity, other food groups (red meat, white meat, vegetable and fruit, eggs, fish, dairy, whole grain, refined grain, and sweets), total sweetened beverages, sugar-type carbonated beverages	*Exposure assessment critique*: A key strength was the timing of exposure assessment that was consistent for aspartame as the major AS in beverages. Key limitations were the retrospective recall using an FFQ; and that there was no specific estimate of aspartame exposure, ASBs used as a proxy (low-calorie colas, low-calorie caffeine-free colas, and other low-calorie carbonated beverages).

Table 2.1   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Chan et al. (2009) (cont.)	alive upon first contact, and could complete a first interview in English; 65 eligible out of area cases met the same criteria but were from counties adjacent to the six Bay Area counties Controls: 1701; control participants were frequency-matched to cases by sex and age within 5-year categories and were selected from the target population using random-digit dialling; controls aged > 65 yr were supplemented by random selection from Health Care Finance Administration lists (now the Centers for Medicare and Medicaid Services); 67% participation rate; no proxy interviews	Pancreas (exocrine, adenocarcinoma), incidence	Daily servings of sugar-free carbonated beverages, men (OR):			Age, energy intake, BMI, race, education, smoking, history of diabetes, physical activity, other food groups (red meat, white meat, vegetable and fruit, eggs, fish, dairy, whole grain, refined grain, and sweets), total sweetened beverages, sugar-type carbonated beverages	*Other strengths*: population-based, large number of cases; detailed in-person interviews/no proxy interviews; models adjusted for BMI and history of diabetes. *Other limitations*: potential for recall bias, possible selection bias of controls (67% response rate), survivor bias; use of random-digit dialling to identify controls may result in non-representative sample.
			0	NR	1		
			< 1/day	NR	1.2 (0.8–1.7)		
			≥ 1/day	NR	1.8 (1.1–2.8)		
			Trend-test *P* value: 0.4				
		Pancreas (exocrine, adenocarcinoma), incidence	Daily servings of sugar-free carbonated beverages, women (OR):				
			0	NR	1		
			< 1/day	NR	0.6 (0.4–1.0)		
			≥ 1/day	NR	1.4 (0.9–2.3)		
			Trend-test *P* value: 0.3				
		Pancreas (exocrine, adenocarcinoma), incidence	Total servings of low-calorie cola (OR):			Age, sex, energy intake, BMI, race, education, smoking, history of diabetes, physical activity, other food groups (red meat, white meat, vegetable and fruit, eggs, fish, dairy, whole grain, refined grain, and sweets), total sweetened beverages, sugar-type carbonated beverages	
			< 1/mo	381	1		
			1–3/mo	34	1.0 (0.7–1.5)		
			1–6/wk	54	0.9 (0.7–1.3)		
			≥ 1/day	57	1.7 (1.2–2.4)		
			Trend-test *P* value: 0.06				

Table 2.1   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Chan et al. (2009) (cont.)	Exposure assessment method: 131-item food questionnaire via in-person interviews, previous 12 mo to assess total sugar-free carbonated beverages/day (low-calorie colas, low-calorie caffeine-free colas, and other low-calorie carbonated beverages)	Pancreas (exocrine, adenocarcinoma), incidence	Total servings of low-calorie cola, men (OR):			Age, energy intake, BMI, race, education, smoking, history of diabetes, physical activity, other food groups (red meat, white meat, vegetable and fruit, eggs, fish, dairy, whole grain, refined grain, and sweets), total sweetened beverages, sugar-type carbonated beverages	
			< 1/mo	NR	1		
			1–3/mo	NR	1.0 (0.6–1.8)		
			1–6/wk	NR	1.1 (0.7–1.7)		
			≥ 1/day	NR	1.8 (1.1–2.9)		
			Trend-test *P* value: 0.06				
		Pancreas (exocrine, adenocarcinoma), incidence	Total servings of low-calorie cola, women (OR):				
			< 1/mo	NR	1		
			1–3/mo	NR	1.0 (0.5–1.9)		
			1–6/wk	NR	0.8 (0.4–1.4)		
			≥ 1/day	NR	1.6 (0.9–2.8)		
			Trend-test *P* value: 0.4				
		Pancreas (exocrine, adenocarcinoma), incidence	Total servings of low-calorie caffeine-free cola (OR):			Age, sex, energy intake, BMI, race, education, smoking, history of diabetes, physical activity, other food groups (red meat, white meat, vegetable and fruit, eggs, fish, dairy, whole grain, refined grain, and sweets), total sweetened beverages, sugar-type carbonated beverages	
			< 1/mo	437	1		
			1–3/mo	24	1.0 (0.6–1.6)		
			1–6/wk	43	0.8 (0.5–1.1)		
			≥ 1/day	22	1.1 (0.7–2.0)		
			Trend-test *P* value: 0.6				

Table 2.1   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
[Chan et al. (2009)](#) (cont.)		Pancreas (exocrine, adenocarcinoma), incidence	Total servings of low-calorie caffeine-free cola, men (OR):			Age, energy intake, BMI, race, education, smoking, history of diabetes, physical activity, other food groups (red meat, white meat, vegetable and fruit, eggs, fish, dairy, whole grain, refined grain, and sweets), total sweetened beverages, sugar-type carbonated beverages	
			< 1/mo	NR	1		
			1–3/mo	NR	1.1 (0.5–2.2)		
			1–6/wk	NR	0.9 (0.5–1.4)		
			≥ 1/day	NR	1.1 (0.5–2.5)		
			Trend-test P value: 0.9				
		Pancreas (exocrine, adenocarcinoma), incidence	Total servings of low-calorie caffeine-free cola, women (OR):				
			< 1/mo	NR	1		
			1–3/mo	NR	0.9 (0.5–1.8)		
			1–6/wk	NR	0.7 (0.4–1.3)		
			≥ 1/day	NR	1.3 (0.6–2.7)		
			Trend-test P value: 0.7				
		Pancreas (exocrine, adenocarcinoma), incidence	Total servings of other low-calorie carbonated beverages (OR):			Age, sex, energy intake, BMI, race, education, smoking, history of diabetes, physical activity, other food groups (red meat, white meat, vegetable and fruit, eggs, fish, dairy, whole grain, refined grain, and sweets), total sweetened beverages, sugar-type carbonated beverages	
			< 1/mo	418	1		
			1–3/mo	40	1.0 (0.7–1.5)		
			1–6/wk	47	0.9 (0.6–1.2)		
			≥ 1/day	21	1.4 (0.8–2.5)		
			Trend-test P value: 0.97				

Table 2.1   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Chan et al. (2009) (cont.)		Pancreas (exocrine, adenocarcinoma), incidence	Total servings of other low-calorie carbonated beverages, men (OR):			Age, energy intake, BMI, race, education, smoking, history of diabetes, physical activity, other food groups (red meat, white meat, vegetable and fruit, eggs, fish, dairy, whole grain, refined grain, and sweets), total sweetened beverages, sugar-type carbonated beverages	
			< 1/mo	NR	1		
			1–3/mo	NR	1.5 (0.9–2.5)		
			1–6/wk	NR	1.0 (0.6–1.6)		
			≥ 1/day	NR	1.8 (0.8–3.8)		
			Trend-test *P* value: 0.2				
		Pancreas (exocrine, adenocarcinoma), incidence	Total servings of other low-calorie carbonated beverages, women (OR):				
			< 1/mo	NR	1		
			1–3/mo	NR	0.7 (0.4–1.3)		
			1–6/wk	NR	0.6 (0.4–1.2)		
			≥ 1/day	NR	1.2 (0.4–3.2)		
			Trend-test *P* value: 0.2				
		Pancreas (exocrine, adenocarcinoma), incidence	Daily servings of sugar-free carbonated beverages, obese (BMI, ≥ 30 kg/m^2) (OR):			Age, sex, energy intake, BMI, race, education, smoking, history of diabetes, physical activity, other food groups (red meat, white meat, vegetable and fruit, eggs, fish, dairy, whole grain, refined grain, and sweets), total sweetened beverages, sugar-type carbonated beverages	
			None	NR	1		
			≥ 1/day	NR	2.6 (0.9–7.7)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level		Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Chan et al. (2009) (cont.)		Pancreas (exocrine, adenocarcinoma), incidence	Daily servings of sugar-free carbonated beverages, overweight (BMI, 25.0–29.9 kg/m^2) (OR):				Age, sex, energy intake, BMI, race, education, smoking, history of diabetes, physical activity, other food groups (red meat, white meat, vegetable and fruit, eggs, fish, dairy, whole grain, refined grain, and sweets), total sweetened beverages, sugar-type carbonated beverages	
			None		NR	1		
			≥ 1/day		NR	1.5 (0.9–2.6)		
		Pancreas (exocrine, adenocarcinoma), incidence	Daily servings of sugar-free carbonated beverages, normal weight (BMI, < 25.0 kg/m^2) (OR):					
			None		NR	1		
			≥ 1/day		NR	1.3 (0.8–2.1)		
		Pancreas (exocrine, adenocarcinoma), incidence	Daily servings of sugar-free carbonated beverages, non-diabetics (OR):					
			None		NR	1		
			≥ 1/day		NR	1.6 (1.1–2.3)		
		Pancreas (exocrine, adenocarcinoma), incidence	Daily servings of sugar-free carbonated beverages, diabetics (OR):				Age, sex, energy intake, BMI, race, education, smoking, physical activity, other food groups (red meat, white meat, vegetable and fruit, eggs, fish, dairy, whole grain, refined grain, and sweets), total sweetened beverages, sugar-type carbonated beverages	
			None		NR	1		
			≥ 1/day		NR	0.9 (0.3–2.6)		

Table 2.1   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Davis et al. (2023) Buffalo, NY, USA 1982–1998 Case–control	Cases: 213; patients with pancreatic cancer who participated in PEDS; predominantly White (97%) and aged 30–89 yr at diagnosis Controls: 852; patients evaluated for pancreatic cancer with non-cancer diagnoses; frequency-matched to cases by sex, 5-year categories of age, and 5-year categories of year the survey was completed Exposure assessment method: questionnaire; exposure to ASBs assessed retrospectively (few years before diagnosis) between 1982 and 1998; frequencies of consumption of diet cola as ASB; no specific assessment of aspartame content in ASB	Pancreas, incidence	Diet cola consumption (OR):			Age, sex, smoking status, BMI categories, total vegetable servings/week, processed meat servings/week, family history of pancreatic cancer, regular cola consumption, regular non-cola soft drinks	*Exposure assessment critique:* Key strengths were that exposure data were collected during the admission process, presumably before diagnosis was known. Key limitations were the retrospective assessment of ASB consumption in a case–control study; the period for diet assessment (before cancer diagnosis in 1982–1998) included early years when no aspartame was found in ASBs (partly irrelevant data for aspartame exposure) and later years when ASBs almost exclusively contained aspartame (very relevant data for aspartame exposure); ascertained diet cola only (not other ASBs); no other sources were considered (although these were more limited).
			Never	144	1		
			Occasional (< 1/day)	36	0.96 (0.62–1.49)		
			Habitual (≥ 1/day)	33	0.92 (0.59–1.43)		
		Pancreas, incidence	Diet cola consumption, never-smokers (OR):			Age, sex, BMI categories, total vegetable servings/ week, processed meat servings/week, family history of pancreatic cancer, regular cola consumption, regular non-cola soft drinks	
			Never	48	1		
			Occasional (< 1/day)	15	1.31 (0.65–2.67)		
			Habitual (≥ 1/day)	14	1.23 (0.60–2.52)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Davis et al. (2023) (cont.)		Pancreas, incidence	Diet cola consumption, former smokers (OR):			Age, sex, BMI categories, total vegetable servings/ week, processed meat servings/week, family history of pancreatic cancer, regular cola consumption, regular non-cola soft drinks	Other comments: in addition to the hospital-based case–control study, the authors also conducted a retrospective cohort mortality study among the 213 patients with pancreatic cancer (203 deaths); for this, they used clinical follow-up data including vital status and date of last contact, obtained from the cancer registry at Roswell Park (last two models).
			Never	72	1		
			Occasional (< 1/day)	16	0.69 (0.37–1.30)		
			Habitual (≥ 1/day)	11	0.54 (0.27–1.10)		
		Pancreas, incidence	Diet cola consumption, current smokers (OR):				
			Never	24	1		
			Occasional (< 1/day)	5	2.71 (0.71–10.35)		
			Habitual (≥ 1/day)	8	3.34 (1.12–9.98)		
		Pancreas, incidence	Diet cola consumption, normal weight (BMI, 18.5–24.9 kg/m^2) (OR):			Age, sex, total vegetable servings/ week, processed meat servings/week, family history of pancreatic cancer, regular cola consumption, regular non-cola soft drinks, smoking status	Other strengths: cases and controls from same catchment area; all evaluated at same hospital for suspected malignancy; models adjusted for BMI. Other limitations: small sample size, especially for stratified analyses, limiting power to detect associations; possibility of selection bias (50% participation rate in controls); potential bias if exposure was related to disease condition of controls.
			Never	60	1		
			Occasional (< 1/day)	8	0.61 (0.26–1.44)		
			Habitual (≥ 1/day)	11	1.29 (0.60–2.78)		
		Pancreas, incidence	Diet cola consumption, overweight/obese (BMI, ≥ 25.0 kg/m^2) (OR):				
			Never	79	1		
			Occasional (< 1/day)	28	1.19 (0.70–2.01)		
			Habitual (≥ 1/day)	21	0.89 (0.51–1.54)		

Table 2.1   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Davis et al. (2023) (cont.)		Pancreas, incidence	Diet cola consumption, women (OR):			Age, total vegetable servings/week, processed meat servings/week, family history of pancreatic cancer, regular cola consumption, regular non-cola soft drinks, smoking status, BMI categories	
			Never	62	1		
			Occasional (< 1/day)	18	1.18 (0.62–2.24)		
			Habitual (≥ 1/day)	18	1.14 (0.61–2.14)		
		Pancreas, incidence	Diet cola consumption, men (OR):				
			Never	82	1		
			Occasional (< 1/day)	18	0.84 (0.46–1.53)		
			Habitual (≥ 1/day)	15	0.79 (0.42–1.49)		
		Pancreas, mortality	Diet cola consumption (HR):			Age, sex, smoking status, BMI categories, total vegetable servings/week, regular cola consumption, regular non-cola soft drinks, histology, stage, surgery status, radiation status	
			Never	137	1		
			Occasional (< 1/day)	35	1.14 (0.75–1.73)		
			Habitual (≥ 1/day)	31	1.05 (0.70–1.59)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
[Palomar-Cros et al. (2023)](#) Spain 2008–2013 Case–control	Cases: 351 (stomach cancer), 1881 (colon and rectal cancer); aged 20–85 yr with newly diagnosed histologically confirmed cancer, resided in catchment area for at least 6 mo; no prior history of their cancer; enrolled as soon as possible after diagnosis; frequency-matched on age, sex, and region to population controls Controls: 3629; randomly selected from administrative records of selected primary health care centres within catchment area Exposure assessment method: self-administered, semiquantitative FFQ, 140 food items, assessing usual dietary intake during the previous year	Stomach, incidence	Consumption of aspartame-containing products (OR):			Age, sex, study centre, education, smoking, radiation exposure, total WCRF score continuous, total energy intake, total sugar intake, other ASs	*Exposure assessment critique*: A key strength was the categorization of intake of ASBs and tabletop sweeteners by type (aspartame vs others) using public data on ingredients in food supply, but it was unclear whether the assumption of aspartame content in products was correct. A key limitation was that beverages and tabletop sweeteners were assessed but there was no consideration of ASs in the rest of the food supply. *Other strengths*: relatively large sample size; histopathological confirmation of cancer cases; extensive assessment of confounding, including from BMI (captured in a combined score based on WCRF/AICR evidence on lifestyle factors; [Romaguera et al., 2017](#)); stratification by diabetes status to evaluate heterogeneity of associations.
			Non-consumers	290	1		
			Medium intake (< 3rd quartile among controls)	42	1.01 (0.69–1.46)		
			High intake (≥ 3rd quartile among controls)	19	1.09 (0.62–1.83)		
			Trend-test *P* value: 0.8				
		Stomach, incidence	Consumption of aspartame-containing products, participants without diabetes (OR):				
			Non-consumers	251	1		
			Medium intake (< 3rd quartile among controls)	28	0.84 (0.53–1.29)		
			High intake (≥ 3rd quartile among controls)	12	0.89 (0.43–1.66)		
			Trend-test *P* value: 0.5				
		Stomach, incidence	Consumption of aspartame-containing products, participants with diabetes (OR):			Age, sex, study centre, education, smoking, radiation exposure, total WCRF score continuous, total energy intake, total sugar intake, other ASs	
			Non-consumers	39	1		
			Medium intake (< 3rd quartile among controls)	14	2.02 (0.92–4.27)		
			High intake (≥ 3rd quartile among controls)	7	2.04 (0.70–5.40)		
			Trend-test *P* value: 0.05				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Palomar-Cros et al. (2023) (cont.)		Stomach, incidence	Consumption of low- or no-calorie soft drinks (OR):			Age, sex, study centre, education, smoking, radiation exposure, total WCRF score continuous, total energy intake, total sugar intake, other sources of sweeteners, total caloric drinks	*Other limitations*: non-prospective study design (case–control); selection bias due to low participation among cases (participation rate was 68% for colorectal; 57% for gastric) and controls (mean participation rate was 53%); recall bias in exposure assessment; potential for bias from exposure measurement error and residual confounding due to other correlates of AS use among those with diabetes; relatively low exposure contrasts for aspartame-containing products; potential for chance findings due to small number in some strata.
			Non-consumers	295	1		
			Medium intake (< 3rd quartile among controls)	36	1.05 (0.70–1.54)		
			High intake (≥ 3rd quartile among controls)	20	1.31 (0.74–2.21)		
			Trend-test *P* value: 0.4				
		Stomach, incidence	Consumption of low- or no-calorie soft drinks, participants without diabetes (OR):				
			Non-consumers	254	1		
			Medium intake (< 3rd quartile among controls)	25	0.93 (0.57–1.46)		
			High intake (≥ 3rd quartile among controls)	12	1.17 (0.57–2.20)		
			Trend-test *P* value: 0.9				
		Stomach, incidence	Consumption of low- or no-calorie soft drinks, participants with diabetes (OR):				
			Non-consumers	41	1		
			Medium intake (< 3rd quartile among controls)	11	2.01 (0.86–4.46)		
			High intake (≥ 3rd quartile among controls)	8	1.86 (0.63–5.01)		
			Trend-test *P* value: 0.08				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Palomar-Cros et al. (2023) (cont.)		Colon and rectum, incidence	Consumption of aspartame-containing products (OR):			Age, sex, study centre, education, smoking, radiation exposure, total WCRF score continuous, total energy intake, total sugar intake, family history of colorectal cancer, night shift work, other ASs	
			Non-consumers	1620	1		
			Medium intake (< 3rd quartile among controls)	172	0.76 (0.62–0.93)		
			High intake (≥ 3rd quartile among controls)	89	0.94 (0.71–1.25)		
			Trend-test *P* value: 0.1				
		Colon and rectum, incidence	Consumption of aspartame-containing products, participants without diabetes (OR):				
			Non-consumers	1358	1		
			Medium intake (< 3rd quartile among controls)	127	0.74 (0.59–0.93)		
			High intake (≥ 3rd quartile among controls)	57	0.87 (0.61–1.21)		
			Trend-test *P* value: 0.04				
		Colon and rectum, incidence	Consumption of aspartame-containing products, participants with diabetes (OR):				
			Non-consumers	262	1		
			Medium intake (< 3rd quartile among controls)	45	0.82 (0.52–1.27)		
			High intake (≥ 3rd quartile among controls)	32	1.09 (0.63–1.87)		
			Trend-test *P* value: 1.0				

Table 2.1   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Palomar-Cros et al. (2023) (cont.)		Colon and rectum, incidence	Consumption of low- or no-calorie soft drinks (OR):			Age, sex, study centre, education, smoking, radiation exposure, total WCRF score continuous, total energy intake, total sugar intake, other sources of sweeteners, total caloric drinks	
			Non-consumers	1659	1		
			Medium intake (< 3rd quartile among controls)	150	0.82 (0.66–1.02)		
			High intake (≥ 3rd quartile among controls)	72	0.77 (0.56–1.05)		
			Trend-test *P* value: 0.02				
		Colon and rectum, incidence	Consumption of low- or no-calorie soft drinks, participants without diabetes (OR):				
			Non-consumers	1387	1		
			Medium intake (< 3rd quartile among controls)	107	0.75 (0.58–0.96)		
			High intake (≥ 3rd quartile among controls)	48	0.71 (0.48–1.01)		
			Trend-test *P* value: < 0.01				

Table 2.1   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Palomar-Cros et al. (2023) (cont.)		Colon and rectum, incidence	Consumption of low- or no-calorie soft drinks, participants with diabetes (OR):			Age, sex, study centre, education, smoking, radiation exposure, total WCRF score continuous, total energy intake, total sugar intake, other sources of sweeteners, total caloric drinks	
			Non-consumers	272	1		
			Medium intake (< 3rd quartile among controls)	43	1.07 (0.68–1.69)		
			High intake (≥ 3rd quartile among controls)	24	0.95 (0.50–1.79)		
			Trend-test *P* value: 1.0				

AS, artificial sweetener; AICR, American Institute of Cancer Research; ASB, artificially sweetened beverage; BMI, body mass index; CI, confidence interval; CPS-II, Cancer Prevention Study II; EPIC, European Prospective Investigation into Cancer and Nutrition; FDA, Food and Drug Administration; FFQ, food frequency questionnaire; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HPFS, Health Professionals Follow-up Study; HR, hazard ratio; MCCS, Melbourne Collaborative Cohort Study; MET, metabolic equivalent tasks; mo, month(s); NHS, Nurses' Health Study; NHS-II, Nurses' Health Study II; NIH-AARP, National Institutes of Health-American Association of Retired Persons; NR, not reported; OR, odds ratio; PEDS, Patient Epidemiologic Data System; PLCO, Prostate, Lung, Colorectal and Ovarian Cancer screening trial; RR, relative risk; SCC, squamous cell carcinoma; SD, standard deviation; SEER, Surveillance, Epidemiology, and End Results; SSB, sugar-sweetened beverage; vs, versus; WCRF, World Cancer Research Fund; wk, week(s); yr, year(s).

[a] The larynx is part of the respiratory system but is included here as reported in McCullough et al. (2022).

duct cancers combined). Results were stratified by diabetes status, determined a priori, because of potentially differing dietary behaviours. ASB consumption of > 2 servings/day was 24% among participants with diabetes, compared with 8% among those without diabetes; 19% of participants with diabetes reported not consuming ASBs, compared with 50% of those without diabetes. The remaining participants reported a consumption of either 1–2 servings/day or < 1 serving/day. The authors tested the proportional hazards assumption across the time scale used in the study (i.e. follow-up time). Because this assumption was found to be violated, the authors presented the associations separately by median follow-up: 0–12 years and ≥ 12 years. Among participants without diabetes, no association with ASB consumption was observed, regardless of follow-up period. Among participants with diabetes, positive associations with risk of liver cancer were reported for several exposure types for ≤ 12 years follow-up (HR per times/day for ASBs overall, 1.13; 95% CI, 1.02–1.25; HR for artificially sweetened soda, 1.13; 95% CI, 1.01–1.27; and HR for artificially sweetened fruit punch, 1.17; 95% CI, 0.92–1.48). All findings were null for ≥ 12 years follow-up. [The strengths of this study included a large sample size from the pooling of two large cohorts to examine the incidence of this relatively rare cancer. Another strength was that the baseline diet assessments in NIH-AARP and PLCO (1995–1996 and 1998, respectively) and the subsequent 12 years of follow-up coincided with a period in which the artificial sweetener used in ASBs was predominantly aspartame. Stratification by diabetes status helped to limit confounding by this important liver cancer risk factor and permitted examination of higher levels of diet soda intake. Diabetes appeared to be an effect modifier of the association, possibly due to greater ASB exposure in participants with diabetes; however, it was unclear whether biological mechanisms specific to individuals with diabetes could play a role in explaining these

findings. The study controlled for multiple liver cancer risk factors but was unable to control for coffee consumption (which has been associated with lower liver cancer risk; IARC, 2018a) or HBV/HCV infection status. Exposure misclassification caused by a single assessment of diet at baseline was also a possibility but was likely to be non-differential.]

In the American Cancer Society's Cancer Prevention Study II (CPS-II) prospective cohort comprising 934 777 men and women in the USA, 2722 deaths from cancers of the liver and intrahepatic bile duct occurred over a median follow-up of 27.7 years (McCullough et al., 2022). A beverage grid on the baseline (1982) questionnaire (which included "diet soda or diet iced tea") queried the number of cups, glasses, or drinks usually consumed per day. If less than once per day, but at least three times per week, participants wrote "½". Overall, the frequency of ASB intake was not associated with liver cancer mortality risk (ICD-10, C22: cancer of the liver and intrahepatic bile duct). However, consumption of ≥ 2 drinks/day versus none was associated with increased risk among non-smoking men (HR, 1.44; 95% CI, 0.99–2.08; P for trend, 0.040). This was attenuated when BMI was included in the model (HR, 1.21; 95% CI, 0.83–1.77; P for trend, 0.335), suggesting some confounding by adiposity. [Mortality is a reasonable proxy for incidence for this cancer, which has a high fatality rate. The study controlled for BMI and sugar-sweetened beverages and excluded participants with a history of diabetes at baseline. However, it was unable to control for HBV/HCV infection status. With only a single baseline assessment of exposure in 1982, 1 year before the approval of aspartame use in beverages, and a median follow-up of 27.7 years, the study had potential for misclassification, which was likely to be non-differential and bias risk estimates towards the null. Although published data from more than 100 000 participants included in both CPS-II and the CPS-II nutrition cohort (a

subcohort of the parent CPS-II mortality cohort) (McCullough et al., 2014) suggested some decrease in ASB consumption between 1982 and 1999 in this population over 17 years, the relative ranking of intake category was consistent during this period.]

A meta-analysis (Jatho et al., 2021) evaluated artificially sweetened soft drink consumption and liver cancer (among other gastrointestinal tract cancers), but the Working Group judged this review to be uninformative for the evaluation of aspartame because it included estimates for regular soft drinks and combined results from prospective and case–control studies.]

2.1.2 Cancers of the colon and rectum

The Working Group identified six cohort studies (comprising seven unique cohorts) that assessed intake of aspartame, by proxy through ASBs, and its association with colon and/or rectal cancer incidence or mortality. These studies included cohorts from the USA (Malik et al., 2019; Hur et al., 2021; McCullough et al., 2022), Australia (Hodge et al., 2018), France (Chazelas et al., 2019), and seven European countries (Mullee et al., 2019). One case–control study assessed the association between artificial sweetener consumption and the incidence of colon and rectal cancer in Italy (Gallus et al., 2007). Another case–control study in Spain looked at associations between ASBs and tabletop sweeteners other than saccharin to represent aspartame intake (Palomar-Cros et al., 2023). A third case–control study from Egypt was also identified (Mahfouz et al., 2014). None of the studies with cancers of the colon or rectum as the outcome used specific aspartame intake as the exposure. [The Working Group noted that all the informative cohort and case–control studies used comprehensive adjustment for confounders, in all cases including adjustment for measures of obesity.]

Four additional studies were not considered to be informative since they did not directly investigate the association between aspartame (or a proxy) and colorectal cancer, or they measured dietary information before the commercial use of aspartame. In the Nurses' Health Study (NHS) and the Health Professionals Follow-up Study (HPFS) cohort, Yuan et al. (2022) estimated the effect of substituting sugar-sweetened beverages with ASBs on the risk of colorectal cancer, whereas Wang et al. (2021) and Nguyen et al. (2020) reported on the association between a dietary pattern (including low-calorie beverages) that promoted the proliferation of sulfur-producing bacteria in the gut and colorectal cancer. Joh et al. (2021) examined the association between intake of ASBs in adolescence and incidence of colorectal adenoma in adult women from the Nurses' Health Study II (NHS-II) cohort. The dietary data for adolescents were retrospectively collected for the period 1960–1982. [The Working Group noted that this period pre-dated the use of aspartame in soft drinks, making the data of questionable value for this investigation. The Working Group noted that colorectal adenoma is a valid outcome as defined in the Preamble to the *IARC Monographs* (see the front matter of the present volume; IARC, 2019), which allows the inclusion of epidemiological studies on benign neoplasms, preneoplastic lesions, malignant precursors, and other end-points when they relate to the agents reviewed.] The case–control study from Egypt (cases and controls were matched on age and sex) was considered to be uninformative because of the small number of cases ($n = 150$) and unclear exposure to aspartame (Mahfouz et al., 2014).

No association was seen between frequency of ASB consumption and colorectal cancer incidence in an Australian cohort (Hodge et al., 2018), or a French cohort (Chazelas et al., 2019). The Australian Melbourne Collaborative Cohort Study (MCCS), a cohort study that included 35 593 men and women followed for cancer incidence

until 30 June 2013, used a food frequency questionnaire (FFQ) to quantify the frequency of consumption of diet soft drinks at baseline in 1990–1994, after the approval of aspartame for use in soft drinks in Australia in 1986 (Hodge et al., 2018). [The Working Group noted that the limitations of this study were the nonspecific exposure and the single baseline assessment of intake.] The French NutriNet-Santé cohort included more than 100 000 men and women enrolled between 2009 to 2017 and followed until 11 January 2018; estimated consumption of 12 different types of ASB was based on multiple 24-hour dietary records collected every 6 months (Chazelas et al., 2019). Baseline diet was the average of all records over the first 2 years of follow-up, and all included participants had at least two records over this period (mean ± SD, 5.6 ± 3.0). [The Working Group considered the use of multiple records as a strength of this study. The inclusion of different beverages with different levels of aspartame and other artificial sweeteners was considered a weakness that could lead to misclassification of aspartame exposure.]

Malik et al. (2019) combined data from the HPFS (37 716 men) and the NHS (80 647 women) to evaluate ASB consumption and colon cancer mortality, finding no association. The main analysis updated ASB consumption every 4 years, whereas the secondary analysis for all-cancer mortality (also conducted in both the NHS and the HPFS) only used the cumulative average intake over time. Follow-up was from 1986 to the end of 2014 for the HPFS, and from 1980 to the end of 2014 for the NHS, thus the first dietary data in the NHS were collected before aspartame was approved for use in beverages. [The Working Group noted that although these cohorts used low-calorie beverages as a proxy for aspartame intake, these were the predominant source of aspartame in the USA, and aspartame was the predominant sweetener used in ASBs, at least for the period from the mid-1980s to the

mid-2000s. The updating of exposure over time was also a strength.]

In the EPIC cohorts, which included 451 743 men and women from seven countries across Europe, data on consumption of artificially sweetened soft drinks defined as "low-calorie or diet fizzy soft drinks" were collected with cohort-specific dietary instruments between 1992 and 2000 (Mullee et al., 2019). In three additional countries included in EPIC (Italy, Spain, and Sweden), data on types of soft drinks were not collected in all recruiting centres, and data from these countries were excluded in this analysis. [The Working Group noted that data were collected during a relevant period for aspartame use in Europe, but the collection of intake data at a single time point, and the variation in products across countries over time may lead to exposure misclassification, which would be expected to be non-differential with respect to cancer outcome.] Cause-specific mortality follow-up was between baseline and 2009–2013 (depending on the study centre). A total of 2095 deaths from colorectal cancer were recorded. The frequency of artificially sweetened soft drink consumption was not associated with colorectal cancer mortality, although the hazard ratio in the group consuming ≥ 1 glasses/day was elevated (HR, 1.22; 95% CI, 0.91–1.64) relative to consumption of < 1 glass/month. [The Working Group noted that the study design, incorporating cohorts from multiple countries with different behaviours with standardized data collection methods, was a strength of this study.]

A study in 95 464 female registered nurses participating in the United States (US) NHS-II cohort investigated the association between consumption of low-calorie carbonated beverages and incidence of early-onset colorectal cancer (Hur et al., 2021). Participants were aged 25–42 years at baseline and self-reported dietary data, including consumption of low-calorie carbonated beverages, using a validated semiquantitative FFQ every 4 years from 1991 until the end of follow-up in June 2015. The

frequency with which low-calorie carbonated beverages were consumed during the previous 12 months was not associated with the incidence of early-onset (before age 50 years) colorectal cancer (Hur et al., 2021). This paper also looked at beverage consumption in adolescence on the basis of a retrospective questionnaire, but this intake occurred between 1960 and 1982, before the approval of aspartame for use in soft drinks.

McCullough et al. (2022) analysed data from the nationwide US CPS-II cohort, which included 934 777 men and women (aged ≥ 28 years) who completed a baseline questionnaire on diet, medical history, and demographic and life-style characteristics in 1982. Participants were followed for cause-specific mortality through 2016. Usual daily consumption of ASB, defined as diet soda or diet iced teas, was ascertained from the dietary questionnaire at baseline. Results showed an inverse association between frequency of consuming ASBs and colorectal cancer mortality, after adjusting for BMI (HR for ≥ 2 drinks/day versus never consuming ASBs, 0.92; 95% CI, 0.86–0.99; P for trend, 0.034), but no association without adjusting for BMI (HR for ≥ 2 drinks/day versus never consuming ASBs was 0.97; 95% CI, 0.90–1.04; P for trend, 0.704). [Data collection occurred the year before aspartame was approved for use in carbonated beverages in the USA. The Working Group considered that with only a single baseline assessment of expo-sure in 1982, 1 year before the approval of aspar-tame use in beverages, and a median follow-up of 27.7 years, the study had the potential for misclassification, which was likely to be non-dif-ferential and bias risk estimates towards the null. However, it was noted that the relative ranking of intake categories of ASB consumption over the 17-year period between 1982 and 1999 among 100 000 participants included in both the CPS-II and the CPS-II nutrition cohort (McCullough et al., 2014) was reasonably consistent over time, such that the reported intakes may partly reflect aspartame intake over the follow-up, during a period when ASBs were the predominant source of aspartame in the USA and aspartame was almost the only sweetener used in ASBs.]

Apart from the studies by Hur et al. (2021) (which used data from the NHS-II cohort) and Malik et al. (2019) (which used data from the combined NHS/HPFS cohort, updating or averaging intake from surveys repeated every 4 years), most other studies assessed intake on only one occasion. Chazelas et al. (2019) used multiple (at least two) 24-hour records to assess intake, but their assessment was based on a single value averaged over the first 2 years. [The Working Group noted that this could contribute to non-differential exposure misclassification if intake had changed over follow-up, and thus bias the results towards the null.]

Three case–control studies reported on the association between artificial sweetener con-sumption and colorectal cancer. [The Working Group noted important limitations common to all case–control studies: the risk of recall bias and the lack of temporal sequence, which may result in reverse causation. The direction of bias was unclear.] Data from a hospital-based unmatched case–control study across four areas of Italy indicated no association between use of artificial sweeteners and either colon (n = 1225) or rectal cancer (n = 728) (Gallus et al., 2007). In contrast to most of the cohort studies that used artificially sweetened soft drinks as the exposure, this study examined use of artificial sweeteners in sachets or as tablets as the exposure (Gallus et al., 2007). The data were collected between 1991 and 2004, as "saccharin" or "other sweet-eners". On the basis of a study of Italian teenagers in 1996 (Leclercq et al., 1999) (which showed that most participants who consumed foods or beverages containing artificial sweeteners were consuming items containing aspartame), "other sweeteners" in the case–control study by Gallus et al. (2007) were assumed to be mainly aspar-tame. [The Working Group noted that it was not clear how valid this assumption was with regard

to sweeteners consumed in this form, although this would not be considered to create a spurious positive association.]

The Spanish multicase–control (MCC-Spain) study (Palomar-Cros et al., 2023) recruited 10 106 people aged 20–85 years with recently diagnosed cancer of the colorectum, breast, stomach, and prostate, and chronic lymphocytic leukaemia (CLL). Cases were frequency-matched to the population controls on age, sex, and region. Recruitment occurred from 2008 through 2013. Controls were randomly selected from primary care centres within the study centre catchment area. Dietary intake data were collected using a validated self-completed FFQ. After exclusion of participants who did not complete the FFQ ($n = 1354$), or who had missing covariate data ($n = 29$), or cases of CLL diagnosed > 1 year before the interview ([$n = 271$; personal communication with the authors]), 8452 cases and controls (including 1881 colorectal cancer cases and 3629 controls) remained across all studied outcomes. [The Working Group noted that exclusion rates by cancer type were not provided.] Aspartame intake was assessed as portions/day of low- or no-calorie soft drinks and tabletop sweeteners that were not saccharin. In a sensitivity analysis, low- or no-calorie soft drinks alone were evaluated. [The Working Group noted that it was unclear how valid was the assumption that it was aspartame that was used in all low- or no-calorie soft drinks and in tabletop sweeteners other than saccharin. Non-differential exposure misclassification was likely.] Intake of aspartame was modelled as non-consumers (reference group), moderate consumers (below the third quartile), and high consumers (third quartile or above). All models were adjusted for a score combining a set of modifiable lifestyle factors, including BMI, which have been established as risk factors for cancer by the World Cancer Research Fund/American Institute of Cancer Research (WCRF/AICR) (Romaguera et al., 2017). Analyses stratified by diabetes status were also performed. Compared with the non-consumers group, the overall odds ratio (OR) for colorectal cancer was 0.76 (95% CI, 0.62–0.93) for the moderate consumers and 0.94 (95% CI, 0.71–1.25) for the high consumers. [The Working Group noted that the results in the moderate consumers group were suggestive of a protective effect.] After stratification by diabetes, the odds ratio for high consumers was 0.87 (95% CI, 0.61–1.21) for people without diabetes and 1.09 (95% CI, 0.63–1.87) in people with diabetes. When only low- or no-calorie soft drinks were used as the exposure, the odds ratio for moderate consumers was 0.82 (95% CI, 0.66–1.02) and that for high consumers was 0.77 (95% CI, 0.56–1.05). [The Working Group noted the wide confidence intervals for most estimates. Limitations included low intake of products potentially containing aspartame, with a mean of 0.12 portions/day, which led to a relatively low exposure contrast, as well as uncertainty about how well these products reflected aspartame consumption. Strengths included the large number of cases and stratification by diabetes status.]

Three meta-analyses that examined the association between colorectal cancer and artificial sweetener consumption were considered uninformative (Jatho et al., 2021; Llaha et al., 2021; Tepler et al., 2021). This was because the exposures studied were not all appropriate proxies for aspartame intake [the Working Group noted, for example, that one study included in the meta-analysis by Jatho et al. (2021) did not separate artificially sweetened and sugar-sweetened beverages]; because the meta-analyses incorrectly combined outcomes from case–control and prospective studies with both cancer incidence and mortality as outcomes (Jatho et al., 2021); because they did not report results for colorectal cancer separately from other gastric luminal cancers (Tepler et al., 2021); or because an insufficient number of studies were identified to perform meta-analysis for colorectal cancer as an outcome (Llaha et al., 2021). A fourth meta-analysis (Yin et al., 2022), which included data

from three cohort studies, reported an overall inverse association between ASB consumption and cancer of the colon and rectum (relative risk, RR for highest versus lowest intake category, 0.78; 95% CI, 0.62–0.99, $P = 0.037$), with low heterogeneity between studies. [The Working Group noted that, although the title suggested that this meta-analysis was focused on artificially sweetened soft drinks as the exposure, studies in which the exposure was low-calorie sweeteners were also included. However, the colorectal cancer meta-analysis included only three cohorts (Hodge et al., 2018; Chazelas et al., 2019; Hur et al., 2021), for which the exposure was ASBs alone. The Working Group noted that data collection for these cohorts occurred over different time periods and did not always coincide with ASBs being the predominant source of aspartame, or with aspartame being the predominant type of artificial sweetener, most notably for the NutriNet-Santé report that did not specifically identify aspartame exposure (Chazelas et al., 2019).] The meta-analysis of ASB intake and colorectal cancer by Pan et al. (2023), which included two studies described above (Hodge et al., 2018; Chazelas et al., 2019) was not considered informative.

2.1.3 Cancers of the pancreas and other digestive system organs (excluding liver and colorectum)

See Table 2.1.

Five prospective cohort studies examined the association between consumption of ASBs or diet soft drinks and risk of cancers of the pancreas (Schernhammer et al., 2005; Bao et al., 2008; Navarrete-Muñoz et al., 2016; Hodge et al., 2018; McCullough et al., 2022) or other digestive organs (Hodge et al., 2018; McCullough et al., 2022). Seven case–control studies examined the association between aspartame exposure or ASB consumption and risk of cancers of the pancreas (Norell et al., 1986; Bosetti et al., 2009; Chan et al., 2009; Davis et al., 2023) or other digestive organs (Mayne et al., 2006; Gallus et al., 2007; Bosetti et al., 2009; Palomar-Cros et al., 2023). Four case–control studies (Norell et al., 1986; Gallus et al., 2007; Bosetti et al., 2009; Palomar-Cros et al., 2023) examined use of tabletop artificial sweetener; two of these studies specifically examined the category of "artificial sweeteners other than saccharin" (Gallus et al., 2007; Bosetti et al., 2009); Norell et al. (1986) examined artificial sweetener users/non-users, and Palomar-Cros et al. (2023) examined tabletop artificial sweeteners other than saccharin together with ASBs as "consumption of aspartame-containing products". The other three case–control studies (Mayne et al., 2006; Chan et al., 2009; Davis et al., 2023) assessed frequency of consumption of diet cola, sugar-free carbonated beverages, and diet carbonated soft drinks, respectively. The study by Larsson et al. (2016) was not considered informative as it was unable to separate consumption of sugar and ASBs.

[After the first small case–control study in 1986, in which artificial sweetener use was categorized as "yes/no" (Norell et al., 1986), advances in study design have included studies with larger sample sizes, prospective design, information on cancer subsite or histological subtype, estimation of ASB intake via repeated exposure assessments over time (one study, Schernhammer et al., 2005), and quantitative assessment of exposure using frequency and dose instead of "yes/no" user. None of the studies presented aspartame separately or estimated aspartame intake from all sources.]

In a study in two USA-based cohorts, the NHS cohort of 88 794 female nurses and the HPFS cohort of 49 364 male health professionals (Schernhammer et al., 2005), 379 cases of pancreatic cancer occurred during the 14–20-year follow-up period beginning in 1980 or 1986, respectively. The study assessed usual consumption of three types of diet soda (diet cola with caffeine, diet cola without caffeine, and other

diet soda), repeatedly every 4 years using a ~130-item FFQ, and the diet assessment was cumulatively updated. The exposure contrast was diet soft drink consumption of > 3 times/week versus < 1 time/month (median, 1.6 versus 0 drinks/day in women and 1.5 versus 0 drinks/day in men). No association with greater consumption of diet soft drinks was observed among men, women, men and women combined, or when stratified by BMI or physical activity. When examined separately by type of diet soda, neither diet cola nor other diet soft drinks was associated with pancreatic cancer risk. [A major strength of this study was the cumulatively updated diet assessment from the time of introduction of aspartame in beverages for up to two decades, potentially capturing lifetime exposure to aspartame from sodas. The study controlled for BMI at baseline and updated diabetes diagnoses during follow-up. Limitations were that diet soft drink intake was not high (highest category, ≥ 3 times/week), and the number of cases was limited in some stratified analyses, with wide confidence intervals, suggesting limited statistical power for some subgroups.]

Bao et al. (2008) followed 487 922 men and women in the NIH-AARP cohort in the USA for a mean of 7.2 years, during which time 1258 participants developed pancreatic cancer. Usual diet in the past 12 months was assessed at baseline between 1995 and 1996 when participants completed a 124-item FFQ. Participants were queried about frequency of consumption of beverages (ranging from "never" to "≥ 6 times per day") and whether they usually drank the sugar-free (diet) or the regular-calorie type of a particular beverage. Beverage consumption was expressed in gram weight. Compared with non-consumers of diet soft drinks, higher quintile consumption at baseline (fifth quintile, median, 816.9 g/day, versus non-consumers) was not associated with pancreatic cancer risk overall in minimally or fully adjusted models, or when stratifying by sex (data not provided), BMI, physical activity, or

smoking status. Increased risk was observed only after excluding the first 2 years of follow-up in the fourth quintile (HR, 1.35; 95% CI, 1.03–1.77) and, with wider confidence intervals, in the fifth quintile (HR, 1.25; 95% CI, 0.94–1.66) versus non-consumers (*P* for trend, 0.19). [The strengths of this analysis included its large size and 90% completeness of case identification via linkage with cancer registries. The study controlled for BMI and excluded participants who reported having diabetes at baseline. The period of follow-up was relevant for diet soft drinks being a major contributor to aspartame in the diet, and for ASB intake reflecting aspartame, since aspartame was almost the only sweetener used in ASBs at that time. The main limitation was the potential for measurement error with the type of sequential assessment of frequency of consumption used in the study (i.e. whether the diet or regular version was usually consumed, instead of asking about beverage types individually), which was likely to result in non-differential exposure misclassification of participants.]

A study in the EPIC cohort (Navarrete-Muñoz et al., 2016) was conducted for seven European countries with information on artificially sweetened soft drink intake at baseline (i.e. it did not include Italy, Spain, and Sweden since some recruitment centres in these countries did not collect this information). Usual diet was assessed at study enrolment between 1992 and 2000 using country-specific diet assessment instruments. Artificially sweetened soft drink intake was assessed asking participants to recall the number of glasses (approximately 250 mL) of "low-calorie or diet fizzy soft drinks" per month, week, or day (Mullee et al., 2019). This quantity was then converted to 330 mL servings per week for the analysis (Navarrete-Muñoz et al., 2016). During a median follow-up of 11.6 years, 586 adenocarcinomas of the exocrine pancreas were verified (follow-up was reported for the whole EPIC cohort, not for the subset with information on ASB consumption). No association between

artificially sweetened soft drink consumption of > 92.2 g/day versus 0.1–2.0 g/day and pancreatic cancer risk was reported for models of men and women combined. However, a 25% increase in risk was observed in continuous models (per 336 g/day) among men but not women (*P* for interaction by sex, 0.004). [Personal communication with the authors confirmed that the correct sex-stratified model was that reported in supplemental Table 5 of their paper (Navarrete-Muñoz et al., 2016), which is the one reported in the text and tables of the present monograph.] Associations did not vary among strata defined by age of diagnosis, BMI, waist circumference, physical activity, and smoking status. [Strengths included the geographically heterogeneous populations with information on cancer subtype, and control for diabetes and BMI. Limitations included the low intake of diet soft drinks (the highest consumption category was > 92.2 g/day). EPIC combined data from multiple European countries, each with its own country-specific dietary questionnaire and potentially its own products with variable aspartame content, largely during a period relevant for aspartame exposure. However, towards the end of the follow-up period for countries that started recruitment later (i.e. towards 2000), co-exposure to other artificial sweeteners was likely. Baseline-only assessment of dietary intake may contribute to non-differential measurement error and bias risk estimates towards the null.]

In the MCCS in Australia, 165 cases of cancer of the gastric cardia occurred among 35 593 participants enrolled between 1990–1994 with up to 23 years of follow-up (Hodge et al., 2018). For artificially sweetened soft drink consumption of ≥ 1 drink/day versus never or < 1 drink/month, there was no association between artificially sweetened soft drink intake and risk of gastric cardia cancer. [Strengths included that the assessment was carried out after the introduction of aspartame into diet soft drinks in Australia (1987) and that the first half of follow-up largely overlapped with the period of aspartame use in Australia). The single baseline assessment and long follow-up probably resulted in non-differential measurement error that could bias results towards the null; the small number of cases (*n* = 10) among the most-exposed participants limited the model stability and ability to detect associations (e.g. the 95% confidence interval was wide; 95% CI, 0.53–1.98). BMI was not adjusted in the model because the study operated on the assumption that the association was mediated by BMI; however, adjustment for waist circumference, a measure of abdominal obesity, was performed.]

In the CPS-II prospective cohort comprising 934 777 men and women in the USA, there were 9196, 1024, 2798, 2727, and 324 deaths from pancreatic, gallbladder, stomach, oesophageal, and small intestine cancer, respectively, over a median follow-up of 27.7 years (McCullough et al., 2022). Deaths from oral, pharyngeal, and laryngeal cancers were combined, making a total of 1852. [The larynx is part of the respiratory system and, in the present monograph, laryngeal cancers are included in Section 2.4 (under "other solid cancers"). The grouping of data on oral, pharyngeal, and laryngeal cancers, as used in this study, was less informative than the individual cancer site-specific results.] ASB consumption of ≥ 2 drinks/day versus never was associated with a 16% increase in risk (95% CI, 1.07–1.26) of death from pancreatic cancer when there was no control for BMI, and an 11% increase in risk (95% CI, 1.02–1.20) when BMI was included in the multivariable-adjusted model. Overall, an increased risk of pancreatic cancer mortality was observed among both men and women, and no interaction with BMI was found (*P* for interaction, 0.678). For ≥ 2 drinks/day of ASB, risk was elevated among never-smokers for men, women, and both combined (HR for men, 1.34; 95% CI, 1.10–1.65; HR for women, 1.12; 95% CI, 0.96–1.30; and HR for men and women combined, 1.19; 95% CI, 1.05–1.34). [These estimates were

provided by personal communication with the author, after the estimates in the original publication (McCullough et al., 2022) were found to be incorrect.] No association was observed between ASB consumption and mortality from cancers of the oesophagus or stomach in categorical or continuous models. This study was unable to distinguish cancers of the gastric cardia from non-cardia cancers, or oesophageal squamous cell carcinoma from adenocarcinoma. [The Working Group noted that the absence of these cancer subtypes may mask associations, since risk factors are known to vary for gastric cardia and non-cardia cancers and for oesophageal squamous cell carcinoma and adenocarcinoma.] A 7% increase in risk was reported for gallbladder cancer mortality only in continuous models (HR per drink/day, 1.07; 95% CI, 1.01–1.14), which was attenuated after controlling for BMI (HR, 1.05; 95% CI, 0.98–1.11), with largely overlapping confidence intervals. Among never-smokers, the point estimate was the same, with slightly wider confidence intervals due to smaller numbers (HR, 1.05; 95% CI, 0.96–1.16; 476 deaths). The hazard ratio for the association between ASBs and small intestine cancer mortality in a continuous model (per drink/day) was 1.12 (95% CI, 1.01–1.23) and was similar when BMI was included in the model (HR, 1.11; 95% CI, 1.00–1.22). Among never-smokers, controlling for BMI, the association of ASB consumption with small intestine cancer mortality was similar, with wider confidence intervals (HR, 1.10; 95% CI, 0.94–1.30; 138 deaths). Among men, the association with mortality from oral, pharyngeal, and laryngeal cancers combined was 1.07 (95% CI, 1.00–1.14) per drink/day, controlling for BMI. The associations for women only and for men and women combined were null in the BMI-adjusted model. [Mortality is a reasonable proxy for incidence for these cancers that have a high fatality rate (pancreas, gallbladder, stomach, and oesophagus). The study controlled for BMI and excluded participants with a history of diabetes

at baseline, minimizing confounding. However, with only baseline assessment of exposures and a median follow-up of 27.7 years, the study had the potential for misclassification and confounding, which was likely to be non-differential and bias risk estimates towards the null. A limitation was that aspartame was not approved in the USA for use in beverages until 1983, 1 year after the baseline exposure assessment for this cohort. The assessment of beverage intake 1 year before the approval of aspartame for addition to beverages increased the possibility of measurement error and confounding, which would most likely bias risk estimates towards the null. As noted above, published data for the 100 000 participants included in both the CPS-II and the CPS-II nutrition cohort (McCullough et al., 2014) suggested that there was some decrease in ASB consumption over the 17 years between 1982 and 1999 in this population, but that the relative ranking of intakes was consistent during this period.]

In a small case–control study conducted in Sweden in 1982–1984 that included 99 cases of cancer of the pancreas and 163 hospital-based and 138 population-based controls, null findings were reported for use of low-calorie sweeteners (sachets) and generic use of artificial sweeteners, respectively (Norell et al., 1986). [Limitations included the assessment of exposure as "use of artificial sweeteners" with non-quantitative responses of "yes" or "no" for past exposure. Participants were asked to recall intake before their illness, if they had changed their diet because of their illness. As aspartame was approved as a tabletop sweetener in Sweden in 1983, it was unlikely that prediagnostic intake reflected aspartame consumption.]

A population-based case–control study in the USA identified 255 cases of gastric cardia adenocarcinoma, 352 cases of non-cardia gastric adenocarcinoma, 206 cases of oesophageal squamous cell carcinoma, 282 cases of oesophageal adenocarcinoma, and 687 population-based controls (Mayne et al., 2006). Participants were

asked about the usual frequency with which they consumed diet soft drinks or soda (per day, week, month, or year) during the 3–5 years before diagnosis (cases) or interview (controls). High (top 20% of intake) versus no consumption of diet carbonated soft drinks was inversely associated with gastric cardia adenocarcinoma (OR, 0.50; 95% CI, 0.31–0.81), non-cardia gastric adenocarcinoma (OR, 0.58; 95% CI, 0.38–0.90), oesophageal adenocarcinoma (OR, 0.52; 95% CI, 0.32–0.83), and oesophageal squamous cell carcinoma (OR, 0.43; 95% CI, 0.23–0.82). Quantile cut-points were not provided for diet carbonated soft drinks. The authors controlled for frequency of reflux symptoms. [A strength of this analysis was the focus on gastric cancer subtype. The limitations included lack of data on duration of exposure. The patients diagnosed with cancer (cases) may have reduced their intake of carbonated beverages because of prediagnostic symptoms; therefore, their recall of their diet 3–5 years before diagnosis may not reflect intake in the distant past.]

In a hospital-based case–control study of 304 cases of oesophageal cancer and 743 controls in Italy (Gallus et al., 2007), no association was found between weekly consumption of sachets or tablets of "sweeteners other than saccharin" (assumed by the authors of the study to be mainly aspartame) and oesophageal cancer. The study also included 598 cases of oral and pharyngeal cancers and 1491 hospital-based controls; no evidence of an association between consumption of sachets or tablets of "sweeteners other than saccharin") and risk of these cancers was provided (Gallus et al., 2007). [The Working Group noted that only for 10 cases of oesophageal cancer and 12 cases of oral or pharyngeal cancer was any use of sachets or tablets per day reported, thus limiting study power. The exposure assessment of > 0 tablets or sachets per day versus non-daily use of tabletop sweeteners had the potential for exposure misclassification, which would tend to bias results towards the null.]

Another hospital-based case–control study based in northern Italy included 230 cases of gastric cancer matched to 547 hospital-based controls and 326 cases of pancreatic cancer matched to 652 controls (Bosetti et al., 2009). No association with increased risk of gastric or pancreatic cancer was observed for users versus non-users of sachets or tablets of "sweeteners other than saccharin", presumed by the author of the study to be mostly aspartame (17 and 35 cases of gastric and pancreatic cancer, respectively, reported use of tablets of "sweeteners other than saccharin"); the other sweetener assessed was saccharin. [Although the cases were histologically confirmed, the analysis presented all histological types combined, probably because of the small sample size. The exposure assessment of users versus non-users of other sweeteners increased the potential for exposure misclassification, which would tend to bias results towards the null.]

A population-based case–control study in the San Francisco Bay Area, USA, which included 532 cases with adenocarcinoma of the exocrine pancreas and 1701 controls, assessed sugar-free carbonated beverages overall and specific types (Chan et al., 2009). Diet was assessed using the Harvard FFQ instrument described above (Schernhammer et al., 2005). Among women and men combined, a higher risk of pancreatic cancer was found among consumers of ≥ 1 drink/day versus none (OR, 1.5; 95% CI, 1.1–2.1; P for trend, 0.2). For specific beverage types, risk was highest for low-calorie cola, with an 80% increase in risk (OR, 1.8; 95% CI, 1.1–2.9) among men, and a 70% increase in risk (OR, 1.7; 95% CI, 1.2–2.4) in men and women combined. Effect modification by diabetes history was reported: risk was elevated among participants without diabetes (OR, 1.6; 95% CI, 1.1–2.3) but not among those with diabetes (OR, 0.9; 95% CI, 0.3–2.6).

A hospital-based case–control study in Buffalo, New York, USA, which included 213 cases of pancreatic cancer and 852 cancer-free

controls, reported null associations between diet cola consumption and pancreatic cancer risk (Davis et al., 2023). Furthermore, no association between prediagnostic diet cola consumption and mortality from pancreatic cancer was observed in a retrospective cohort analysis of the 213 pancreatic cancer cases (203 pancreatic cancer deaths).

The MCC-Spain study enrolled 351 cases of stomach cancer and 3629 population-based controls between 2008 and 2013 and assessed consumption of aspartame-containing products as low- or no-calorie soft drinks and tabletop sweeteners other than saccharin (Palomar-Cros et al., 2023). Overall, there was no association between aspartame-containing products and the risk of stomach cancer. However, among participants with diabetes, high consumption of aspartame-containing products (sex-specific third quartile or above among controls) compared with non-consumers was associated with a higher risk of stomach cancer (OR, 2.04; 95% CI, 0.70–5.40; P for trend, 0.05), as was consumption of low- or no-calorie soft drinks (OR, 1.86; 95% CI, 0.63–5.01; P for trend, 0.08). [The limitations of the study by Palomar-Cros et al. (2023) included that the assumption that aspartame was used during this time period was questionable, since a mix of sweeteners may have been represented.]

[The strengths of these case–control studies included adjustment for BMI (Mayne et al., 2006; Gallus et al., 2007; Bosetti et al., 2009; Chan et al., 2009; Davis et al., 2023; Palomar-Cros et al., 2023) and adjustment or control for diabetes (Bosetti et al., 2009; Chan et al., 2009; Palomar-Cros et al., 2023) in some studies. A potential limitation of all included case–control studies was recall bias, which would be expected to bias estimates upward if cases were more likely than controls to recall artificial sweetener consumption, or downward if controls were more health-conscious and artificial sweetener use was viewed as healthy. Some of the case–control studies may have been subject to selection bias because of the low participation rate for controls – 67% in Chan et al. (2009); 50% in Davis et al. (2023); 70.2% in Mayne et al. (2006) (reported in Gammon et al., 1997); 53% in Palomar-Cros et al. (2023) (reported in Castaño-Vinyals et al., 2015) – if those who chose to participate varied from the source population in terms of sweetener use. Use of random-digit dialling to identify controls may result in a population that is not representative of the source population (Mayne et al., 2006; Chan et al., 2009). Cases were identified from population-based tumour registries (Mayne et al., 2006) or from patients admitted to specific hospitals or cancer centres (Norell et al., 1986; Gallus et al., 2007; Bosetti et al., 2009; Chan et al., 2009; Davis et al., 2023; Palomar-Cros et al., 2023) Artificial sweetener intake may have been enriched in the cases if diabetes status influenced hospital admission; however, diabetes status did not vary by case or control status in the study by Chan et al. (2009). In the hospital-based studies (Gallus et al., 2007; Bosetti et al., 2009; Davis et al., 2023), any potential associations between artificial sweetener use and the medical conditions of controls may lead to underestimation in associations with cancer if sweetener use (as a marker of diabetes or excess body weight, or other conditions) were higher in the controls (for example, non-traumatic orthopaedic conditions potentially related to excess body weight) than in the cases. The use of only tabletop sweeteners as potential sources of aspartame in the studies by Gallus et al. (2007), Bosetti et al. (2009), and Norell et al. (1986), may lead to misclassification of participants with regard to aspartame exposure. In the studies of Bosetti et al. (2009) and Gallus et al. (2007), diet soft drinks were not included, but the low frequency of consumption of diet soft drinks in middle-aged and elderly Italian participants at that time made their contribution less likely. Davis et al. (2023) examined only diet cola, which may have led to misclassification of exposure.]

Six meta-analyses of the association between ASBs and risk of cancers of the pancreas and other parts of the digestive tract were published (Jatho et al., 2021; Llaha et al., 2021; Tepler et al., 2021; Yin et al., 2022; and Pan et al., 2023). None reported a positive association with cancers of the digestive tract (excluding colorectal cancer).

One meta-analysis (Tepler et al., 2021) evaluated separately the associations between artificial sweeteners and risk of gastrointestinal luminal (i.e. organs with a lumen, an internal tubular channel, such as the intestine) and non-luminal (i.e. pancreatic) cancers. The study used the guidelines of the Cochrane Handbook (Higgins et al., 2023) and Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA), and evaluated study quality using the Newcastle–Ottawa Scale for quality and risk of bias assessment (all included studies were rated as "good"). The authors identified eight studies (four prospective, four case–control) of artificial sweetener consumption (exposed) or no consumption (non-exposed) in relation to gastrointestinal luminal or non-luminal cancers. The meta-analysis for non-luminal cancers included estimations only for pancreatic cancer, which was not associated with "artificial sweetener" consumption. This included three prospective studies and six case–control estimates (four estimates from the same case–control study). [The Working Group judged this meta-analysis to be uninformative because it included estimates for saccharin (Bosetti et al., 2009), which limited relevance for the agent under consideration, and it included four separate estimates of different and overlapping categories of ASB consumption from the same study (Chan et al., 2009) in the meta-analysis.]

Another meta-analysis (Jatho et al., 2021) that evaluated artificially sweetened soft drink consumption and gastrointestinal cancer included 38 studies (21 case–control and 17 cohort studies). [The Working Group judged this review to be uninformative for the evaluation of aspartame because all meta-analyses included estimates for regular soft drinks and combined results from prospective and case–control studies.]

A meta-analysis of sweetened beverages and cancer risk by Llaha et al. (2021) was conducted according to PRISMA guidelines. The only digestive cancer meta-analysed was pancreatic cancer (three cohort studies, two case–control studies). For high versus low intake of ASB, the relative risk was 1.07 (95% CI, 0.77–1.48). The I^2 (43.6) and $P = 0.13$ from the Cochran Q-test suggested between-study heterogeneity. In stratified analyses, estimates remained null by study type (RR for cohort studies, 1.05; 95% CI, 0.92–1.21; RR for case–control studies, 0.66; 95% CI, 0.37–1.17); by region [RR for USA, 1.08; 95% CI, 0.67–1.74; RR for Europe, 0.99; 95% CI, 0.61–1.60]; and by reference intake category (RR for high versus low, 0.93; 95% CI, 0.55–1.56; RR for high versus none, 1.27; 95% CI, 0.19–8.50). [The Working Group noted that not all studies included in the pancreatic cancer meta-analysis met the definition of the exposure in this monograph (one case–control study conducted in the 1970s assessed diet soda consumption before aspartame approval in the USA; Gold et al., 1985), limiting its informativeness.]

A dose–response meta-analysis of ASB consumption and cancer risk from prospective studies (Yin et al., 2022) used the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) approach to assess certainty of evidence. The only digestive cancer meta-analysed separately was pancreatic cancer (four cohorts, RR, 1.10; 95% CI, 0.92–1.31; $P = 0.307$; $I^2 = 0.0\%$), with no linear or nonlinear relation suggested. The four cohorts, including EPIC (Navarrete-Muñoz et al., 2016), NIH-AARP (Bao et al., 2008), and NHS and HPFS (Schernhammer et al., 2005), were assigned as having moderate risk of bias, with the most common reason being lack of direct measurement of exposure (e.g. questionnaires). [Measurement

error from use of an FFQ would be expected to bias associations towards the null.]

A meta-analysis by Pan et al. (2023) included three cohort studies (HPFS, NHS, and NIH-AARP) on pancreatic cancer and did not report an association (RR, 1.03; 95% CI, 0.96–1.10). The authors did not detect a linear dose–response relation between ASB intake per 250 mL and pancreatic cancer risk. [The meta-analysis did not include all available studies. The authors used GRADE criteria to rate the certainty of evidence for pancreatic cancer as "low" primarily due to observational design and wide confidence intervals.]

2.2 Cancers of the urinary tract

See Table 2.2.

Four cohort and four case–control studies investigating the role of artificial sweetener consumption and cancers of the urinary tract are presented below according to the main type of exposure under study, and separately for kidney cancer, bladder cancer, and lower urinary tract cancers combined. Most studies focused on overall consumption of ASBs without specific assessment of aspartame (Hodge et al., 2018; Heath et al., 2021; McCullough et al., 2022; Ringel et al., 2023), and only two studies considered duration of use (Nomura et al., 1991; Andreatta et al., 2008). One small, hospital-based case–control study in men (54 cases only) was conducted in 2002–2008 in Lebanon and examined frequency of use of artificial sweeteners and bladder cancer risk (Kobeissi et al., 2013). [The Working Group noted that the study was subject to several potential biases, including selection bias (only 23% of the eligible cases participated), unclear exposure assessment, and a very small number of cases (n = 54), and was therefore considered to be uninformative (results were null).]

[The main development in the design of the studies was the evolution from case–control studies to cohort studies. There was little change,

however, with regard to use of ASB consumption as a proxy for aspartame consumption (most studies), to more detailed assessments of surrogate definitions of aspartame use (i.e. ASB consumption, or consumption of sachets or tablets/week of artificial sweeteners) including duration of use, which was more common in the case–control studies (Nomura et al., 1991; Gallus et al., 2007; Andreatta et al., 2008).]

Four recent cohort studies, two from the USA (McCullough et al., 2022; Ringel et al., 2023), one from Australia (Hodge et al., 2018), and the other from Europe (Heath et al., 2021), provided information on ASB consumption and risk of kidney cancer. Both US studies (McCullough et al., 2022; Ringel et al., 2023) also assessed bladder cancer. Whereas McCullough et al. (2022) examined mortality only, Heath et al. (2021) presented results for both cancer mortality and incidence.

The cohort study from Australia, the MCCS, included 35 593 participants and 146 incident kidney cancer cases (Hodge et al., 2018). Baseline consumption of artificially sweetened soft drinks was assessed using FFQs. The results demonstrated lack of a positive association between consumption of artificially sweetened soft drinks and incident kidney cancer (linear model for increasing daily frequency of consumption, HR, 0.78; 95% CI, 0.40–1.55; P for trend, 0.48). [The Working Group noted that a weakness of the study was the small proportion of individuals consuming artificially sweetened soft drinks at least once daily (5.8%).]

The EPIC cohort study was conducted in 10 European countries and comprised 521 000 baseline participants and a mean follow-up of 15 years (for cancer incidence) and 16 years (for mortality) (Heath et al., 2021). Analyses on sugar-sweetened soft drinks and ASBs were restricted to 281 483 baseline participants with available information on types of soft drink, including 589 incident cases of renal cell carcinoma and 265 deaths from renal cell carcinoma (participants from Italy, Spain, and Umeå, Sweden

Table 2.2 Epidemiological studies on consumption of aspartame and cancers of the urinary tract

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Hodge et al. (2018) Australia Enrolment, 1990–1994/ follow-up until 30 June 2013 Cohort	35 593; MCCS – a prospective cohort study of men and women aged 40–69 yr at recruitment and free of cancer, angina, heart attack, or diabetes at baseline; participants with extreme baseline energy intake were excluded Exposure assessment method: self-administered 121-item FFQ with separate questions on frequency of consumption in the past year of diet (artificially sweetened) soft drinks	Kidney, incidence	Artificially sweetened soft drink consumption (HR): Never or < 1/mo 1–3/mo 1–6/wk ≥ 1/day Continuous (per beverage/ day) Trend-test *P* value: 0.48	114 11 12 9 146	1 0.71 (0.38–1.36) 0.66 (0.36–1.22) 0.92 (0.46–1.84) 0.78 (0.40–1.55)	Age, sex, socioeconomic index, country of birth, alcohol intake, smoking status, physical activity, Mediterranean diet score, sugar-sweetened soft drink consumption, waist circumference	*Exposure assessment critique*: Key strengths were that it was a prospective study, that assessment was after aspartame introduction in diet soft drinks in Australia (1987), and that first half of follow-up largely overlapped with period of aspartame use in Australia. Key limitations were the FFQ assessment with no specific estimate of aspartame exposure, ASB as a proxy, and exposure data at baseline only; small number of consumers. *Other strengths*: adjustment for key confounders, including a measure of obesity (waist circumference). *Other limitations*: Likely bias from non-differential exposure misclassification given single baseline assessment and long follow-up.

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Heath et al. (2021) 10 European countries Enrolment, 1991–2000/ follow-up, mean, 15 yr (incidence), 16 yr (mortality) Cohort	281 483; members of the EPIC prospective cohort study (> 520 000 participants aged 30–70 yr) with complete data (389 220) and excluding participants from Italy, Spain, and Umeå (Sweden). Exposure assessment method: questionnaire; exposure to ASB soft drinks overall assessed once at baseline between 1991 and 2000 through country-specific validated tools (mainly FFQ) covering the usual diet over the past year; no specific assessment of aspartame or AS content of ASB	Kidney (RCC), incidence	Artificially sweetened soft drink intake (HR): Continuous (per 100 g/day) Trend-test *P* value: 0.61	589	1.02 (0.96–1.08)	Sex, country, age, education, smoking status, alcohol consumption, physical activity, juice intake and sugar-sweetened soda intake, BMI, energy intake	*Exposure assessment critique*: A key strength was the prospective assessment of ASB consumption (several types of beverages) in several western Europe countries at a period relevant for aspartame exposure (between 1991 and 2000). Key limitations were that no other sources of aspartame were considered; uncertainty regarding aspartame content in ASBs in every country; and there was only one assessment at baseline. Other information: context of low ASB consumption in middle-aged adults. *Other strengths*: large cohort study; results adjusted for BMI; several relevant sensitivity analyses were considered. *Other limitations*: power was low in some sensitivity analyses.
		Kidney (RCC), incidence	Artificially sweetened soft drink intake (HR): Continuous (per 100 g/day) Trend-test *P* value: 0.59	589	1.02 (0.96–1.08)	Sex, country, age, education, smoking status, alcohol consumption, physical activity, juice intake and sugar-sweetened soda intake, BMI, energy intake, fruit and vegetable intake	
		Kidney (RCC), incidence	Artificially sweetened soft drink intake, excluding the first 2 yr of follow-up (HR): Continuous (per 100 g/day) Trend-test *P* value: 0.87	528	0.99 (0.93–1.06)	Sex, country, age, education, smoking status, alcohol consumption, physical activity, juice intake and sugar-sweetened soda intake, BMI, energy intake	

Table 2.2   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
[Heath et al. (2021)](#) (cont.)		Kidney (RCC), incidence	Artificially sweetened soft drink intake, excluding participants with self-reported diabetes at baseline (HR):			Sex, country, age, education, smoking status, alcohol consumption, physical activity, juice intake and sugar-sweetened soda intake, BMI, energy intake	
			Continuous (per 100 g/day)	562	1.02 (0.96–1.09)		
			Trend-test *P* value: 0.46				
		Kidney (RCC), incidence	Artificially sweetened soft drink intake, men (HR):			Country, age, education, smoking status, alcohol consumption, physical activity, juice intake and sugar-sweetened soda intake, BMI, energy intake	
			Continuous (per 100 g/day)	347	0.99 (0.91–1.08)		
			Trend-test *P* value: 0.85				
		Kidney (RCC), incidence	Artificially sweetened soft drink intake, women (HR):				
			Continuous (per 100 g/day)	242	1.05 (0.96–1.14)		
			Trend-test *P* value: 0.28				
		Kidney (RCC), mortality	Artificially sweetened soft drink intake (HR):			Sex, country, age, education, smoking status, alcohol consumption, physical activity, juice intake and sugar-sweetened soda intake, BMI, energy intake	
			Continuous (per 100 g/day)	265	1.06 (0.99–1.14)		
			Trend-test *P* value: 0.11				

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Heath et al. (2021) (cont.)		Kidney (RCC), mortality	Artificially sweetened soft drink intake (HR):			Sex, country, age, education, smoking status, alcohol consumption, physical activity, juice intake and sugar-sweetened soda intake, BMI, energy intake, fruit and vegetable intake	
			Continuous (per 100 g/day)	265	1.06 (0.99–1.14)		
			Trend-test *P* value: 0.10				
		Kidney (RCC), mortality	Artificially sweetened soft drink intake, excluding the first 2 yr of follow-up (HR):			Sex, country, age, education, smoking status, alcohol consumption, physical activity, juice intake and sugar-sweetened soda intake, BMI, energy intake	
			Continuous (per 100 g/day)	256	1.05 (0.97–1.13)		
			Trend-test *P* value: 0.26				
		Kidney (RCC), mortality	Artificially sweetened soft drink intake, excluding participants with self-reported diabetes at baseline (HR):				
			Continuous (per 100 g/day)	256	1.06 (0.99–1.15)		
			Trend-test *P* value: 0.10				
		Kidney (RCC), mortality	Artificially sweetened soft drink intake, men (HR):			Country, age, education, smoking status, alcohol consumption, physical activity, juice intake and sugar-sweetened soda intake, BMI, energy intake	
			Continuous (per 100 g/day)	142	1.04 (0.93–1.15)		
			Trend-test *P* value: 0.49				

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Heath et al. (2021) (cont.)		Kidney (RCC), mortality	Artificially sweetened soft drink intake, women (HR):			Country, age, education, smoking status, alcohol consumption, physical activity, juice intake and sugar-sweetened soda intake, BMI, energy intake	
			Continuous (per 100 g/day)	123	1.08 (0.98–1.19)		
			Trend-test *P* value: 0.11				
		Kidney (RCC), incidence	Artificially sweetened soft drink intake (HR):			Sex, country, age, education, smoking status, alcohol consumption, physical activity, juice intake and sugar-sweetened soda intake, BMI, energy intake	
			0 g/day	NR	1		
			50 g/day	NR	1.18 (0.99–1.41)		
			100 g/day	NR	1.28 (0.98–1.66)		
			200 g/day	NR	1.29 (0.98–1.69)		
			300 g/day	NR	1.25 (0.95–1.63)		
			400 g/day	NR	1.21 (0.91–1.61)		
		Kidney (RCC), mortality	Artificially sweetened soft drink intake (HR):				
			0 g/day	NR	1		
			50 g/day	NR	1.13 (0.87–1.46)		
			100 g/day	NR	1.21 (0.82–1.78)		
			200 g/day	NR	1.28 (0.85–1.93)		
			300 g/day	NR	1.33 (0.90–1.96)		
			400 g/day	NR	1.38 (0.93–2.05)		

Table 2.2   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level		Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2022) USA Enrolment, 1982/follow-up, through 2016 (median, 27.7 yr) Cohort	934 777 (416 313 men, 518 464 women); CPS-II prospective cohort; adults aged ≥ 28 yr; excluded participants with personal history at baseline of diabetes or cancer other than nonmelanoma skin cancer, men aged > 90 yr or women aged > 95 yr at enrolment, and those reporting only prior but not current consumption of either SSBs or ASBs Exposure assessment method: questionnaire; exposure to ASB assessed in 1982 through a question about the number of drinks/day of diet soda or ice teas (one pooled item) and potential changes over the past 10 yr; no specific assessment of aspartame content in ASB	Kidney, mortality	ASB consumption (HR):				Age, sex, race/ ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption	*Exposure assessment critique*: A key strength was the prospective assessment of ASB consumption. Key limitations included a single dietary assessment at baseline in 1982 that was before the use of aspartame in ASBs, hence the relevance regarding aspartame exposure depends on the stability of ASB consumption over up to 34 yr of follow-up, but such information was not directly available; no other sources were considered (although these were more limited); and uncertainty regarding aspartame content in ASBs after the mid-2000s. Other comments: exclusion of participants who reported only prior but not current consumption of either SSBs or ASBs at baseline. *Other strengths*: large cohort with long follow-up; ability to examine multiple cancer types, stratify by sex or BMI, and limit to never-smokers; comprehensive adjustment for confounders, including SSB consumption. *Other limitations*: likely bias from non-differential exposure misclassification.
			Never	NR		1		
			< 1 drink/ day	NR		0.98 (0.87–1.11)		
			1 drink/day	NR		1.08 (0.94–1.24)		
			≥ 2 drinks/ day	NR		1.04 (0.90–1.20)		
			Continuous (per drink/ day)	3129		1.01 (0.97–1.05)		
			Trend-test *P* value: 0.412					
		Kidney, mortality	ASB consumption, BMI adjusted (HR):				Age, sex, race/ ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR		1		
			< 1 drink/ day	NR		0.92 (0.82–1.04)		
			1 drink/day	NR		1.01 (0.88–1.16)		
			≥ 2 drinks/ day	NR		0.95 (0.82–1.10)		
			Continuous (per drink/ day)	3129		0.99 (0.95–1.03)		
			Trend-test *P* value: 0.468					

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2022) (cont.)		Urinary bladder, mortality	ASB consumption (HR):			Age, sex, race/ ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption	
			Never	NR	1		
			< 1 drink/ day	NR	0.94 (0.83–1.06)		
			1 drink/day	NR	0.99 (0.86–1.14)		
			≥ 2 drinks/ day	NR	1.04 (0.90–1.20)		
			Continuous (per drink/ day)	3419	1.00 (0.95–1.04)		
			Trend-test *P* value: 0.794				
		Urinary bladder, mortality	ASB consumption, BMI adjusted (HR):			Age, sex, race/ ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR	1		
			< 1 drink/ day	NR	0.93 (0.82–1.05)		
			1 drink/day	NR	0.98 (0.85–1.13)		
			≥ 2 drinks/ day	NR	1.02 (0.88–1.18)		
			Continuous (per drink/ day)	3419	0.99 (0.95–1.03)		
			Trend-test *P* value: 0.922				

Table 2.2   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Ringel et al. (2023) USA Enrolment, 1993–1998/ follow-up, until 28 February 2020 Cohort	80 388; Women's Health Initiative Observational Study participants (aged 50–79 yr at enrolment) who completed a questionnaire regarding ASBs and did not self-report a prior diagnosis of urinary tract cancer Exposure assessment method: questionnaire; exposure to ASB assessed in 1996–2001 through nine frequencies of consumption of diet drinks; no specific assessment of aspartame content in ASB	Kidney (RCC), incidence	ASB consumption (HR):			Age, race, ethnicity, neighbourhood SES (used as a proxy for the likelihood of environmental exposures), smoking, BMI, history of hypertension, and diet quality	*Exposure assessment critique*: A key strength was the prospective assessment of ASB consumption at a relevant period for aspartame exposure from ASBs (the USA between 1996 and 2001). Key limitations were that no other sources were considered (although these were more limited); there was only one dietary assessment at baseline and no consideration of potential variations of ASB consumption over up to 24 yr of follow-up); and uncertainty regarding aspartame content in ASBs after the mid-2000s. *Other strengths*: prospective large cohort study, detailed outcome assessment, long follow-up period. *Other limitations*: potential for residual confounding.
			Never or < 1 serving/ wk	188	1		
			1–6 servings/wk	91	1.34 (1.03–1.75)		
			≥ 1 serving/ day	48	1.14 (0.80–1.62)		
		Urinary bladder, incidence	ASB consumption (HR):			Age, race, ethnicity, neighbourhood SES (used as a proxy for the likelihood of environmental exposures), smoking, water consumption	
			Never or < 1 serving/ wk	295	1		
			1–6 servings/wk	106	0.99 (0.78–1.26)		
			≥ 1 serving/ day	47	0.75 (0.53–1.06)		

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Nomura et al. (1991) USA, Hawaii (Oahu) 1977–1986 Case–control	Cases: 261; population-based, identified at seven largest community hospitals on Oahu that capture 96% of diagnosed cases (235 urinary bladder, 19 renal pelvis, 7 ureter) Controls: 522; population-based, identified from state survey; matched to cases by age (within 5 yr), sex, ethnic group (Caucasian [White] or Japanese), residence on Oahu; 1:2 ratio Exposure assessment method: questionnaire; diet history of 29 food items consumed during a usual week, usual diet 1 yr before diagnosis. assessing ASBs, such as diet or low-calorie sodas	Lower urinary tract (urinary bladder, renal pelvis, ureter), incidence	Use of ASs from diet beverages, men (OR): Non-users / Users	151 / 44	1 / 1.4 (0.9–2.2)	Age, current residence on Oahu, ethnicity, pack-years of cigarette smoking	*Exposure assessment critique*: Key limitations were the retrospective recall; that there was no specific estimate of aspartame exposure, and ASBs as a proxy only (diet or low-calorie sodas). Limited value as exposure was collected in 1977–1986, but study participants could not have consumed aspartame before it was approved in 1983. *Other strengths*: captured almost all cases in catchment area (Oahu). *Other limitations*: adjustment only for pack-years of cigarette smoking (but used conditional logistic regression to account for matching factors);potential for bias from differential misclassification, as interviewers were not blinded to case–control status.
		Lower urinary tract (urinary bladder, renal pelvis, ureter), incidence	Use of ASs from diet beverages, men (OR): Non-users 1–2 can-years 3+ can-years Trend-test *P* value: 0.29	151 25 19	1 1.5 (0.8–2.6) 1.3 (0.7–2.5)		
		Lower urinary tract (urinary bladder, renal pelvis, ureter), incidence	Use of ASs from diet beverages, women (OR): Non-users / Users	52 / 14	1 / 1.2 (0.5–2.7)		
		Lower urinary tract (urinary bladder, renal pelvis, ureter), incidence	Use of ASs from diet beverages, women (OR): Non-users 1–2 can-years 3+ can-years Trend-test *P* value: 0.31	52 10 4	1 2.0 (0.8–5.2) 0.4 (0.1–1.7)		

Table 2.2   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Gallus et al. (2007) Italy 1991–2004 Case–control	Cases: 767; multiple hospital-based case–control studies Controls: 1534; patients admitted to the same hospitals for acute, non-neoplastic disorders Exposure assessment method: questionnaire; assessment of use of tabletop sweeteners containing either saccharin or other sweeteners as sachets or tablets per week in the 2 yr before cancer diagnosis	Kidney (RCC), incidence	Consumption of ASs other than saccharin (OR):			Age, sex, study centre, education, alcohol use, tobacco smoking, BMI, total energy intake, consumption of hot beverages	*Exposure assessment critique*: Key limitations were that there was no specific assessment of aspartame, aspartame was considered as "other sweeteners" but with unclear actual contribution; only one source considered (tabletop sweeteners); retrospective assessment in a case–control study (potential for differential misclassification). Other information: according to the authors, there was low consumption of sources of sweeteners (including ASBs) in the study population, middle-aged adults in Italy between 1991 and 2004). *Other strengths*: high response rates (< 5% refusals) for cases and controls reduce the potential for selection bias; large sample size with large case numbers for rarer cancers; control for key potential confounders, including BMI. *Other limitations*: potential for recall bias and reverse causation; low statistical power.
			Non-consumers	710	1		
			> 0 sachets or tablets/ day	57	1.03 (0.73–1.46)		

Table 2.2   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Andreatta et al. (2008) Argentina 1999–2006 Case–control	Cases: 197; incident cases of histologically confirmed urinary tract tumours of transitional-cell types of the renal pelvis, ureter, and/or urinary bladder admitted to 10 public and private hospitals in the greater Cordoba region Controls: 397; with acute, non-neoplastic, and non-urinary tract diseases, selected from the respective hospital's admission registry at the same time as the case diagnosis Exposure assessment method: questionnaire; assessment of ever use and duration of use of AS in infusions (tea, coffee, mate) in Argentina between 1999 and 2006 (5 yr before cancer diagnosis)	Urinary tract (urinary bladder, renal pelvis, ureter), incidence	Duration of AS consumption (OR): Never Short-term (1–9 yr) Long-term (≥ 10 yr) Trend-test P value: < 0.02	146 21 30	1 1.10 (0.61–2.00) 2.18 (1.22–3.89)	Age, sex, BMI, social status, years of tobacco use	*Exposure assessment critique*: Key limitations were that there was no specific assessment of aspartame; only one source of exposure was considered (ASs added to infusions) and limited use of aspartame; no quantitative assessment (ever use and duration of use); retrospective assessment in a case–control study (potential for differential misclassification). *Other strengths*: both men and women enrolled; large catchment area in Argentina. *Other limitations*: AS varieties were classified into two subgroups (according to prevalence of sale at time of assessment period): saccharin/ cyclamate and aspartame/ acesulfame-K; results on duration of use were mainly driven by saccharin/cyclamate users and may not reflect exposure to aspartame.

AS, artificial sweetener; ASB, artificially sweetened beverage; BMI, body mass index; CI, confidence interval; CPS-II, Cancer Prevention Study II; EPIC, European Prospective Investigation into Cancer and Nutrition; FDA, Food and Drug Administration; FFQ, food frequency questionnaire; HR, hazard ratio; MCCS, Melbourne Collaborative Cohort Study; mo, month(s); NR, not reported; OR, odds ratio; RCC, renal cell carcinoma; SES, socioeconomic status; SSB, sugar-sweetened beverage; vs, versus; wk, week(s); yr, year(s).

were excluded). Statistical analyses were adjusted for a large number of potential confounders (age, sex, country, educational attainment, smoking status, alcohol consumption, physical activity, juice intake, sugar-sweetened soft drink consumption) and additionally for energy intake and BMI. Consumption of artificially sweetened soft drinks was not associated with the risk of either being diagnosed with renal cell carcinoma (HR, 1.02; 95% CI, 0.96–1.08) or dying from renal cell carcinoma (HR per 100 g/day, 1.06; 95% CI, 0.99–1.14). Analyses using restricted cubic splines that evaluated artificially sweetened soft drink consumption of 400 g/day versus 0 g/day did not suggest an elevated risk of renal cell carcinoma incidence (HR, 1.21; 95% CI, 0.91–1.61) or renal cell carcinoma mortality (HR, 1.38; 95% CI, 0.93–2.05). [The Working Group noted that power was low in some of the sensitivity analyses. Noted strengths were that sensitivity analyses were conducted, additionally adjusting for fruit and vegetable consumption, excluding the first 2 years of follow-up, and excluding participants with self-reported diabetes at baseline; none of these produced different results. The Working Group further noted that adjustment for BMI attenuated the findings, leaving the confidence intervals in the cubic spline graph (in the original publication, supplementary Fig. 2, bottom right) overlapping with 1. No distinction was made between aspartame and other types of artificial sweetener in ASBs, probably increasing non-differential exposure misclassification and leading to bias towards the null.]

A cohort study using data from the CPS-II in the USA included 934 777 participants and investigated the association between ASB consumption, measured at baseline via an FFQ, and subsequent cancer risk (McCullough et al., 2022). During follow-up (median, 27.7 years), 3129 kidney cancer deaths and 3419 bladder cancer deaths were recorded. Overall, the study found no association between ASB consumption and kidney cancer deaths. Results of

multivariable-adjusted analyses (including age, sex, race/ethnicity, smoking, marital status, education, consumption of red and processed meat, fruit and vegetables, alcohol, sugar-sweetened beverage consumption) were presented without adjustment for BMI (RR for ≥ 2 drinks/day versus never, 1.04; 95% CI, 0.90–1.20) and with further adjustment for BMI (RR for ≥ 2 drinks/day versus never, 0.95; 95% CI, 0.82–1.10). Similar to the results for kidney cancer, no association was found between ASB consumption and risk of bladder cancer (HR for ≥ 2 ASBs/day versus never, 1.02; 95% CI, 0.88–1.18). [With only a single baseline assessment of exposure in 1982, 1 year before the approval of aspartame use in beverages, and a median follow-up of 27.7 years, the study had the potential for misclassification, which was likely to be non-differential and bias risk estimates towards the null. Although published data from more than 100 000 participants included in both the CPS-II and the CPS-II nutrition cohort (McCullough et al., 2014) suggested some decrease in ASB consumption over the 17 years between 1982 and 1999 in this population, the relative ranking of intake category was consistent during this period.]

The Women's Health Initiative Observational Study (WHI-OS) accrued a total of 804 cancers of the urinary tract among 80 388 eligible women in the USA (Ringel et al., 2023). Consumption of ASBs was assessed once, 3 years after enrolment, which took place between 1993 and 1998. Compared with no or < 1 serving/week, no increased risk for urinary bladder cancer (total of 448 cases) was noted for higher consumption of ASBs (HR for 1–6 servings/week, 0.99; 95% CI, 0.78–1.26; and HR for ≥ 1 serving/day, 0.75; 95% CI, 0.53–1.06), whereas for kidney cancer (total of 327 cases), an increased risk was observed in the 1–6 servings/week group (HR, 1.34; 95% CI, 1.03–1.75), but not in the ≥ 1 serving/day group (HR, 1.14; 95% CI, 0.80–1.62). [The Working Group noted as weaknesses of the study the one-time assessment of beverage consumption,

potential for residual confounding, and measurement error in the exposure assessment, which probably biased estimates towards the null. Strengths pertained to its large size and long follow-up.]

A case–control study conducted in Hawaii, USA, by Nomura et al. (1991) investigated the association between diet beverages and cancers of the lower urinary tract. In men, the odds ratio for any diet beverage consumption was 1.4 (95% CI, 0.9–2.2; 44 cases), compared with non-consumers, and an exposure–response relation (cumulative use) was not observed (*P* for trend, 0.29). Similarly, in women, an odds ratio of 1.2 (95% CI, 0.5–2.7; *P* for trend, 0.31; 14 cases) was reported when consumers were compared with non-consumers. [The Working Group noted that the time frame of this study (which included cancers diagnosed between 1977 and 1987) was of questionable relevance for aspartame exposure, considering that aspartame use in ASBs was authorized in the USA in 1983. Besides the retrospective assessment of diet after diagnosis, interviewers were not blinded to case–control status, which increased the potential for differential exposure misclassification.]

One hospital-based case–control study was conducted in 1991–2004 in four areas in Italy and combined cases of cancer at multiple organ sites with a selection of controls from the same overall pool of controls (Gallus et al., 2007). The study examined weekly consumption of saccharin and sweeteners other than saccharin, expressed in sachets or tablets/week. The authors stated that the consumption of "sweeteners other than saccharin" mainly comprised aspartame consumption. The study included 767 cases of kidney cancer and 1534 controls. After adjustment for a large number of potential confounders (including sex, age, alcohol, tobacco, BMI, energy intake, and hot beverages), there was no association between exposure to artificial sweeteners other than saccharin (> 0 sachets or tablets/day) and renal cell carcinoma risk (OR, 1.03;

95% CI, 0.73–1.46). Results were not presented separately by sex. [The Working Group noted as key limitations the uncertainty surrounding the authors' assumption with regard to aspartame intake, defined as "other sweeteners" (not distinguishing between aspartame and other artificial sweeteners other than saccharin); low power; and the low consumption of sweeteners in this Italian population. These limitations affected precision and probably introduced bias towards the null. A strength of this case–control study was the relatively high participation rates: fewer than 5% of both cases and controls who were contacted refused to participate.]

One case–control study, conducted in 1999–2006 in Argentina, examined the association between several definitions of aspartame use and (lower) urinary tract tumours (Andreatta et al., 2008). Compared with non-users, long-term (≥ 10 years) artificial sweetener consumption referring exclusively to use as an additive in infusions (tea, coffee, mate), without consideration of consumption from other sources such as soft drinks or dietetic foods, was associated with an increased risk of urinary tract tumours (OR, 2.18; 95% CI, 1.22–3.89). [The Working Group noted that the assessment of artificial sweetener consumption distinguished between saccharin/cyclamate and aspartame/acesulfame-K, but most artificial sweetener consumers were saccharin/cyclamate users, with 40 cases occurring in the saccharin/cyclamate group, and only 11 cases in the aspartame/acesulfame-K group. Results on duration of use, which did not distinguish between saccharin/cyclamate and aspartame/acesulfame-K, were therefore mainly driven by saccharin/cyclamate users and the results may not reflect exposure to aspartame.]

One meta-analysis on non-sugar sweeteners and health outcomes, which summarized eight studies (one of which was included twice) with a variety of types of non-sugar sweetener including but not limited to aspartame, reported a combined estimate (odds ratio) for bladder

cancer risk of 1.03 (95% CI, 0.84–1.25) ([Toews et al., 2019](#)). [The Working Group considered this meta-analysis to be uninformative, because the most recent study included was from 1994 ([Momas et al., 1994)](#), and the exposure assessment was unclear for the inclusion of any type of non-sugar sweetener.]

2.3 Cancers of the breast and prostate

See [Table 2.3](#).

Six cohort studies ([Hodge et al., 2018](#); [Malik et al., 2019](#); [Mullee et al., 2019](#); [Romanos-Nanclares et al., 2021](#); [Debras et al., 2022b](#); [McCullough et al., 2022](#)) investigating the association between consumption of aspartame and artificial sweeteners primarily containing aspartame and cancer of the breast are presented below according to main type of exposure under study. Results are presented separately for post- and premenopausal versus overall breast cancer. Five of these cohort studies also reported on prostate cancer outcomes ([Hodge et al., 2018](#); [Malik et al., 2019](#); [Mullee et al., 2019](#); [Debras et al., 2022b](#); [McCullough et al., 2022](#)). Whereas [McCullough et al. (2022)](#), [Malik et al. (2019)](#), and [Mullee et al. (2019)](#) examined mortality only, all other studies reported on breast or prostate cancer incidence.

Most studies focused on overall consumption of ASBs without separately assessing aspartame consumption. In addition to the cohort studies, three case–control studies on breast cancer ([Ewertz and Gill, 1990](#); [Gallus et al., 2007](#); [Palomar-Cros et al., 2023](#)) and two on prostate cancer ([Gallus et al., 2007](#); [Palomar-Cros et al., 2023](#)) are described below.

[The main development in the design of the studies was the evolution from case–control studies to cohort studies. There has been little advancement, however, on the exposure assessment of aspartame intake, since most studies evaluated consumption of ASBs.]

The Australian MCCS cohort study included 946 incident postmenopausal breast cancer cases among 21 492 women and 433 aggressive prostate cancer cases among 14 101 men ([Hodge et al., 2018](#)). The study had up to 23 years of follow-up from 1990–1994 until 30 June 2013. Consumption of artificially sweetened soft drinks was classified into four categories, from < 1 drink/month to ≥ 1 drink/day. Overall, the study reported no association between consumption of artificially sweetened soft drinks and breast cancer risk (HR for ≥ 1 drink/day compared with < 1 drink/month, 0.95; 95% CI, 0.73–1.25). There was no association between consumption of artificially sweetened soft drinks and prostate cancer risk (HR for ≥ 1 drink/day compared with < 1 drink/month, 0.81; 95% CI, 0.49–1.33). [The Working Group noted that a potential limitation of this study was that a very large proportion of the study population reported no consumption of artificially sweetened soft drinks or < 1 drink/month (74.9%), and only 5.8% reported consuming artificially sweetened soft drinks at least daily. The single baseline assessment of beverage consumption, given the long follow-up, was likely to have introduced bias towards the null. The Working Group also noted that the same cohort was used to examine the association between consumption of artificially sweetened soft drinks and premenopausal breast cancer risk (181 cases) ([Bassett et al., 2020](#)). The results were presented combined for all "non-obesity-related cancers", but not for premenopausal breast cancer separately, and the study was therefore not informative for the evaluation of breast cancer risk.]

[Malik et al. (2019)](#) reported results from the NHS cohort, which included 121 700 women aged 30–55 years at study entry (1976), and found no association between consumption of ASBs and breast cancer mortality (HR for ≥ 2 servings/day versus < 1 serving/month, 1.14; 95% CI, 0.92–1.40). Additionally, [Malik et al. (2019)](#) reported results from the prospective HPFS cohort, which comprised 37 716 men

Table 2.3 Epidemiological studies on consumption of aspartame and cancers of the breast and prostate

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Hodge et al. (2018) Australia Enrolment, 1990–1994/ follow-up until 30 June 2013 Cohort	35 593 (14 101 men, 21 492 women); MCCS – a prospective cohort study of men and women aged 40–69 yr at recruitment and free of cancer, angina, heart attack, or diabetes at baseline; participants with extreme baseline energy intake were excluded Exposure assessment method: self-administered 121-item FFQ with separate questions on frequency of consumption in the past year of diet (artificially sweetened) soft drinks	Breast (postmenopausal, invasive only), incidence	Artificially sweetened soft drink consumption (HR):			Age, socioeconomic index, country of birth, alcohol intake, smoking status, physical activity, Mediterranean diet score, sugar-sweetened soft drink consumption, waist circumference	*Exposure assessment critique*: Key strengths were that it was a prospective study, that assessment was after aspartame introduction in diet soft drinks in Australia (1987), and that first half of follow-up largely overlapped with period of aspartame use in Australia. Key limitations were the FFQ assessment with no specific estimate of aspartame exposure, ASBs as a proxy, and exposure data at baseline only; small number of consumers. *Other strengths*: adjustment for key confounders, including a measure of obesity (waist circumference). *Other limitations*: Likely bias from non-differential exposure misclassification given single baseline assessment and long follow-up.
			Never or < 1/mo	716	1		
			1–3/mo	69	0.94 (0.73–1.22)		
			1–6/wk	101	0.90 (0.72–1.12)		
			≥ 1/day	60	0.95 (0.73–1.25)		
			Continuous (per beverage/day)	946	0.92 (0.71–1.18)		
			Trend-test *P* value: 0.51				
		Prostate (aggressive/ advanced), incidence	Artificially sweetened soft drink consumption (HR):				
			Never or < 1/mo	333	1		
			1–3/mo	33	0.94 (0.65–1.36)		
			1–6/wk	50	1.09 (0.80–1.48)		
			≥ 1/day	17	0.81 (0.49–1.33)		
			Continuous (per beverage/day)	433	0.91 (0.60–1.38)		
			Trend-test *P* value: 0.66				

Table 2.3 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Malik et al. (2019) USA Enrolment, 1976 (NHS), 1986 (HPFS/follow-up, 1980–2014 (NHS), 1986–2014 (HPFS) Cohort	37 716 men and 80 647 women; female registered nurses aged 30–55 yr in the NHS and male health professionals aged 40–75 yr in the HPFS; excluding those with history of diabetes, cardiovascular disease, or cancer at baseline, or with implausible dietary intake Exposure assessment method: prospective assessment of ASB consumption through repeated FFQs between 1980–1986 and 2010	Breast, mortality	ASB intake, women (NHS) (HR):			Age, race, smoking, alcohol intake, postmenopausal hormone use, physical activity, family history of diabetes; family history of myocardial infarction, family history of cancer, multivitamin use, aspirin use, baseline history of hypertension and hypercholesterolemia, intake of whole grains, fruit, vegetables, or red and processed meat, total energy, BMI, SSB intake	*Exposure assessment critique*: A key strength was the prospective assessment of ASB consumption from repeated, validated diet assessments every 4 yr, the majority at a very relevant period for aspartame exposure from ASBs (the USA between the 1980s and 2010) potentially capturing lifetime exposure to aspartame. Key limitations were that other sources of aspartame were not considered (although these were more limited); and uncertainty regarding aspartame content in ASBs after the mid-2000s. *Other strengths*: large cohort with long follow-up. *Other limitations*: likely bias from non-differential misclassification of exposure to aspartame; stratified numbers of deaths not provided for specific cancer sites.
			< 1 serving/mo	NR	1		
			1–4 servings/mo	NR	1.06 (0.91–1.23)		
			2–6 servings/wk	NR	0.90 (0.78–1.05)		
			1 to < 2 servings/ day	NR	0.92 (0.76–1.13)		
			≥ 2 servings/day	NR	1.14 (0.92–1.40)		
			Continuous (per serving/day)	NR	1.01 (0.95–1.08)		
			Trend-test *P* value: 0.57				

Table 2.3 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Malik et al. (2019) (cont.)		Prostate, mortality	ASB intake, men (HPFS) (HR):			Age, race, smoking, alcohol intake, physical activity, family history of diabetes; family history of myocardial infarction, family history of cancer, multivitamin use, aspirin use, baseline history of hypertension and hypercholesterolemia, intake of whole grains, fruit, vegetables, or red and processed meat, total energy, BMI, SSB intake	
			< 1 serving/mo	NR	1		
			1–4 servings/mo	NR	0.80 (0.61–1.06)		
			2–6 servings/wk	NR	1.02 (0.84–1.25)		
			1 to < 2 servings/ day	NR	0.93 (0.66–1.32)		
			≥ 2 servings/day	NR	1.01 (0.67–1.52)		
			Continuous (per serving/day)	NR	1.02 (0.91–1.14)		
			Trend-test P value: 0.92				

Table 2.3 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Mullee et al. (2019) Europe Enrolment, 1992–2000/ follow-up, through 2009– 2013 (depending on study centre; mean, 16.4 yr) Cohort	451 743 (321 081 women, 130 662 men); EPIC cohort study participants from 10 European countries (Denmark, France, Greece, Germany, Italy, Netherlands, Norway, Spain, Sweden, and the United Kingdom); men and women excluding those with prevalent cancer, heart disease, stroke, or diabetes or with implausible dietary intake; for artificially sweetened soft drinks, participants from Italy, Spain, and Sweden were not included; 1402 breast cancer deaths; 907 prostate cancer deaths	Breast, mortality	Consumption of artificially sweetened soft drink (glass, 250 mL) (HR): < 1 glass/mo 1–4 glasses/mo > 1 to 6 glasses/ wk ≥ 1 glass/day Trend-test *P* value: 0.38	NR NR NR NR	1 0.79 (0.63–0.98) 0.90 (0.74–1.10) 0.85 (0.59–1.22)	Age, centre, BMI, physical activity index, educational status, alcohol consumption, smoking status, smoking intensity, smoking duration, ever use of contraceptive pill, menopausal status, ever use of menopausal hormone therapy, intakes of total energy, red and processed meat, fruits and vegetables, coffee, fruit and vegetable juice, sugar-sweetened soft drinks	*Exposure assessment critique*: A key strength was the prospective assessment of ASB consumption (several types of beverage) in several western Europe countries at a period relevant for aspartame exposure (between 1991 and 2000). Key limitations were that no other sources of aspartame were considered; uncertainty regarding aspartame content in ASBs in every country; and there was only one assessment at baseline. Other information: context of low ASB consumption in middle-aged adults. Other comments: in this study, total soft drink consumption (regardless of type of sweetener) was associated with higher (overall) mortality.
		Prostate, mortality	Consumption of artificially sweetened soft drink (glass, 250 mL) (HR): < 1 glass/mo 1–4 glasses/mo > 1 to 6 glasses/ wk ≥ 1 glass/day Trend-test *P* value: 0.53	NR NR NR NR	1 1.23 (0.95–1.60) 1.36 (1.05–1.78) 1.05 (0.64–1.75)	Age, centre, BMI, physical activity index, educational status, alcohol consumption, smoking status, smoking intensity, smoking duration, intakes of total energy, red and processed meat, fruits and vegetables, coffee, fruit and vegetable juice, sugar-sweetened soft drinks	

Table 2.3   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Mullee et al. (2019) (cont.)	Exposure assessment method: questionnaire; exposure to artificially sweetened soft drinks overall assessed once at baseline through country-specific validated tools (mainly FFQ) covering the usual diet over the past year; no specific assessment of aspartame or AS content of the artificially sweetened soft drinks						*Other strengths*: population-based cohort spanning multiple countries with different behaviours; large number of cases; results adjusted for appropriate potential confounders, including BMI.

Table 2.3 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Romanos-Nanclares et al. (2021) USA Enrolment, 1976 (NHS), 1989 (NHS-II)/follow-up, 1980–2016 (NHS), 1991–2017 (NHS-II) Cohort	82 713 (NHS) and 93 085 (NHS-II); NHS and NHS-II participants; female nurses aged 30–55 yr (NHS) or 25–42 yr (NHS-II) at enrolment; excluding women with prevalent cancer or implausible total energy intake Exposure assessment method: prospective and repeated FFQs assessing ASB consumption between 1980–1991 and 2016–2017	Breast, incidence	Cumulative average intake of ASBs (HR): < 1/mo ≥ 1 to ≤ 4/mo > 1 to < 7/wk ≥ 1/day Continuous (per 1 serving/day) Trend-test *P* value: 0.08	3177 1531 4197 2474 11 379	1 1.01 (0.95–1.07) 0.98 (0.94–1.03) 0.96 (0.91–1.02) 0.99 (0.97–1.01)	Age, calendar year, SSB intake, race, age at menarche, age at menopause, postmenopausal hormone use, oral contraceptive use, parity and age at first birth, breastfeeding history, family history of breast cancer, history of benign breast disease, height, cumulatively updated alcohol and total caloric intake, physical activity, BMI at age 18 yr, modified AHEI score (with SSBs and alcohol removed), SES, change in weight since age 18 yr, cohort	*Exposure assessment critique*: A key strength was the prospective assessment of ASB consumption as a cumulative average from repeated, validated diet assessments every 4 yr, the majority at a very relevant period for aspartame exposure from ASBs, potentially capturing lifetime exposure to aspartame. Key limitations were that other sources of aspartame were not considered although these were more limited); and uncertainty regarding aspartame content in ASBs after the mid-2000s.

Table 2.3 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Romanos-Nanclares et al. (2021) (cont.)		Breast, incidence	Cumulative average intake of ASBs, NHS (HR):			Age, calendar year, SSB intake, race, age at menarche, age at menopause, postmenopausal hormone use, oral contraceptive use, parity and age at first birth, breastfeeding history, family history of breast cancer, history of benign breast disease, height, cumulatively updated alcohol and total caloric intake, physical activity, BMI at age 18 yr, modified AHEI score (with SSBs and alcohol removed), SES, change in weight since age 18 yr	*Other strengths*: long-running, large cohort studies, complete assessment and control for potential confounders, including weight changes. *Other limitations*: relative lack of statistical power in certain substrata; potential for bias from non-differential exposure misclassification.
			< 1/mo	2242	1		
			≥ 1 to ≤ 4/mo	1123	1.00 (0.93–1.08)		
			> 1 to < 7/wk	2870	0.97 (0.92–1.03)		
			≥ 1/day	1260	0.99 (0.92–1.07)		
			Continuous (per serving/day)	7495	1.00 (0.97–1.04)		
			Trend-test *P* value: 0.87				

Table 2.3   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Romanos-Nanclares et al. (2021) (cont.)		Breast, incidence	Cumulative average intake of ASBs, NHS-II, without adjustment for change in weight since age 18 yr (HR):			Age, calendar year, SSB intake, race, age at menarche, age at menopause, postmenopausal hormone use, oral contraceptive use, parity and age at first birth, breastfeeding history, family history of breast cancer, history of benign breast disease, height, cumulatively updated alcohol and total caloric intake, physical activity, BMI at age 18 yr, modified AHEI score (with SSBs and alcohol removed), SES	
			< 1/mo	935	1		
			≥ 1 to ≤ 4/mo	408	1.03 (0.91–1.16)		
			> 1 to < 7/wk	1327	0.99 (0.91–1.08)		
			≥ 1/day	1214	0.93 (0.84–1.01)		
			Continuous (per 1 serving/day)	3884	0.98 (0.95–1.01)		
			Trend-test P value: 0.04				

Table 2.3 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Romanos-Nanclares et al. (2021) (cont.)		Breast, incidence	Cumulative average intake of ASBs, NHS-II (HR):			Age, calendar year, SSB intake, race, age at menarche, age at menopause, postmenopausal hormone use, oral contraceptive use, parity and age at first birth, breastfeeding history, family history of breast cancer, history of benign breast disease, height, cumulatively updated alcohol and total caloric intake, physical activity, BMI at age 18 yr, modified AHEI score (with SSBs and alcohol removed), SES, change in weight since age 18 yr	
			< 1/mo	935	1		
			≥ 1 to ≤ 4/mo	408	1.02 (0.91–1.15)		
			> 1 to < 7/wk	1327	0.98 (0.89–1.07)		
			≥ 1/day	1214	0.91 (0.83–1.00)		
			Continuous (per 1 serving/day)	3884	0.98 (0.95–1.00)		
			Trend-test P value: 0.02				

Table 2.3   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Debras et al. (2022b) France Enrolment, 2009–2021/ follow-up until 22 January 2021 (median, 7.8 yr) Cohort	102 865 (80 711 women, 22 154 men); population-based cohort NutriNet-Santé (web-based); men and women aged ≥ 18 yr Exposure assessment method: questionnaire; participants are asked every 6 mo to complete a series of three validated web-based 24 h dietary records randomly assigned over a 2-wk period (2 weekdays, 1 weekend day); at least two 24 h dietary records during the first 2 yr of follow-up considered in analyses (mean ± SD, 5.6 ± 3.0)	Breast: incidence	Aspartame intake (HR): Non-consumers Lower consumers (< 15.39 mg/day) Higher consumers (≥ 15.39 mg/day) Trend-test *P* value: 0.036	647 176 156	1 1.09 (0.92–1.29) 1.22 (1.01–1.48)	Age, BMI, height, percentage weight gain during follow-up, physical activity, smoking status, number of smoked cigarettes in pack-years, educational level, number of 24 h dietary records, family history of cancer, prevalent diabetes, energy intake without alcohol, daily intakes of alcohol, sodium, saturated fatty acids, fibre, sugar, fruit and vegetables, whole-grain foods, and dairy products, other AS intake, age at menarche, age at first childbirth, number of biological children, baseline menopausal status, and oral contraceptive use and hormonal treatment for menopause at baseline and during follow-up	*Exposure assessment critique:* A key strength was it was a prospective study using dietary records with detailed, quantitative assessment of aspartame based on food composition data updates for food supply changes over time. A key limitation was the baseline assessment in the main analysis, but a sensitivity analysis was conducted using all 24 h dietary records available during follow-up. *Other strengths*: large cohort; large number of cases; sensitivity analyses excluded prevalent diabetes or used all available 24 h dietary records throughout follow-up; explored menopause-related heterogeneity for breast cancer.

Table 2.3   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Debras et al. (2022b) (cont.)		Breast, incidence	Aspartame intake, participants without diabetes (HR):			Age, BMI, height, percentage weight gain during follow-up, physical activity, smoking status, number of smoked cigarettes in pack-years, educational level, number of 24 h dietary records, family history of cancer, energy intake without alcohol, daily intakes of alcohol, sodium, saturated fatty acids, fibre, sugar, fruit and vegetables, whole-grain foods, and dairy products, other AS intake, age at menarche, age at first childbirth, number of biological children, baseline menopausal status, and oral contraceptive use and hormonal treatment for menopause at baseline and during follow-up	*Other limitations*: low aspartame use in the cohort (28%); self-selection may limit generalizability; potential for bias from residual confounding and reverse causation.
			Non-consumers	457	1		
			Lower consumers (< 15.39 mg/day)	170	1.09 (0.91–1.29)		
			Higher consumers (≥ 15.39 mg/day)	147	1.21 (0.99–1.46)		
			Trend-test *P* value: 0.052				

Table 2.3   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Debras et al. (2022b) (cont.)		Breast, incidence	Aspartame intake (time-dependent) (HR):			Age, BMI, height, percentage weight gain during follow-up, physical activity, smoking status, number of smoked cigarettes in pack-years, educational level, number of 24 h dietary records, family history of cancer, prevalent diabetes, energy intake without alcohol, daily intakes of alcohol, sodium, saturated fatty acids, fibre, sugar, fruit and vegetables, whole-grain foods, and dairy products, other AS intake, age at menarche, age at first childbirth, number of biological children, baseline menopausal status, and oral contraceptive use and hormonal treatment for menopause at baseline and during follow-up	
			Non-consumers	647	1		
			Lower consumers (< 15.39 mg/day)	176	1.05 (0.89–1.23)		
			Higher consumers (≥ 15.39 mg/day)	156	1.18 (0.97–1.42)		
			Trend-test *P* value: 0.106				

Table 2.3   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Debras et al. (2022b) (cont.)		Breast (premenopausal), incidence	Aspartame intake (HR):			Age, BMI, height, percentage weight gain during follow-up, physical activity, smoking status, number of smoked cigarettes in pack-years, educational level, number of 24 h dietary records, family history of cancer, prevalent diabetes, energy intake without alcohol, daily intakes of alcohol, sodium, saturated fatty acids, fibre, sugar, fruit and vegetables, whole-grain foods, and dairy products, other AS intake, age at menarche, age at first childbirth, number of biological children, baseline menopausal status, and oral contraceptive use and hormonal treatment for menopause at baseline and during follow-up	
			Non-consumers	261	1		
			Lower consumers (< 16.44 mg/day)	73	1.08 (0.83–1.42)		
			Higher consumers (≥ 16.44 mg/day)	61	1.07 (0.79–1.46)		
			Trend-test *P* value: 0.564				
		Breast (postmenopausal), incidence	Aspartame intake (HR):				
			Non-consumers	386	1		
			Lower consumers (< 12.16 mg/day)	100	1.13 (0.91–1.42)		
			Higher consumers (≥ 12.16 mg/day)	98	1.24 (0.98–1.57)		
			Trend-test *P* value: 0.060				

Table 2.3   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Debras et al. (2022b) (cont.)		Prostate, incidence	Aspartame intake (HR):			Age, BMI, height, percentage weight gain during follow-up, physical activity, smoking status, number of smoked cigarettes in pack-years, educational level, number of 24 h dietary records, family history of cancer, prevalent diabetes, energy intake without alcohol, daily intakes of alcohol, sodium, saturated fatty acids, fibre, sugar, fruit and vegetables, whole-grain foods, and dairy products, other AS intake	
			Non-consumers	310	1		
			Lower consumers (< 14.45 mg/day)	49	0.95 (0.70–1.30)		
			Higher consumers (≥ 14.45 mg/day)	44	1.28 (0.91–1.79)		
			Trend-test *P* value: 0.280				

Table 2.3 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Debras et al. (2022b) (cont.)		Prostate, incidence	Aspartame intake, participants without diabetes (HR):			Age, BMI, height, percentage weight gain during follow-up, physical activity, smoking status, number of smoked cigarettes in pack-years, educational level, number of 24 h dietary records, family history of cancer, energy intake without alcohol, daily intakes of alcohol, sodium, saturated fatty acids, fibre, sugar, fruit and vegetables, whole-grain foods, and dairy products, other AS intake	
			Non-consumers	297	1		
			Lower consumers (< 14.45 mg/day)	46	0.97 (0.71–1.33)		
			Higher consumers (≥ 14.45 mg/day)	37	1.24 (0.87–1.77)		
			Trend-test P value: 0.367				
		Prostate, incidence	Aspartame intake (time-dependent) (HR):				
			Non-consumers	310	1		
			Lower consumers (< 14.45 mg/day)	49	1.12 (0.87–1.45)		
			Higher consumers (≥ 14.45 mg/day)	44	1.21 (0.87–1.71)		
			Trend-test P value: 0.194				
McCullough et al. (2022) USA Enrolment, 1982/follow-up, through 2016 (median, 27.7 yr) Cohort	934 777 (416 313 men, 518 464 women); CPS-II prospective cohort; adults aged ≥ 28 yr; excluded participants with personal history at baseline of diabetes or cancer other than nonmelanoma skin cancer, men aged > 90 yr or	Breast (postmenopausal), mortality	ASB consumption (HR):			Age, race/ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, parity, age at menarche, estrogen use, oral contraceptive use, age at first live birth, menopausal status	*Exposure assessment critique*: A key strength was the prospective assessment of ASB consumption. Key limitations were that only one dietary assessment was conducted at baseline in 1982 that was before the use of aspartame in ASBs, hence the relevance regarding aspartame exposure depends on the stability
			Never	NR	1		
			< 1 drink/day	NR	1.02 (0.94–1.10)		
			1 drink/day	NR	0.97 (0.88–1.06)		
			≥ 2 drinks/day	NR	1.10 (1.00–1.20)		
			Continuous (per drink/day)	6074	1.03 (1.00–1.06)		
			Trend-test P value: 0.153				

Table 2.3 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2022) (cont.)	women aged > 95 yr at enrolment, and those reporting only prior but not current consumption of either SSBs or ASBs; breast cancer results limited to women postmenopausal at baseline (367 978) Exposure assessment method: questionnaire; exposure to ASB assessed in 1982 through a question about the number of drinks per day of diet soda or ice teas (one pooled item) and potential changes over the past 10 yr; no specific assessment of aspartame content in ASB	Breast (postmenopausal), mortality	ASB consumption, BMI adjusted (HR):			Age, race/ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, parity, age at menarche, estrogen use, oral contraceptive use, age at first live birth, menopausal status, BMI	of ASB consumption over up to 34 yr of follow-up, but such information was not directly available; no other sources were considered (although these were more limited); and uncertainty regarding aspartame content in ASBs after the mid-2000s. Other comments: exclusion of participants who reported only prior but not current consumption of either SSBs or ASBs at baseline. Other strengths: large cohort with long follow-up; ability to examine multiple cancer types, stratify by sex or BMI, and limit to never-smokers; comprehensive adjustment for confounders, including SSB consumption.
			Never	NR	1		
			< 1 drink/day	NR	0.97 (0.90–1.05)		
			1 drink/day	NR	0.92 (0.84–1.01)		
			≥ 2 drinks/day	NR	1.03 (0.94–1.13)		
			Continuous (per drink/day)	6074	1.01 (0.98–1.04)		
			Trend-test P value: 0.838				
		Prostate, mortality	ASB consumption (HR):			Age, race/ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption	
			Never	NR	1		
			< 1 drink/day	NR	0.99 (0.92–1.07)		
			1 drink/day	NR	0.91 (0.83–1.00)		
			≥ 2 drinks/day	NR	0.98 (0.89–1.08)		
			Continuous (per drink/day)	9381	0.99 (0.96–1.02)		
			Trend-test P value: 0.258				

Table 2.3   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2022) (cont.)		Prostate, mortality	ASB consumption, BMI adjusted (HR):			Age, race/ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	*Other limitations*: likely bias from non-differential exposure misclassification.
			Never	NR	1		
			< 1 drink/day	NR	0.99 (0.92–1.07)		
			1 drink/day	NR	0.91 (0.83–1.00)		
			≥ 2 drinks/day	NR	0.98 (0.89–1.08)		
			Continuous (per drink/day)	9381	0.99 (0.96–1.02)		
			Trend-test *P* value: 0.258				
		Prostate, mortality	ASB consumption, normal weight (BMI, 18.5 to < 25 kg/m^2) (HR):				
			Never	NR	1		
			< 1 drink/day	NR	0.94 (0.81–1.08)		
			1 drink/day	NR	0.78 (0.64–0.95)		
			≥ 2 drinks/day	NR	0.88 (0.71–1.08)		
			Continuous (per drink/day)	3461	0.96 (0.91–1.02)		
			Trend-test *P* value: 0.022				
		Prostate, mortality	ASB consumption, overweight (BMI, 25 to < 30 kg/m^2) (HR):				
			Never	NR	1		
			< 1 drink/day	NR	0.99 (0.90–1.10)		
			1 drink/day	NR	0.88 (0.78–1.00)		
			≥ 2 drinks/day	NR	0.92 (0.81–1.05)		
			Continuous (per drink/day)	4781	0.96 (0.93–1.00)		
			Trend-test *P* value: 0.062				

Table 2.3 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2022) (cont.)		Prostate, mortality	ASB consumption, obese (BMI, ≥ 30 kg/m²) (HR):			Age, race/ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR	1		
			< 1 drink/day	NR	1.01 (0.81–1.26)		
			1 drink/day	NR	1.15 (0.90–1.48)		
			≥ 2 drinks/day	NR	1.23 (0.98–1.53)		
			Continuous (per drink/day)	861	1.06 (1.00–1.12)		
			Trend-test *P* value: 0.052				
Ewertz and Gill (1990) Denmark 1983–1984 (1 yr) population-based Case–control	Cases: 1474; women aged < 70 yr with incident breast cancer identified from the Danish Cancer Registry and the nationwide clinical trial of the Danish Breast Cancer Cooperative Group Controls: 1322; age-stratified random sample of the general female population, selected from the Central Population Register, excluding those with a prior breast cancer diagnosis	Breast, incidence	AS intake in coffee or tea (OR):			Age at diagnosis and place of residence	*Exposure assessment critique*: Key limitations were the retrospective recall using an FFQ; no specific estimate of aspartame exposure, only sweetener use in tea and coffee as proxy; limited value due to timing of exposures; the assessment period was March 1982 to February 1983; aspartame was approved in Denmark in 1983 but whether it was available in January and February was questionable; saccharin and cyclamate were mainly used, therefore the likelihood that this reflects aspartame use was limited. *Other strengths*: large sample size.
			No	1216	1		
			Yes	147	0.94 (0.73–1.20)		

Table 2.3   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Ewertz and Gill (1990) (cont.)	Exposure assessment method: self-administered, semiquantitative FFQ with additional questions on consumption of tea, coffee, sugar, and AS						
Gallus et al. (2007) Italy 1991–2004 Case–control	Cases: 2569 (female breast), 1294 (prostate); hospital-based; median age, 55 yr (female breast) or 66 yr (prostate) Controls: 2588 (female breast), 1451 (prostate); patients admitted to the same hospitals as the cases, though for acute, non-neoplastic disorders; median age, 56 yr (female breast) or 63 yr (prostate)	Breast, incidence Prostate, incidence	Consumption of AS other than saccharin, women (OR): Non-consumers > 0 sachets or tablets/day Consumption of AS other than saccharin (OR): Non-consumers > 0 sachets/ tablets per day	2350 219 1217 77	1 0.80 (0.65–0.97) 1 1.23 (0.86–1.76)	Age, study centre, education, alcohol use, tobacco smoking, BMI, total energy intake, consumption of hot beverages, parity, and menopausal status/age at menopause Age, study centre, education, alcohol use, tobacco smoking, BMI, total energy intake, consumption of hot beverages	*Exposure assessment critique*: Key limitations were that there was no specific assessment of aspartame, aspartame was considered as "other sweeteners" but with unclear actual contribution; only one source was considered (tabletop sweeteners); the retrospective assessment in a case–control study (potential for differential misclassification). Other information: according to the authors, there was limited consumption of sources of sweeteners (including ASBs) in the study population (middle-aged adults in Italy between 1991 and 2004).

Table 2.3 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Gallus et al. (2007) (cont.)	Exposure assessment method: questionnaire; assessment of use of tabletop sweeteners containing either saccharin or other sweeteners as sachets or tablets per week in the 2 yr before cancer diagnosis						*Other strengths*: high response rates (< 5% refusals) for cases and controls reduced the potential for selection bias; large sample size with large case numbers for rarer cancers; control for key potential confounders, including BMI. *Other limitations*: somewhat limited adjustment for confounders as far as breast cancer risk factors are concerned (e.g. age at menarche, hormone use, family history of breast cancer); potential for recall bias and reverse causation.

Table 2.3   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Palomar-Cros et al. (2023) Spain 2008–2013 Case–control	Cases: 1510 (breast cancer), 972 (prostate cancer); aged 20–85 yr with newly diagnosed histologically confirmed cancer, resided in catchment area for at least 6 mo; no prior history of their cancer; enrolled as soon as possible after diagnosis; frequency-matched on age, sex, and region to population controls	Breast, incidence	Consumption of aspartame-containing products (OR):			Age, study centre, education, smoking, radiation exposure, total WCRF score continuous, total energy intake, total sugar intake, family history of breast cancer, night shift work, menopause, nulliparous, age at first child, use of contraceptive, other AS	*Exposure assessment critique*: A key strength was the categorization of intake of ASBs and tabletop sweeteners by type (aspartame vs others) using public data on ingredients in food supply, but it was unclear whether the assumption of aspartame content in products was correct. A key limitation was that the study assessed beverages and tabletop sweeteners but there was no consideration of ASs in the rest of the food supply.
			Non-consumers	1146	1		
			Medium intake (< 3rd quartile among controls)	265	0.82 (0.67–1.01)		
			High intake (≥ 3rd quartile among controls)	99	0.94 (0.69–1.28)		
			Trend-test *P* value: 0.2				
		Breast, incidence	Consumption of aspartame-containing products, participants without diabetes (OR):				
			Non-consumers	1065	1		
			Medium intake (< 3rd quartile among controls)	242	0.81 (0.66–1.01)		
			High intake (≥ 3rd quartile among controls)	91	1.04 (0.75–1.45)		
			Trend-test *P* value: 0.4				

Table 2.3 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Palomar-Cros et al. (2023) (cont.)	Controls: 3629 (1674 for breast cancer, 1308 for prostate cancer); randomly selected from administrative records of selected primary health care centres within catchment area Exposure assessment method: self-administered, semiquantitative FFQ, 140 food items, assessing usual dietary intake during the previous year	Breast, incidence	Consumption of aspartame-containing products, participants with diabetes (OR): Non-consumers Medium intake (< 3rd quartile among controls) High intake (≥ 3rd quartile among controls) Trend-test *P* value: 0.03	81 23 8	1 0.73 (0.33–1.57) 0.28 (0.08–0.83)	Age, study centre, education, smoking, radiation exposure, total WCRF score continuous, total energy intake, total sugar intake, family history of breast cancer, night shift work, menopause, nulliparous, age at first child, use of contraceptive, other AS	*Other strengths*: large sample size for some outcomes; histopathological confirmation of cancer cases; extensive assessment of confounding, including from BMI (captured in a combined score based on WCRF/AICR evidence on lifestyle factors; Romaguera et al., 2017); stratification by diabetes status to evaluate heterogeneity of associations.
		Prostate, incidence	Consumption of aspartame-containing products (OR): Non-consumers Medium intake (< 3rd quartile among controls) High intake (≥ 3rd quartile among controls) Trend-test *P* value: 0.4	814 110 48	1 0.81 (0.61–1.07) 0.96 (0.63–1.46)	Age, study centre, education, smoking, radiation exposure, total WCRF score continuous, total energy intake, total sugar intake, family history of prostate cancer, night shift work, other AS	

Table 2.3 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Palomar-Cros et al. (2023) (cont.)		Prostate, incidence	Consumption of aspartame-containing products, participants without diabetes (OR):			Age, study centre, education, smoking, radiation exposure, total WCRF score continuous, total energy intake, total sugar intake, family history of prostate cancer, night shift work, other AS	*Other limitations*: non-prospective study design (case–control); selection bias due to low participation among cases (participation rate was 71% for breast, 72% for prostate) and controls (mean participation rate, 53%); recall bias in exposure assessment; potential for exposure measurement error and residual confounding due to other correlates of AS use among those with diabetes; relatively low exposure contrasts for aspartame-containing products; potential for chance findings due to small number in some strata.
			Non-consumers	704	1		
			Medium intake (< 3rd quartile among controls)	87	0.84 (0.61–1.15)		
			High intake (≥ 3rd quartile among controls)	30	0.82 (0.48–1.36)		
			Trend-test *P* value: 0.2				
		Prostate, incidence	Consumption of aspartame-containing products, participants with diabetes (OR):				
			Non-consumers	110	1		
			Medium intake (< 3rd quartile among controls)	23	0.85 (0.45–1.56)		
			High intake (≥ 3rd quartile among controls)	18	1.91 (0.87–4.2)		
			Trend-test *P* value: 0.3				
		Prostate (low grade; Gleason score, < 7), incidence	Consumption of aspartame-containing products (OR):				
			Non-consumers	374	1		
			Medium intake (< 3rd quartile among controls)	49	0.8 (0.55–1.15)		
			High intake (≥ 3rd quartile among controls)	22	0.97 (0.55–1.65)		
			Trend-test *P* value: 0.4				

Table 2.3 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Palomar-Cros et al. (2023) (cont.)		Prostate (low grade; Gleason score, < 7), incidence	Consumption of aspartame-containing products, participants without diabetes (OR):			Age, study centre, education, smoking, radiation exposure, total WCRF score continuous, total energy intake, total sugar intake, family history of prostate cancer, night shift work, other AS	
			Non-consumers	332	1		
			Medium intake (< 3rd quartile among controls)	44	0.95 (0.63–1.40)		
			High intake (≥ 3rd quartile among controls)	12	0.68 (0.32–1.35)		
			Trend-test *P* value: 0.3				
		Prostate (low grade, Gleason score, < 7), incidence	Consumption of aspartame-containing products, participants with diabetes (OR):				
			Non-consumers	42	1		
			Medium intake (< 3rd quartile among controls)	5	0.47 (0.15–1.24)		
			High intake (≥ 3rd quartile among controls)	10	2.46 (0.93–6.38)		
			Trend-test *P* value: 0.3				
		Prostate (high grade, Gleason score, ≥ 7), incidence	Consumption of aspartame-containing products (OR):				
			Non-consumers	[428]	1		
			Medium intake (< 3rd quartile among controls)	[58]	0.83 (0.59–1.17)		
			High intake (≥ 3rd quartile among controls)	[26]	1.07 (0.64–1.76)		
			Trend-test *P* value: 0.7				

Table 2.3 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Palomar-Cros et al. (2023) (cont.)		Prostate (high grade; Gleason score, ≥ 7), incidence	Consumption of aspartame-containing products, participants without diabetes (OR):			Age, study centre, education, smoking, radiation exposure, total WCRF score continuous, total energy intake, total sugar intake, family history of prostate cancer, night shift work, other AS	
			Non-consumers	361	1		
			Medium intake (< 3rd quartile among controls)	41	0.77 (0.51–1.16)		
			High intake (≥ 3rd quartile among controls)	18	1.00 (0.53–1.81)		
			Trend-test *P* value: 0.5				
		Prostate (high grade, Gleason score, ≥ 7), incidence	Consumption of aspartame-containing products, participants with diabetes (OR):				
			Non-consumers	67	1		
			Medium intake (< 3rd quartile among controls)	17	1.11 (0.54–2.24)		
			High intake (≥ 3rd quartile among controls)	8	1.56 (0.54–4.19)		
			Trend-test *P* value: 0.4				

AHEI, Alternate Healthy Eating Index; AICR, American Institute of Cancer Research; AS, artificial sweetener; ASB, artificially sweetened beverage; BMI, body mass index; CI, confidence interval; CPS-II, Cancer Prevention Study II; EPIC, European Prospective Investigation into Cancer and Nutrition; FDA, Food and Drug Administration; FFQ, food frequency questionnaire; HPFS, Health Professionals Follow-up Study; HR, hazard ratio; MCCS, Melbourne Collaborative Cohort Study; mo, month(s); NHS, Nurses' Health Study; NHS-II, Nurses' Health Study II; NR, not reported; OR, odds ratio; SD, standard deviation; SES, socioeconomic status; SSB, sugar-sweetened beverage; vs, versus; WCRF, World Cancer Research Fund; wk, week(s); yr, year(s).

aged 40–75 years at baseline in 1986, and found no association between consumption of ASBs and prostate cancer mortality (HR for ≥ 2 servings/day versus < 1 serving/month, 1.01; 95% CI, 0.67–1.52). [The Working Group noted that the main strengths of the study were the repeated exposure assessments during a time when aspartame had already been approved for use in ASBs (follow-up started between 1980 (NHS) and 1986 (HPFS), and continued through 2014); the focus on lethal prostate cancers; and the complete control for potential confounders. Nonetheless, the potential for residual confounding could not be ruled out. A weakness of note was the potential for measurement error, which would result in a bias towards the null.]

The EPIC cohort study was conducted in 10 European countries and at baseline included 321 081 female and 130 662 male participants, among whom 1402 breast cancer and 907 prostate cancer deaths were diagnosed during follow-up (mean follow-up, 16.4 years) (Mullee et al., 2019). The statistical analyses (which excluded participants from Italy, Spain, and Sweden because information on type of soft drink consumption was not collected in all participating centres) did not report a positive association between consumption of artificially sweetened soft drinks and breast cancer mortality (HR for ≥ 1 glass/day of artificially sweetened soft drink compared with < 1 glass/month, 0.85; 95% CI, 0.59–1.22; *P* for trend, 0.38). The findings were null for prostate cancer mortality. [The Working Group noted that a major strength of the cohort was its large size and that it spanned several European countries. A main limitation of the analyses was the single, baseline-only assessment of beverage consumption, which took place between 1992 and 2000. Exposure misclassification was likely to be non-differential, biasing results towards the null.]

Another study based in the USA was conducted in two long-running prospective cohort studies (NHS and NHS-II), with repeated diet exposure updates throughout follow-up, and reported on breast cancer incidence (Romanos-Nanclares et al., 2021). The NHS cohort comprises 121 700 registered nurses who have been followed for major disease outcomes via biennial questionnaires since study inception in 1976; the NHS-II cohort comprises 116 429 registered nurses who have been similarly followed since its establishment in 1986. In both cohort studies, diet was assessed with a validated FFQ (administered in the NHS in 1980, 1984, 1986, and every 4 years thereafter, and in the NHS-II in 1991 and every 4 years thereafter). ASBs were defined as caffeinated, noncaffeinated, and noncarbonated low-calorie or diet beverages. Questions included the frequency of consumption over the past year for a standard 355 mL (12 oz) serving (1 glass/can/bottle) of each beverage. Cumulative averages of dietary data were based on repeated measures from FFQs. During follow-up (1980–2016 for NHS and 1991–2017 for NHS-II), 11 379 incident breast cancer cases were documented across both cohorts (among 82 713 NHS and 93 085 NHS-II participants free of cancer at baseline and with valid data). The pooled hazard ratio comparing extreme categories of ASB consumption (≥ 1/day compared with < 1/month) was 0.96 (95% CI, 0.91–1.02). Although no association with ASB consumption was observed in the NHS, intake was inversely associated with the risk of breast cancer in the NHS-II (HR for ≥ 1/day compared with < 1/month, 0.93; 95% CI, 0.84–1.01; *P* for trend, 0.04; and HR with additional adjustment for weight change since age 18 years, 0.91; 95% CI, 0.83–1.00; *P* for trend, 0.02). Overall, the findings were not suggestive of an increase in breast cancer risk among women with higher consumption of ASBs. [The Working Group noted that the strengths of the NHS and NHS-II cohort studies outweighed their weaknesses; strengths included the cohorts' large size, repeated exposure assessment over a long follow-up period, and complete assessment and control for potential confounders. Main weaknesses pertained to

the relative lack of power in certain substrata. It was also noted that the findings from the breast cancer incidence analyses were consistent with those from the breast cancer mortality analyses (Malik et al., 2019). The potential for non-differential exposure misclassification also existed, probably attenuating results.]

NutriNet-Santé, a very detailed and large population-based cohort study in France, included 80 711 women and 979 total incident breast cancer cases (median follow-up, 7.8 years; 156 cases among the higher-consumers of aspartame) (Debras et al., 2022b; reported earlier in Chazelas et al., 2019 for ASB consumption). Among 22 154 men, a total of 403 incident prostate cancer cases (same follow-up; 44 cases among the higher-consumers of aspartame) were also included. The NutriNet-Santé cohort is a web-based cohort that started in 2009 and included 102 865 participants who are followed every 6 months by three non-consecutive web-based 24-hour dietary records, randomly assigned over 15 days (2 weekdays and 1 weekend day). Artificial sweetener intake was assessed through 24-hour dietary records, in which brands and commercial names of industrial products were routinely collected with the aim of assessing exposure to food additives (Chazelas et al., 2021). The study assessed exposure to several artificial sweeteners: acesulfame-K (European food additive identification number E950), aspartame (E951), cyclamates (E952), saccharin (E954), sucralose (E955), thaumatin (E957), dihydrochalcone (E959), steviol glycosides (E960), and salt of aspartame-acesulfame (E962); the quantities consumed of all these artificial sweeteners were summed to calculate the variable "total artificial sweeteners". The study reported on the association between cancer risk and exposure to total artificial sweeteners, aspartame, acesulfame-K and sucralose. In the main analysis, the study used the average of all available (up to 15) 24-hour dietary records during the first 2 years of follow-up (sensitivity analyses

were also presented using all available dietary records during the whole follow-up period). The definition of aspartame intake accounted for all dietary sources, and the study also reported on other most frequently consumed artificial sweeteners. Among artificial sweetener consumers, higher-consumers (above the sex-specific median consumption for total sweeteners) had a mean aspartame intake of 47.42 mg/day versus only 3.24 mg/day for lower-consumers, illustrating the interindividual variability in intake in this cohort. Results were reported overall and for pre- and postmenopausal breast cancer separately. The results suggested an increased risk of breast cancer overall (HR, 1.22; 95% CI, 1.01–1.48; *P* for trend, 0.036) for women with higher consumption of aspartame, i.e. above the sex-specific median among aspartame users compared with non-users. Hazard ratios for pre- and postmenopausal women were as follows, with no statistical heterogeneity detected (formally tested): for premenopausal women, 1.07 (95% CI, 0.79–1.46; *P* for trend, 0.564); and for postmenopausal women, 1.24 (95% CI, 0.98–1.57; *P* for trend, 0.060). The study found no significantly increased risk of prostate cancer associated with higher consumption of aspartame, i.e. above the median among male aspartame users versus non-users (multivariable-adjusted HR, 1.28; 95% CI, 0.91–1.79; *P* for trend, 0.280) although the hazard ratio point estimate was quantitatively similar to that reported for breast cancer risk in the same cohort. Similar results were observed in sensitivity analyses of breast and prostate cancer excluding participants with a history of diabetes at baseline. No interaction between aspartame and BMI was detected for breast (*P* for interaction, 0.796) or prostate (*P* for interaction, 0.884) cancer. The study provided additional information, including whether differences in risk were observed between sugar or artificial sweeteners. [The Working Group noted that the investigators for the NutriNet-Santé cohort study had planned before the data-collection phase to investigate

several food additives and consequently made a special, and so far quite unique, investment in collecting detailed food consumption information on the type, and where possible the brand, of many foods and drinks, and building a database on the food additive content of hundreds of foods. To date, the NutriNet-Santé study seemed to be the only cohort study that had built the methodology required for separating with some level of precision the consumption of foods and drinks specifically containing aspartame. The main analysis also considered intake over the first 2 years of follow-up, and additional analysis used repeated assessments throughout the follow-up period. Nonetheless, the potential for non-differential exposure misclassification remained.]

The CPS-II cohort study in the USA assessed the association between ASB consumption evaluated at baseline in 1982 (median follow-up, 27.7 years) and mortality from postmenopausal breast cancer and prostate cancer (McCullough et al., 2022). For breast cancer, among the large cohort comprising 367 978 postmenopausal (at baseline) women and 6074 postmenopausal breast cancer deaths, multivariable-adjusted analyses were conducted considering BMI separately, and reported no association after adjustment for several potential confounders and BMI; the results were compatible with a 3% increase in risk (HR, 1.03; 95% CI, 1.00–1.06) of breast cancer mortality per 1 drink/day of ASBs, before adjusting for BMI. For prostate cancer, among the cohort comprising 416 313 men and 9381 prostate cancer deaths, multivariable-adjusted analyses were conducted considering BMI separately, and described a null finding before and after adjustment for BMI. There was a suggestive interaction with BMI and the consumption of ASBs (*P* for interaction, 0.013), with a positive association only among obese participants (≥ 2 drinks/day versus never: HR for individuals with BMI of 18.5 to < 25 kg/m², 0.88; 95% CI, 0.71–1.08; HR for BMI 25–30 kg/m², 0.92; 95% CI, 0.81–1.05; HR for BMI > 30 kg/m², 1.23;

95% CI, 0.98–1.53). [The Working Group noted that the dietary assessment preceded by 1 year the approval of aspartame for use in beverages in the USA; therefore, over the 27 years of follow-up, non-differential misclassification was likely, which would be expected to bias results towards the null. However, published data among participants included in both the CPS-II and the CPS-II nutrition cohort (McCullough et al., 2014) suggested that the relative ranking of intake category was consistent during the first 17-year period.]

Three case–control studies reported on the association between artificial sweetener use and breast cancer risk (two of these also reported on prostate cancer risk).

One population-based case–control study in Denmark examined associations between breast cancer and artificial sweetener consumption in coffee or tea (Ewertz and Gill, 1990). This study was fairly large, with 1474 breast cancer cases and 1322 controls, and reported a null finding. [The Working Group noted that the questionnaire assessment of artificial sweetener consumption referred to the period March 1982 to February 1983, and that aspartame was only approved for use in Denmark in 1983; therefore, this study was considered to be uninformative.]

One hospital-based case–control study, which combined multiple hospital-based case–control studies conducted in 1991–2004 across four areas in Italy, examined weekly consumption of sachets/tablets of "sweeteners other than saccharin" (assumed by the study authors to mainly comprise aspartame) and breast and prostate cancer risk (Gallus et al., 2007). This large study (2569 breast cancer cases and 2588 controls) reported an inverse association between breast cancer and consumption of artificial sweeteners other than saccharin. The overall association for prostate cancer was null in this study, which included 1294 prostate cases and 1451 controls. [The Working Group noted that a strength of this case–control study was the

relative high participation rates: fewer than 5% of both cases and controls contacted for participation refused; weaknesses pertained to the exposure assessment, which did not distinguish between aspartame and artificial sweeteners other than saccharin, probably leading to a bias towards the null; and recall bias, given its case–control design.]

The MCC-Spain study, a case–control study considering multiple cancer sites and conducted between 2008 and 2013 (Palomar-Cros et al., 2023), investigated associations between use of artificial sweeteners (assessed via FFQ), separated into aspartame and other artificial sweeteners both derived from ASBs, tabletop sweeteners, and consumption of "gaseosa" (an artificially sweetened soft drink commonly consumed in Spain), and their association with risk of breast (1510 cases) and prostate (972 cases) cancer, using 3629 population controls. Overall, no increased risk of breast or prostate cancer was observed among those with high aspartame consumption (defined as third quartile or above). For breast cancer, among the study participants with diabetes, compared with non-consumers, there was an inverse association (OR, 0.28; 95% CI, 0.08–0.83; P for trend, 0.03), whereas for prostate cancer, the odds ratio was 1.91 (95% CI, 0.87–4.20; P for trend, 0.3). [The Working Group noted that the study's strengths were its size and ability to adjust for a large variety of potential confounders, including BMI, captured in the WCFR/AICR score (Romaguera et al., 2017), and that it stratified by diabetes status. A further strength was that prostate cancer cases were stratified by low-grade (Gleason score, < 7) versus high-grade aggressive prostate cancer (Gleason score, ≥ 7). Limitations included the incomplete exposure assessment and the uncertainty of the assumption that all low-calorie or no-calorie sweetened beverages contained aspartame only, as well as the potential for recall bias that is inherent to case–control studies.]

A recent meta-analysis (Yin et al., 2022) included four studies on ASB use (Hodge et al., 2018; Chazelas et al., 2019; Romanos-Nanclares et al., 2021; Debras et al., 2022b) and breast cancer risk, with a combined estimate of 0.99 (95% CI, 0.90–1.08; P = 0.75; I^2 = 50.3%) for highest versus lowest category of ASB consumption, although these four studies were deemed of very low certainty (using the risk of bias in non-randomized studies of exposures, ROBINS-E, grading scheme). This assessment was mainly based on the criterion "indirectness", the "inconsistency" of the results of these four studies, and a "moderate" risk of bias. No dose–response analyses were carried out for breast cancer separately. Two studies (Chazelas et al., 2019; Debras et al., 2022b), both with overlapping data from the NutriNet-Santé cohort, were included in a meta-analysis for prostate and breast cancer risk (Yin et al., 2022) that showed no association with ASB consumption. [One additional meta-analysis also reported on ASB consumption and prostate cancer risk (Llaha et al., 2021), but because of the incomplete summary of studies and small body of evidence considered, it was not considered informative by the Working Group.] The most recent meta-analysis, by Pan et al. (2023), examined the combined association between ASBs and breast and prostate cancer risk, reporting no association between higher consumption of ASBs (per increase of 250 mL/day) versus none, and breast cancer risk (HR, 0.95; 95% CI, 0.80–1.12) or prostate cancer risk (HR, 0.93; 95% CI, 0.69–1.26). [The Working Group noted that in this meta-analysis (Pan et al., 2023) only three studies contributed to the breast cancer findings and two studies to prostate cancer findings, and they determined that this study did not contribute additional information.]

2.4 Cancers of the brain, thyroid, and uterus, and other solid cancers

See Table 2.4.

Seven cohort studies (including eight cohorts) reported on aspartame or ASBs and cancers of the brain (Lim et al., 2006; McCullough et al., 2022), uterus (Inoue-Choi et al., 2013; Hodge et al., 2018; McCullough et al., 2022), thyroid (Zamora-Ros et al., 2022), ovary (Hodge et al., 2018; McCullough et al., 2022), or lung (Malik et al., 2019; McCullough et al., 2022; You et al., 2022).

Five case–control studies reported on ASBs or tabletop sweeteners and cancers of the brain (Gurney et al., 1997; Cabaniols et al., 2011), uterus (Bosetti et al., 2009), thyroid (Singh et al., 2020), larynx (Gallus et al., 2007), and ovary (Gallus et al., 2007).

One cohort (Lim et al., 2006) and two case–control studies (Gurney et al., 1997; Cabaniols et al., 2011) described their exposure assessment as being specific to aspartame intake, indicating that it was derived from data on aspartame contained in or added to drinks and foods. [Although the methodology described in the two publications left some uncertainty about the actual specificity of the reported exposure to aspartame, which could have resulted in exposure misclassification, the Working Group considered that the assessment of aspartame consumption from ASBs and tabletop sweeteners in Lim et al. (2006) covered a time period that was highly relevant for aspartame exposure from this source in the USA.] All other studies reported on the exposure to artificially sweetened drinks and foods and the use of sweeteners in sachet and tablet form, but without any further specification of the type of artificial sweetener to which the study participants had been exposed.

All studies estimated average daily consumption of aspartame or artificial sweeteners as part of usual diet. In cohort studies, the estimate referred to the time of participant enrolment in the study, whereas in case–control studies, it referred to a period of time preceding cancer diagnoses for cancer cases and to an equivalent period for controls. One study additionally considered age at start of consumption (Gurney et al., 1997).

Although most of the studies were not specifically designed to investigate the possible carcinogenic effect of aspartame, all the studies included in this section measured artificial sweetener consumption in the general context of measuring usual diet and included questions to separately identify sugar-sweetened and artificially sweetened drinks.

Within the USA-based NIH-AARP cohort, Lim et al. (2006) analysed data from 473 984 study participants with baseline validated 124-item FFQ and reported the association between aspartame consumption and brain cancer risk. In this study, particular attention was paid to improve the measurement of diet by requesting that all participants fill in an FFQ that included specific questions on the consumption of beverages, from which aspartame content was later estimated. The 124-item FFQ included questions on three beverages that potentially contained aspartame: soda ("soft drinks, soda, pop"), fruit drinks ("such as Hi-C, lemonade, and Kool-Aid"), and iced tea; it had additional questions on the consumption of artificial sweeteners, including aspartame, in the form of sachets and tablets added to cups of coffee or hot tea. From these responses, a "daily consumption of aspartame" was computed, taking into account aspartame content, portion size, and intake frequency of each beverage. The aspartame content per 100 g beverage was estimated to be 50 mg for diet soda (i.e. 180 mg/12 oz can), 14.95 mg for diet fruit drink, 25.55 mg for diet iced tea, and 35 mg per tabletop packet added to each cup of coffee/ hot tea, based on the information provided by the Nutrition Data System for Research of the

Table 2.4 Epidemiological studies on consumption of aspartame and cancers of the brain, thyroid, and uterus, and other solid cancers

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Lim et al. (2006) USA Enrolment, 1995–1996/ follow-up, through 2000 Cohort	473 984 (285 079 men and 188 905 women); NIH-AARP Diet and Health Study: AARP members aged 50–71 yr residing in the study area (California, Florida, Pennsylvania, New Jersey, North Carolina, Louisiana, Atlanta, Detroit) excluding those with prevalent cancer, invalid BMI data, outliers on reported energy intake, and proxy responders; 315 gliomas including 231 glioblastomas Exposure assessment method: FFQ at baseline, 5 yr of follow-up; consumption frequency of three types of soft drink (soda, fruit drinks, and iced tea) + frequency of consumption of diet vs regular versions + three portion sizes; use of tabletop sweetener packets; standard doses of aspartame assigned to beverages and packets	Brain (glioma), incidence	Aspartame intake (RR): None > 0 to < 100 mg/day ≥ 100 to < 200 mg/day ≥ 200 to < 400 mg/day ≥ 400 mg/day Trend-test P value: 0.05	162 83 32 16 22	1 0.99 (0.75–1.29) 0.70 (0.48–1.03) 0.66 (0.39–1.12) 0.73 (0.46–1.15)	Age at entry, sex, ethnicity, BMI, history of diabetes	*Exposure assessment critique*: A key strength was the prospective and quantitative assessment of aspartame exposure from various ASBs and tabletop packets in a relevant period for aspartame (1995–1996 in the USA). Key limitations were the sequential assessment of the frequency of consumption firstly of soft drinks and then of diet/sugar-free versions with imprecise frequencies inducing inaccuracies; other sources of aspartame were not considered (although these were more limited); exposure misclassification due to older age at enrolment and a lack of data before study entry (aspartame in beverages introduced in the USA 10 yr before baseline).
		Brain (glioma: glioblastoma), incidence	Aspartame intake (RR): None > 0 to < 100 mg/day ≥ 100 to < 200 mg/day ≥ 200 to < 400 mg/day ≥ 400 mg/day Trend-test P value: 0.05	NR NR NR NR NR	1 NR NR NR 0.64 (0.37–1.10)	Age at entry, sex, ethnicity, BMI, history of diabetes	

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Lim et al. (2006) (cont.)							*Other strengths*: large prospective cohort; case ascertainment based on cancer registries with high (> 90%) completeness; large numbers of cases. *Other limitations*: likely bias from non-differential exposure misclassification.
Inoue-Choi et al. (2013) USA (Iowa Women's Health Study) Enrolment, 1986/follow-up, through 2010 Cohort	23 039; women aged 55–69 yr randomly selected from Iowa drivers' licence list who responded to a mailed questionnaire in 1986 (42% response rate); women with a history of cancer except nonmelanoma skin cancer, who were not postmenopausal, or who reported extreme dietary intake were excluded Exposure assessment method: semiquantitative FFQ at baseline in 1986 assessing sugar-free beverage consumption with standard serving size	Uterus/uterine corpus (type I endometrial cancer), incidence	Consumption of sugar-free beverages (HR): 0 servings/wk > 0–0.0002 servings/wk 0.0003–0.4 servings/wk 0.5–2.8 servings/wk 2.8–64.1 servings/wk Trend-test *P* value: 0.31	152 36 93 125 100	1 0.69 (0.48–1.00) 0.85 (0.65–1.11) 1.03 (0.80–1.32) 0.77 (0.59–1.01)	Age, smoking status, physical activity, estrogen use, alcohol use, age at menarche, age at menopause, number of live births, history of diabetes, coffee intake, BMI	*Exposure assessment critique*: A key strength was the prospective assessment of ASB consumption at a very relevant period for aspartame exposure from ASBs (1986 in the USA). Key limitations were that no other sources were considered (although these were more limited); there was only one assessment at baseline, not considering potential variations in consumption over up to 24 yr of follow-up; and uncertainty of aspartame content in ASBs after the mid-2000s.

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level		Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Inoue-Choi et al. (2013) (cont.)		Uterus/uterine corpus (type I endometrial cancer), incidence	Consumption of sugar-free beverages, excluding women with a history of diabetes (HR):				Age, smoking status, physical activity, estrogen use, alcohol use, age at menarche, age at menopause, number of live births, coffee intake, BMI	*Other strengths*: sample size, prospective design, cancer registry linkage for outcome assessment, adjustment for BMI and other relevant confounders. *Other limitations*: likely bias from non-differential exposure misclassification.
			0 servings/wk		NR	1		
			> 0–0.0002 servings/wk		NR	0.73 (0.50–1.06)		
			0.0003–0.4 servings/wk		NR	0.86 (0.65–1.13)		
			0.5–2.8 servings/wk		NR	0.99 (0.76–1.28)		
			2.8–64.1 servings/wk		NR	0.80 (0.60–1.06)		
			Trend-test *P* value: 0.35					
		Uterus/uterine corpus (type II endometrial cancer), incidence	Consumption of sugar-free beverages (HR):				Age, smoking status, physical activity, estrogen use, alcohol use, age at menarche, age at menopause, number of live births, history of diabetes, coffee intake, BMI	
			0 servings/wk		27	1		
			> 0–0.0002 servings/wk		8	0.78 (0.34–1.79)		
			0.0003–0.4 servings/wk		13	0.66 (0.33–1.30)		
			0.5–2.8 servings/wk		21	1.09 (0.61–1.95)		
			2.8–64.1 servings/wk		17	0.89 (0.48–1.68)		
			Trend-test *P* value: 0.95					

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Inoue-Choi et al. (2013) (cont.)		Uterus/uterine corpus (type II endometrial cancer), incidence	Consumption of sugar-free beverages (serving/week), excluding women with a history of diabetes (HR):			Age, smoking status, physical activity, estrogen use, alcohol use, age at menarche, age at menopause, number of live births, coffee intake, BMI	
			0 servings/wk	NR	1		
			> 0–0.0002 servings/wk	NR	0.81 (0.35–1.86)		
			0.0003–0.4 servings/wk	NR	0.63 (0.31–1.28)		
			0.5–2.8 servings/wk	NR	1.16 (0.64–2.09)		
			2.8–64.1 servings/wk	NR	0.87 (0.45–1.69)		
			Trend-test P value: 0.97				
Hodge et al. (2018) Australia Enrolment, 1990–1994/ follow-up until 30 June 2013 Cohort	35 593 (21 492 women); MCCS – a prospective cohort study of men and women aged 40–69 yr at recruitment and free of cancer, angina, heart attack, or diabetes at baseline; participants with extreme baseline energy intake were excluded	Uterus/ uterine corpus: endometrium, incidence	Artificially sweetened soft drink consumption (HR):			Age, socioeconomic index, country of birth, alcohol intake, smoking status, physical activity, Mediterranean diet score, sugar-sweetened soft drink consumption, waist circumference	*Exposure assessment critique*: Key strengths were that it was a prospective study, that assessment was after aspartame introduction in diet soft drinks in Australia (1987), and that first half of follow-up largely overlapped with period of aspartame use in Australia.
			Never or < 1/mo	125	1		
			1–3/mo	9	0.58 (0.29–1.16)		
			1–6/wk	23	1.11 (0.70–1.77)		
			≥ 1/day	10	0.81 (0.42–1.55)		
			Continuous (per beverage/day)	167	0.92 (0.52–1.65)		
			Trend-test P value: 0.78				

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Hodge et al. (2018) (cont.)	Exposure assessment method: self-administered 121-item FFQ with separate questions on frequency of consumption in the past year of diet (artificially sweetened) soft drinks	Ovary, incidence	Artificially sweetened soft drink consumption (HR): Never or < 1/mo	75	1	Age, socioeconomic index, country of birth, alcohol intake, smoking status, physical activity, Mediterranean diet score, sugar-sweetened soft drink consumption, waist circumference	Key limitations were the FFQ assessment with no specific estimate of aspartame exposure, ASBs used as a proxy, and exposure data at baseline only; small number of consumers. *Other strengths*: adjustment for key confounders, including a measure of obesity (waist circumference). *Other limitations*: likely bias from non-differential exposure misclassification given single baseline assessment and long follow-up.
			1–3/mo	20	0.80 (0.38–1.69)		
			1–6/wk	24	1.39 (0.83–2.34)		
			≥ 1/day	11	1.37 (0.72–2.61)		
			Continuous (per beverage/day)	130	1.51 (0.84–2.73)		
			Trend-test *P* value: 0.17				

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Malik et al. (2019) USA Enrolment, 1976 (NHS), 1986 (HPFS/ follow-up, 1980–2014 (NHS), 1986–2014 (HPFS) Cohort	37 716 men and 80 647 women; female registered nurses aged 30–55 yr in the NHS and male health professionals aged 40–75 yr in the HPFS; excluding those with history of diabetes, cardiovascular disease, or cancer at baseline, or with implausible dietary intake Exposure assessment method: prospective assessment of ASB consumption through repeated FFQs between 1980–1986 and 2010	Lung, mortality	ASB intake (HR): < 1 serving/mo 1–4 servings/mo 2–6 servings/wk 1 to < 2 servings/day ≥ 2 servings/day Continuous (per serving/day) Trend-test P value: 0.20	NR NR NR NR NR NR	1 0.96 (0.85–1.08) 0.85 (0.76–0.95) 0.93 (0.80–1.08) 0.92 (0.77–1.09) 0.98 (0.92–1.08)	Age, race, smoking, alcohol intake, postmenopausal hormone use (NHS), physical activity, family history of diabetes; family history of myocardial infarction, family history of cancer, multivitamin use, aspirin use, baseline history of hypertension and hypercholesterolemia, intake of whole grains, fruit, vegetables, or red and processed meat, total energy, BMI, SSB intake	*Exposure assessment critique*: A key strength was the prospective assessment of ASB consumption from repeated diet assessments every 4 yr, the majority at a very relevant period for aspartame exposure from ASBs (the USA between the 1980s and 2010) potentially capturing lifetime exposure to aspartame. A key limitation was that other sources of aspartame were not considered (although these were more limited); and uncertainty of aspartame content in ASBs after the mid-2000s. *Other strengths*: large, prospective cohort with long follow-up; detailed and validated assessments were updated at each survey round to account for changes in intake over time.

Table 2.4   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Malik et al. (2019) (cont.)							*Other limitations*: bias from non-differential misclassifications of exposure to aspartame likely; stratified numbers of deaths not provided for specific cancer sites.
McCullough et al. (2022) USA Enrolment, 1982/follow-up, through 2016 (median, 27.7 yr) Cohort	934 777 (416 313 men, 518 464 women); CPS-II prospective cohort; adults aged ≥ 28 yr; excluded participants with personal history at baseline of diabetes or cancer other than nonmelanoma skin cancer, men aged > 90 yr or women aged > 95 yr at enrolment, and those reporting only prior but not current consumption of either SSBs or ASBs; uterine/ovarian cancer results exclude women with a history of hysterectomy, uterine surgery, surgically induced menopause, or oophorectomy (ovarian cancer only)	Uterus/uterine corpus, mortality	ASB consumption (HR): Never < 1 drink/day 1 drink/day ≥ 2 drinks/day Continuous (per drink/day) Trend-test *P* value: 0.049	NR NR NR NR 1693	1 1.04 (0.90–1.19) 1.07 (0.90–1.26) 1.18 (1.00–1.40) 1.04 (0.99–1.09)	Age, race/ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, parity, age at menarche, estrogen use, oral contraceptive use, age at first live birth, menopausal status	*Exposure assessment critique*: A key strength was the prospective assessment of ASB consumption. Key limitations were that only one dietary assessment was carried out at baseline in 1982 that was before the use of aspartame in ASBs, hence the relevance regarding aspartame exposure depends on the stability of ASB consumption over up to 34 yr of follow-up, but such information was not directly available; no other sources were considered (although these were more limited); and uncertainty of aspartame content in ASBs after the mid-2000s.

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2022) (cont.)	Exposure assessment method: questionnaire; exposure to ASBs assessed in 1982 through a question about the number of drinks/day of diet soda or ice teas (one pooled item) and potential changes over the past 10 yr; no specific assessment of aspartame content in ASBs	Uterus/uterine corpus, mortality	ASB consumption, BMI-adjusted (HR): Never	NR	1	Age, race/ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, parity, age at menarche, estrogen use, oral contraceptive use, age at first live birth, menopausal status, BMI	Other information: exclusion of participants who reported only prior but not current consumption of either SSBs or ASBs at baseline. *Other strengths*: large, prospective cohort with long follow-up; ability to examine multiple cancer types, stratify by sex or BMI, and limit to never-smokers; comprehensive adjustment for confounders, including SSB consumption. *Other limitations*: likely bias from non-differential exposure misclassification.
			< 1 drink/day	NR	0.93 (0.81–1.07)		
			1 drink/day	NR	0.95 (0.80–1.13)		
			≥ 2 drinks/day	NR	1.01 (0.85–1.21)		
			Continuous (per drink/day)	1693	0.99 (0.95–1.05)		
			Trend-test *P* value: 0.878				
		Uterus/uterine corpus, mortality	ASB consumption, never-smokers (HR): Never	NR	1	Age, race/ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, parity, age at menarche, estrogen use, oral contraceptive use, age at first live birth, menopausal status	
			< 1 drink/day	NR	1.14 (0.96–1.36)		
			1 drink/day	NR	1.07 (0.85–1.33)		
			≥ 2 drinks/day	NR	1.15 (0.91–1.45)		
			Continuous (per drink/day)	1025	1.02 (0.95–1.05)		
			Trend-test *P* value: 0.167				

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2022) (cont.)		Uterus/uterine corpus, mortality	ASB consumption, never-smokers, BMI-adjusted (HR):			Age, race/ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, parity, age at menarche, estrogen use, oral contraceptive use, age at first live birth, menopausal status, BMI	
			Never	NR	1		
			< 1 drink/day	NR	1.03 (0.86–1.22)		
			1 drink/day	NR	0.95 (0.76–1.18)		
			≥ 2 drinks/day	NR	0.98 (0.77–1.24)		
			Continuous (per drink/day)	1025	0.97 (0.91–1.04)		
			Trend-test P value: 0.781				
		Uterus/uterine corpus, mortality	ASB consumption, BMI, 18.5 to < 25 kg/m^2 (HR):				
			Never	NR	1		
			< 1 drink/day	NR	0.89 (0.72–1.11)		
			1 drink/day	NR	0.93 (0.71–1.21)		
			≥ 2 drinks/day	NR	1.03 (0.78–1.37)		
			Continuous (per drink/day)	762	1.00 (0.93–1.09)		
			Trend-test P value: 0.883				
		Uterus/uterine corpus, mortality	ASB consumption, BMI, 25 to < 30 kg/m^2 (HR):				
			Never	NR	1		
			< 1 drink/day	NR	1.07 (0.85–1.36)		
			1 drink/day	NR	0.97 (0.72–1.32)		
			≥ 2 drinks/day	NR	1.19 (0.89–1.59)		
			Continuous (per drink/day)	520	1.00 (0.92–1.1)		
			Trend-test P value: 0.316				

Table 2.4   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2022) (cont.)		Uterus/uterine corpus, mortality	ASB consumption, BMI, ≥ 30 kg/m² (HR):			Age, race/ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, parity, age at menarche, estrogen use, oral contraceptive use, age at first live birth, menopausal status, BMI	
			Never	NR	1		
			< 1 drink/day	NR	0.84 (0.61–1.14)		
			1 drink/day	NR	0.91 (0.64–1.29)		
			≥ 2 drinks/day	NR	0.78 (0.54–1.14)		
			Continuous (per drink/day)	325	0.96 (0.86–1.07)		
			Trend-test *P* value: 0.183				
		Brain, mortality	ASB consumption, BMI-adjusted (HR):			Age, sex, race/ ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR	1		
			< 1 drink/day	NR	1.06 (0.96–1.18)		
			1 drink/day	NR	0.99 (0.87–1.12)		
			≥ 2 drinks/day	NR	0.97 (0.84–1.11)		
			Continuous (per drink/day)	3402	0.98 (0.95–1.02)		
			Trend-test *P* value: 0.737				

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2022) (cont.)		Brain, mortality	ASB consumption, men, BMI-adjusted (HR):			Age, race/ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR	1		
			< 1 drink/day	NR	1.02 (0.86–1.21)		
			1 drink/day	NR	1.06 (0.87–1.29)		
			≥ 2 drinks/day	NR	0.96 (0.77–1.18)		
			Continuous (per drink/day)	1772	0.99 (0.93–1.05)		
			Trend-test P value: 0.928				
		Brain, mortality	ASB consumption, women, BMI-adjusted (HR):				
			Never	NR	1		
			< 1 drink/day	NR	1.10 (0.96–1.26)		
			1 drink/day	NR	0.95 (0.79–1.13)		
			≥ 2 drinks/day	NR	0.98 (0.82–1.18)		
			Continuous (per drink/day)	1630	0.98 (0.93–1.04)		
			Trend-test P value: 0.805				
		Lung, mortality	ASB consumption, BMI-adjusted (HR):			Age, sex, race/ ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR	1		
			< 1 drink/day	NR	0.92 (0.88–0.95)		
			1 drink/day	NR	0.91 (0.86–0.95)		
			≥ 2 drinks/day	NR	0.90 (0.86–0.95)		
			Continuous (per drink/day)	34 381	0.97 (0.96–0.99)		
			Trend-test P value: < 0.0001				

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2022) (cont.)		Lung, mortality	ASB consumption, men, BMI-adjusted (HR):			Age, race/ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR	1		
			< 1 drink/day	NR	0.93 (0.88–0.99)		
			1 drink/day	NR	0.91 (0.85–0.98)		
			≥ 2 drinks/day	NR	0.93 (0.87–1.00)		
			Continuous (per drink/day)	20 121	0.98 (0.96–1.00)		
			Trend-test P value: 0.002				
		Lung, mortality	ASB consumption, women, BMI-adjusted (HR):				
			Never	NR	1		
			< 1 drink/day	NR	0.89 (0.85–0.94)		
			1 drink/day	NR	0.88 (0.83–0.94)		
			≥ 2 drinks/day	NR	0.84 (0.79–0.90)		
			Continuous (per drink/day)	14 260	0.95 (0.94–0.97)		
			Trend-test P value: < 0.0001				
		Lung, mortality	ASB consumption, never-smokers, BMI-adjusted (HR):			Age, sex, race/ ethnicity, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR	1		
			< 1 drink/day	NR	0.92 (0.82–1.03)		
			1 drink/day	NR	1.01 (0.89–1.16)		
			≥ 2 drinks/day	NR	0.99 (0.86–1.14)		
			Continuous (per drink/day)	3546	1.00 (0.96–1.04)		
			Trend-test P value: 0.775				

Table 2.4   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2022) (cont.)		Lung, mortality	ASB consumption, men, never-smokers, BMI-adjusted (HR):			Age, race/ethnicity, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR	1		
			< 1 drink/day	NR	0.95 (0.78–1.16)		
			1 drink/day	NR	1.06 (0.85–1.31)		
			≥ 2 drinks/day	NR	1.08 (0.86–1.36)		
			Continuous (per drink/day)	1545	1.02 (0.96–1.09)		
			Trend-test P value: 0.519				
		Lung, mortality	ASB consumption, women, never-smokers, BMI-adjusted (HR):				
			Never	NR	1		
			< 1 drink/day	NR	0.89 (0.78–1.02)		
			1 drink/day	NR	0.98 (0.83–1.15)		
			≥ 2 drinks/day	NR	0.92 (0.76–1.11)		
			Continuous (per drink/day)	2001	0.98 (0.93–1.04)		
			Trend-test P value: 0.276				

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2022) (cont.)		Ovary, mortality	ASB consumption, women, BMI-adjusted (HR):			Age, race/ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, parity, age at menarche, estrogen use, oral contraceptive use, age at first live birth, menopausal status, BMI	
			Never	NR	1		
			< 1 drink/day	NR	1.05 (0.95–1.16)		
			1 drink/day	NR	1.16 (1.03–1.30)		
			≥ 2 drinks/day	NR	1.01 (0.89–1.15)		
			Continuous (per drink/day)	3225	1.00 (0.97–1.04)		
			Trend-test P value: 0.284				
You et al. (2022) USA Enrolment, 1993–2001/ follow-up through 2009 (median, 11.3 yr) Cohort	92 997; PLCO cancer screening trial participants, men and women aged 55–74 yr in 10 study centres; participants with history of cancer or diabetes were excluded Exposure assessment method: diet history questionnaire: FFQ with 156 items	Lung, incidence	Type of soft drink consumption (HR):			Age, sex, race, study centre, arm, total energy intake, alcohol consumption, smoking status, BMI categories (baseline), physical activity, education, red meat intake, amounts of fruits and vegetables, coffee, family history of lung cancer	*Exposure assessment critique*: A key strength was the timing of exposure consistent for aspartame as major AS in beverages; ASBs were major source of aspartame in this country and time frame.
			No soft drink consumption	173	1		
			Regular only	657	0.87 (0.73–1.03)		
			Diet only	487	0.89 (0.75–1.07)		
			Both	200	0.79 (0.64–0.97)		

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
You et al. (2022) (cont.)		Lung, incidence	Type of soft drink consumption, men (HR):			Age, race, study centre, arm, total energy intake, alcohol consumption, smoking status, BMI categories (baseline), physical activity, education, red meat intake, amounts of fruits and vegetables, coffee, family history of lung cancer	Key limitations were that there was no specific estimate of aspartame exposure, ASBs were used as a proxy; only consumption vs non-consumption was considered (not dose), and exposure data were reported at baseline only.
			No soft drink consumption	77	1		
			Regular only	431	0.98 (0.76–1.26)		
			Diet only	234	1.08 (0.82–1.42)		
			Both	140	0.96 (0.72–1.28)		
		Lung, incidence	Type of soft drink consumption, women (HR):			Age, race, study centre, arm, total energy intake, alcohol consumption, smoking status, BMI categories (baseline), physical activity, education, red meat intake, amounts of fruits and vegetables, coffee, family history of lung cancer, estrogen use	Other information: PLCO is a trial of lung cancer screening but in this study was considered a prospective cohort; allocation to control or intervention arms was not associated with soft drink choice. *Other strengths*: prospective analysis. *Other limitations*: likely bias from non-differential exposure misclassification.
			No soft drink consumption	[96]	1		
			Regular only	[226]	0.80 (0.62–1.02)		
			Diet only	[253]	0.78 (0.61–0.99)		
			Both	[60]	0.62 (0.45–0.87)		

Table 2.4   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
You et al. (2022) (cont.)		Lung, incidence	Type of soft drink consumption, never-smokers (HR):			Age, sex, race, study centre, arm, total energy intake, alcohol consumption, BMI categories (baseline), physical activity, education, red meat intake, amounts of fruits and vegetables, coffee, family history of lung cancer	
			No soft drink consumption	17	1		
			Regular only	47	0.79 (0.45–1.40)		
			Diet only	37	0.65 (0.36–1.18)		
			Both	24	0.85 (0.45–1.61)		
		Lung, incidence	Type of soft drink consumption, ever/current smokers (HR):				
			No soft drink consumption	156	1		
			Regular only	610	0.90 (0.75–1.08)		
			Diet only	450	0.82 (0.68–0.99)		
			Both	176	0.71 (0.57–0.89)		
		Lung (small cell/oat cell), incidence	Type of soft drink consumption (HR):			Age, sex, race, study centre, arm, total energy intake, alcohol consumption, smoking status, BMI categories (baseline), physical activity, education, red meat intake, amounts of fruits and vegetables, coffee, family history of lung cancer	
			No soft drink consumption	26	1		
			Regular only	91	0.78 (0.49–1.22)		
			Diet only	72	0.91 (0.57–1.46)		
			Both	25	0.71 (0.40–1.24)		

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Zamora-Ros et al. (2022) Europe Enrolment, 1992–2000/ follow-up, mean ± SD, 13.9 ± 4.0 yr Cohort	450 064; EPIC study participants, men and women, mostly aged 35–70 yr, from 9 European countries (Denmark, France, Germany, Italy, Netherlands, Norway, Spain, Sweden, and the United Kingdom); excluding participants with prevalent cancer other than nonmelanoma skin cancer at baseline or with extreme energy intake/expenditure; those with missing date of follow-up or lack information on lifestyle factors (Greece did not provided data for this study); data on ASBs was not available for participants from Florence, Ragusa, Turin, Umeå, and Varese; overall 712 thyroid cancers (573 papillary, 108 follicular, 31 otherwise specified)	Thyroid (differentiated: papillary, follicular, otherwise), incidence	ASB consumption (HR): Non-consumers (0 mL/day) 1st tertile (> 0–5.8 mL/day) 2nd tertile (5.9–42.9 mL/ day) 3rd tertile (43.0–3389.5 mL/ day) Continuous (per 100 mL/day) Trend-test *P* value: 0.26	392 29 32 42 495	1 0.88 (0.53–1.46) 0.83 (0.55–1.24) 1.16 (0.80–1.69) 1.00 (0.91–1.11)	Sex, centre, age at recruitment, BMI, smoking status, physical activity, educational level, alcohol and energy intake, and in women also for menopausal status, oral contraceptive use, and infertility problems	*Exposure assessment critique*: A key strength was the prospective assessment of ASB consumption (several types of beverages) in several western European countries at a period relevant for aspartame exposure (between 1991 and 2000). Key limitations were that no other sources of aspartame were considered; uncertainty regarding the aspartame content in ASBs in every country; and there was only one assessment at baseline. Other information: context of low ASB consumption in middle-aged adults. *Other strengths*: prospective large study. *Other limitations*: likely bias from non-differential exposure misclassification.

Table 2.4   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Zamora-Ros et al. (2022) (cont.)	Exposure assessment method: questionnaire; exposure to artificially sweetened soft drinks overall assessed once at baseline through country-specific validated tools (mainly FFQ) covering the usual diet over the past year; no specific assessment of aspartame or AS content of the artificially sweetened soft drinks	Thyroid (papillary), incidence	ASB consumption (HR):			Sex, centre, age at recruitment, BMI, smoking status, physical activity, educational level, alcohol and energy intake, and in women also for menopausal status, oral contraceptive use, and infertility problems	
			Non-consumers (0 mL/day)	385	1		
			1st tertile (> 0–6.6 mL/day)	22	0.95 (0.54–1.67)		
			2nd tertile (6.7–42.9 mL/day)	20	0.73 (0.44–1.19)		
			3rd tertile (43.0–3389.5 mL/day)	42	1.51 (1.04–2.19)		
			Continuous (per 100 mL/day)	469	1.05 (0.95–1.15)		
			Trend-test *P* value: 0.16				
Gurney et al. (1997) USA (Los Angeles, San Francisco) 1984–1991 Case–control	Cases: 56; age ≥ 19 yr; born in 1981 or after, primary brain tumour diagnosed in 1984–1991 Controls: 94; age ≥19 yr; born in 1981 or after, control subjects were recruited using random-digit dialling, frequency-matched on age at diagnosis, year of birth, sex, and study site	Brain, incidence	Consumption of aspartame in any food or drink (OR):			Sex, age at diagnosis, study site, birth year	*Exposure assessment critique*: A key strength was the lifetime exposure of children captured in a relevant period for aspartame (1981–1991 in the USA). Key limitations were the retrospective assessment in a case–control study; only frequency and duration of exposure were assessed (no dose); and unclear sources of aspartame.
			No consumption	39	1		
			Any consumption	17	1.1 (0.5–2.6)		
		Brain, incidence	Age at first consumption of aspartame in any food or drink (OR):			Sex, age at diagnosis, study site, birth year	
			No consumption	NR	1		
			< 3 yr	7	1.0 (0.3–3.1)		
			3–7 yr	10	1.2 (0.4–3.6)		
		Brain, incidence	Duration of consumption of aspartame in any food or drink (OR):			Sex, age at diagnosis, study site, birth year	
			No consumption	NR	1		
			< 2 yr	9	1.2 (0.4–3.3)		
			≥ 2 yr	8	1.1 (0.3–3.4)		

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Gurney et al. (1997) (cont.)	Exposure assessment method: questionnaire; retrospective assessment through in-person interviews of mothers regarding the period and frequency of consumption by their children/during their pregnancy of several sources of aspartame	Brain, incidence	Frequency of consumption of aspartame in any food or drink (OR):			Sex, age at diagnosis, study site, birth year	*Other strengths*: data from food questionnaire; dose–response analysis; in utero exposure. *Other limitations*: small sample size; potential for bias from differential exposure misclassification.
			No consumption	NR	1		
			< 1 time/wk	7	1.6 (0.5–5.2)		
			≥ 1 time/wk	10	0.9 (0.3–2.4)		
		Brain, incidence	Consumption of aspartame in diet drinks (OR):				
			No consumption	47	1		
			Any consumption	9	0.9 (0.3–2.4)		
		Brain, incidence	Age at first consumption of aspartame in diet drinks (OR):				
			No consumption	NR	1		
			< 3 yr	4	0.8 (0.2–3.1)		
			≥ 3 yr	5	1.0 (0.3–3.4)		
		Brain, incidence	Duration of consumption of aspartame in diet drinks (OR):				
			No consumption	NR	1		
			< 2 yr	4	0.8 (0.2–3.1)		
			≥ 2 yr	5	0.9 (0.3–3.4)		
		Brain, incidence	Frequency of consumption of aspartame in diet drinks (OR):				
			No consumption	NR	1		
			< 1 time/wk	5	1.2 (0.3–4.5)		
			≥ 1 time/wk	4	0.6 (0.2–2.3)		

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Gurney et al. (1997) (cont.)		Brain, incidence	Consumption of aspartame in any food or drink during pregnancy or breastfeeding (effect in offspring, OR):			Sex, age at diagnosis, study site, birth year	
			No consumption	40	1		
			Any consumption	9	0.7 (0.3–1.7)		
		Brain, incidence	Consumption of aspartame in any food or drink during pregnancy (effect in offspring, OR):				
			No consumption	NR	1		
			Any consumption	7	0.6 (0.2–1.7)		
		Brain, incidence	Consumption of aspartame in any food or drink during breastfeeding (effect in offspring, OR):				
			No consumption	NR	1		
			Any consumption	5	0.7 (0.2–2.0)		
		Brain, incidence	Consumption of aspartame in diet drinks during pregnancy or breastfeeding (effect in offspring, OR):				
			No consumption	44	1		
			Any consumption	5	0.9 (0.3–2.8)		
		Brain, incidence	Consumption of aspartame in diet drinks during pregnancy (effect in offspring, OR):				
			No consumption	NR	1		
			Any consumption	3	0.7 (0.2–2.7)		
		Brain, incidence	Consumption of aspartame in diet drinks during breastfeeding (effect in offspring, OR):				
			No consumption	NR	1		
			Any consumption	4	1.1 (0.3–4.0)		

Table 2.4   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Gallus et al. (2007) Italy 1991–2004 Case–control	Cases: 460 (larynx), 1031 (ovary); histologically confirmed cancers of larynx (415 men, 45 women; median age, 61 yr), or ovary (1031; median age, 55 yr); > 95% participation Controls: 1088 (larynx), 2411 (ovary); controls admitted to same network of general and teaching hospitals as cases for acute, non-neoplastic disorders; total of 7028 patients (3301 men and 3727 women; 4838 included in more than one study); 24% trauma, 31% other nontraumatic orthopaedic conditions, 17% acute surgical disorders, 28% miscellaneous other diseases; > 95% participation	Larynx, incidence	Consumption of ASs other than saccharin (OR):			Age, sex, study centre, education, tobacco smoking, alcohol drinking, BMI, total energy intake, consumption of hot beverages	*Exposure assessment critique*: Key limitations were that there was no specific assessment of aspartame, aspartame was considered as "other sweeteners" but with unclear actual contribution; only one source considered (tabletop sweeteners); retrospective assessment in a case–control study. Other information: according to the authors, limited consumption of sources of sweeteners (including ASBs) in the study population (middle-aged adults in Italy between 1991 and 2004). *Other strengths*: high response rates (< 5% refusals) for cases and controls reduce the potential for selection bias; large sample size with large case numbers for rarer cancers; control for key potential confounders, including BMI.
			Non-consumers	439	1		
			> 0 sachets or tablets/day	21	1.62 (0.84–3.14)		
		Ovary, incidence	Consumption of ASs other than saccharin (OR):			Age, study centre, education, tobacco smoking, alcohol drinking, BMI, total energy intake, consumption of hot beverages, parity, menopausal status/ age at menopause	
			Non-consumers	958	1		
			> 0 sachets or tablets/day	73	0.75 (0.56–1.00)		

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Gallus et al. (2007) (cont.)	Exposure assessment method: questionnaire; assessment of use of tabletop sweeteners containing either saccharin or other sweeteners as sachets or tablets per week in the 2 yr before cancer diagnosis						*Other limitations*: low AS use; potential for recall bias and reverse causation.
Bosetti et al. (2009) Italy 1997–2007 Case–control	Cases: 454; hospital-based, greater Milan area; cases had histologically confirmed endometrial cancer (median age, 60 yr) Controls: 908; controls selected from same network of general and teaching hospitals as cases for acute, non-neoplastic disorders, frequency-matched on age, sex, and study centre; 25% traumas, 32% nontraumatic orthopaedic conditions, 15% acute surgical disorders, 27% miscellaneous other diseases; > 95% control participation	Uterus/ uterine corpus (endometrium), incidence	Consumption of low-calorie sweeteners other than saccharin (OR): Non-users Users	394 58	1 1.37 (0.96–1.95)	Age, study centre	*Exposure assessment critique*: Key limitations were that there was no specific assessment of aspartame, aspartame was included "other sweeteners" but with unclear actual contribution; only one source considered (tabletop sweeteners); retrospective assessment in a case–control study (potential for differential misclassification). Other information: according to the authors, limited consumption of sources of sweeteners (including ASBs) in the study population (middle-aged adults in Italy between 1991 and 2007).
		Uterus/ uterine corpus (endometrium), incidence	Consumption of low-calorie sweeteners other than saccharin (OR): Non-users Users	394 58	1 1.07 (0.71–1.61)	Age, study centre, year of interview, education, BMI, tobacco smoking, history of diabetes, consumption of hot beverages, total energy intake	

Table 2.4   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Bosetti et al. (2009) (cont.)	Exposure assessment method: questionnaire; assessment of use of tabletop sweeteners containing either saccharin or other sweeteners as several sachets or tablets per week in the 2 yr before cancer diagnosis; exposure to "other sweeteners" considered as ever users vs non-users only						*Other strengths*: > 95% participation rate among controls; adjustment for several key confounders, including BMI and diabetes status. *Other limitations*: exposure assessment limited to users vs non-users which increases the potential for exposure misclassification; low frequency of consumers; potential for reverse causation, recall bias.

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Cabaniols et al. (2011) France (Marseille and Toulon) 2005 Case–control	Cases: 122; recruitment of consecutive new cases of primary malignant brain (glioma grades II–IV) tumour in patients aged > 18 yr Controls: 122; controls, matched on age (± 5 yr) and sex, randomly selected from patients hospitalized for reasons unrelated to cancer in the neurosurgery department Exposure assessment method: self-administered questionnaire with question on aspartame consumption frequency for the past 5 yr	Brain (glioma, grades II–IV), incidence	Aspartame consumption (OR): < 1 time/wk ≥ 1 time/wk	92 30	1 1.02 (0.57–1.85)	Age, sex	*Exposure assessment critique*: Key limitations were the retrospective recall using an unknown question on frequency of aspartame use (question and validity not reported); unclear sources considered. *Other limitations*: small sample size, recall bias; study methodology not well described.

Table 2.4   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Singh et al. (2020) USA 2004–2014 Case–control	Cases: 50; hospital-based study (Mount Sinai, Queens hospital centre, Elmhurst hospital centre); cases were patients undergoing total thyroidectomy for well-differentiated thyroid cancer; all were aged > 18 yr Controls: 50; controls (aged > 18 yr) chosen among patients diagnosed with thyroid nodules through needle aspiration; matching and control recruitment not mentioned Exposure assessment method: telephone based, self-report questionnaire on use of ASs before diagnosis	Thyroid (well-differentiated), incidence	AS consumption (OR): None Any consumption	12 38	1 10.1 (4.01–25.10)	None	*Exposure assessment critique*: Key limitations were the retrospective recall using a questionnaire. no specific assessment of aspartame, only total ASs and only in beverages, multiple sweeteners in products, with aspartame a major contributor but contribution could not be quantified). *Other limitations*: small sample size, the exposure period was unclear with respect to the diagnosis, unclear control selection, control for confounders not reported, control group participants had thyroid nodules; study methodology not well described; statistical methods appear to be non-standard.

AS, artificial sweetener; ASB, artificially sweetened beverage; BMI, body mass index; CI, confidence interval; CPS-II, Cancer Prevention Study-II; EPIC, European Prospective Investigation into Cancer and Nutrition; FDA, Food and Drug Administration; FFQ, food frequency questionnaire; HPFS, Health Professionals Follow-up Study; HR, hazard ratio; MCCS, Melbourne Collaborative Cohort Study; mo, month(s); NHS, Nurses' Health Study; NIH-AARP, National Institutes of Health-American Association of Retired Persons; NR, not reported; OR, odds ratio; PLCO, Prostate, Lung, Colorectal and Ovarian Cancer screening trial; SD, standard deviation; SSB, sugar-sweetened beverage; vs, versus; wk, week(s); yr, year(s).

University of Minnesota ([Nutrition Coordinating Center, 2023](#)). The multivariable data analyses on incident cancer cases accumulated during follow-up (1995–1996 to 2000) found that aspartame was not positively associated with glioma overall (RR for ≥ 400 mg/day versus none, 0.73; 95% CI, 0.46–1.15; *P* for trend, 0.05) or with glioblastoma, the largest subtype of brain tumours (RR for ≥ 400 mg/day versus none, 0.64; 95% CI, 0.37–1.10; *P* for trend, 0.05; 231 cases). The associations were null when the lowest level of aspartame intake was set as the reference category (data not reported in [Table 2.4](#)). [The Working Group noted that, although the researchers applied exposure assessment methods aimed at more specifically estimating consumption of aspartame (separately from that of other artificial sweeteners) and the period of assessment coincided with a relevant period of aspartame exposure from ASBs in the USA, aspartame in beverages in the USA was introduced 10 years before the baseline questionnaire administration; therefore, a certain degree of exposure misclassification may have been introduced by the lack of data before study entry periods. As in other prospective studies, participants were potentially exposed to various and changing types of artificial sweetener during adulthood in the 1980s to 2000s, depending on the evolution of drinks and food products on the market, and these variations were not measured. Therefore, some random measurement error leading to underestimation of a potential association between aspartame and cancer risk was likely. Additionally, this study was limited by a rather short follow-up (5 years).]

The Iowa Women's Health Study Cohort included 23 039 women aged 55–69 years in 1986, who were randomly selected from driver's licence lists in Iowa, USA, and who responded to a mailed questionnaire ([Inoue-Choi et al., 2013](#)). During follow-up (1986–2010), 592 cases of endometrial cancer (type I, 506; type II, 86) were diagnosed. The article reported the BMI-adjusted association between quintiles of sugar-free beverage consumption and risk of endometrial cancer (type I and II separately), before and after exclusion of participants with a history of diabetes. There was no association for endometrial cancer risk in all women or in women without history of diabetes. [The Working Group noted that there was no specific assessment of aspartame, since only ASB consumption was measured. The assessment of ASB consumption (1986) covered a relevant period in the USA for the use of aspartame in beverages. In addition, ASB consumption may have varied at the individual level over time, thus the study was limited by the single baseline assessment. All these uncertainties in exposure assessment were likely to result in non-differential misclassification of aspartame exposure.]

The MCCS included 35 593 participants (14 101 men and 21 492 women) aged 40–69 years ([Hodge et al., 2018](#)). The baseline diet questionnaire, with 121 drinks and food items, included questions about consumption of diet soft drinks (artificially sweetened) by number of times per day or per week. During follow-up (1990–1994 to 2013), 167 cancers of the endometrium and 130 cancers of the ovary were identified. No association was found between frequency of consumption of artificially sweetened soft drinks and risk of endometrial cancer (HR for ≥ 1/day versus < 1/month, 0.81; 95% CI, 0.42–1.55). Risk of ovarian cancer was positively associated with artificially sweetened soft drink consumption; however, with a wide confidence interval (HR for ≥ 1/day versus < 1/month, 1.37; 95% CI, 0.72–2.61). In the combined cohort of the NHS (follow-up, 1980–2014) and the HPFS (follow-up, 1986–2014), there was no association between ASB consumption and lung cancer mortality (HR per 1 serving/day increment, 0.98; 95% CI, 0.92–1.08) ([Malik et al., 2019](#)). [The Working Group noted that the study provided no specific estimate of aspartame exposure. Only ASB consumption was investigated, which was considered by the Working Group as a proxy for aspartame. [Malik et al. (2019)](#) assessed ASB exposure during a time

period that was relevant for aspartame being used as the main artificial sweetener in the USA, although there was more uncertainty about the use of aspartame as the only artificial sweetener in beverages between the mid-2000s and 2010. The dietary questionnaire collected information on consumption frequency only, without portion sizes. Therefore, non-differential misclassification of the exposure to aspartame was likely.]

The CPS-II cohort study included 416 313 men and 518 464 women aged ≥ 28 years at baseline (follow-up, 1982–2016) (McCullough et al., 2022). The baseline questionnaire included a grid that asked how many cups, glasses, or drinks of ASBs were usually drunk per day and for how many years, with write-in reporting by frequency and duration. Diet soda and diet iced teas were considered as ASB, whereas "non-diet colas" and "other non-diet soft drinks" were considered sugar-sweetened beverages. The CPS-II cohort study used cancer mortality as the end-point. The multivariable-adjusted analysis additionally adjusting for BMI found no association between intake of artificial sweeteners and risk of dying from cancer of the brain (HR for ≥ 2 drinks/day versus none, 0.97; 95% CI, 0.84–1.11) or the lung (for the entire cohort, HR per drink of ASB consumed per day, 0.97; 95% CI, 0.96–0.99; and for never-smokers, HR per drink per day, 1.00; 95% CI, 0.96–1.04). The cohort also included 1693 deaths from cancer of the uterus and 3225 deaths from cancer of the ovary. No association was found with the consumption of ASBs after adjustment for BMI. For uterine cancer, the HR for ≥ 2 drinks/day versus never was 1.18 (95% CI, 1.00–1.40; P for trend, 0.049) in the model without adjustment for BMI, and 1.01 (95% CI, 0.85–1.21; P for trend, 0.878) in the model controlling for BMI. The BMI-adjusted overall relative risk expressed per drink per day was 0.99 (95% CI, 0.95–1.05) for endometrial cancer and 1.00 (95% CI, 0.97–1.04) for ovarian cancer.

[The Working Group noted that the exposure assessment took place in 1982, 1 year before the approval of aspartame use in food or beverages in the USA. The Working Group considered that this study was relevant for aspartame exposure, given the published data on the participants of the CPS-II cohort who were also in the CPS-II nutrition cohort, which showed consistent relative ranking of consumption of ASBs over 17 years of follow-up (McCullough et al., 2014). However, there was large potential for random error in the exposure measurement and in confounders, with only one exposure measurement at baseline and a median follow-up of 27.7 years.]

The PLCO cohort (follow-up, 1993–2001 to 2009) reported on diet soft drink consumption without further specification of the type of artificial sweetener or type of drink, using an FFQ containing 156 items (You et al., 2022). The overall analyses (including both men and women, and both smokers and never-smokers, adjusted for smoking, sex, and other potential confounders) found a hazard ratio of 0.89 (95% CI, 0.75–1.07) for consuming only diet soft drink compared with no consumption of any soft drink. [The Working Group noted that the relative risk for consumption of only regular soft drinks versus no consumption of any soft drinks was very similar (RR, 0.87; 95% CI, 0.73–1.03).] Separate analyses restricted to never-smokers, to men only, and to women only, found similar estimates of relative risk, all close to or below 1 (see Table 2.4), suggesting no association between lung cancer risk and the consumption of diet soft drinks compared with no soft drink consumption. [The Working Group noted that the assessment of consumption of ASBs in this study was consistent with a period when aspartame was used as the major sweetener in ASBs in the USA; however, the lack of information on intake before baseline may result in potential non-differential exposure misclassification. The lack of assessment of consumption of drinks and foods potentially containing aspartame and other artificial

sweeteners may also result in non-differential exposure misclassification.]

The EPIC study examined the association between artificial sweeteners and thyroid cancer (Zamora-Ros et al., 2022). The investigation included 450 064 adults from 10 European countries. Dietary intake was assessed using validated country-specific dietary questionnaires. After a mean follow-up time of 14 years, 712 first primary differentiated thyroid cancers were diagnosed, but the analysis on ASBs included 495 thyroid cancer cases, because data on ASB consumption were not available in some centres. The main purpose of this study was to analyse the association between dietary patterns and thyroid cancer risk. In addition, results were presented for specific food groups, including sugar-sweetened beverages and ASBs. Overall, the study found little evidence of an association between consumption of ASBs and thyroid cancer risk. The hazard ratio comparing the higher tertile of consumption of ASBs with no consumption was 1.16 (95% CI, 0.80–1.69).

[The Working Group noted that the study measured ASBs, but there was no specific methodology aimed at the assessment of aspartame consumption. However, despite uncertainty regarding the aspartame content of artificially sweetened soft drinks, the study was conducted during a time period that was relevant for aspartame exposure from ASBs. Potential variations in the aspartame content of available artificially sweetened soft drinks over time was not measured, since the assessment was performed at baseline only; therefore, there was potential for non-differential misclassification of aspartame exposure.]

One case–control study from USA investigated the risk of brain tumours in childhood in relation to consumption of aspartame during infancy and childhood (Gurney et al., 1997). Information was collected from the biological mothers of the cases and the matched controls. The study also investigated the association between consumption of aspartame during pregnancy and subsequent risk of brain tumours in the offspring. Overall, the results indicated no association between aspartame consumption and brain tumour risk in childhood (OR, 1.1; 95% CI, 0.5–2.6), or between aspartame consumption during pregnancy and brain tumour risk in the offspring (OR, 0.7; 95% CI, 0.3–1.7). [The Working Group noted that only information on frequency of consumption and duration of exposure was ascertained, but portion sizes were not collected. Exposure referred to the period between 1981 and 1991 in the USA, potentially capturing lifetime exposure to aspartame in the USA. However, the lack of specificity regarding food source and the retrospective assessment may have resulted in measurement errors. Therefore, there was potential for inadequate extrapolation to exposure to aspartame, and possible differential misclassification because of retrospective assessment in a case–control study.]

A case–control study of 460 cases of laryngeal cancer and 1031 cases of ovarian cancer were conducted in 1991–2004 in Italy, with controls selected from the same network of general and teaching hospitals (Gallus et al., 2007). After adjustment for age, sex, study centre, education, tobacco smoking, alcohol drinking, BMI, total energy intake, and consumption of hot beverages, the study found a positive but imprecise association between the consumption of artificial sweeteners other than saccharin and laryngeal cancer risk (OR, 1.62; 95% CI, 0.84–3.14), and an inverse association with ovarian cancer risk (OR, 0.75; 95% CI, 0.56–1.00) in models further adjusted for parity, menopausal status, and age at menopause).

Another case–control study was conducted in Italy (Bosetti et al., 2009), similarly to the hospital-based case–control research programme described above (Gallus et al., 2007). The study included 454 cases of histologically confirmed endometrial cancer and 908 controls with acute, non-neoplastic disorders and selected from same

network of general and teaching hospitals as the cases. Statistical analyses adjusting for age, study centre, year of interview, education, BMI, tobacco smoking, history of diabetes, consumption of hot beverages, and total energy intake found no association between use of artificial sweeteners other than saccharin and endometrial cancer risk (OR, 1.07; 95% CI, 0.71–1.61). [The Working Group noted that for both studies, Bosetti et al. (2009) and Gallus et al. (2007), aspartame intake was estimated on the basis of use of tabletop sweetener sachets or tablets other than saccharin. The assumption that these sweeteners were actually aspartame was based on data from teenagers in 2000 (Arcella et al., 2004) and 1996 (Leclercq et al., 1999) that may not be valid, since the consumption of foods by adults and teenagers may differ. In addition, it was not clear whether aspartame was widely used in Italy over the whole data collection period, and there was no consideration of exposure from ASBs, although the authors claimed that such exposure was limited in middle-aged elderly Italian people at the time of study assessment.]

A small case–control study on brain cancer, conducted in France in 2005, was primarily focused on personal habits and psychological stress (Cabaniols et al., 2011). The study reported no association with aspartame. [The Working Group noted that the overall methodology of the study was only very briefly described in the article, and it did not specify how aspartame exposure was measured or how it was separated from generic exposure to artificial sweeteners.]

A small case–control study carried out in 2004–2014 in the USA included 50 patients diagnosed with well-differentiated thyroid cancer and 50 control patients diagnosed with benign thyroid nodules (Singh et al., 2020). Artificial sweetener consumption was assessed by a telephone interview and included questions on amount and duration of artificial sweetener consumption. A crude odds ratio was reported (OR, 10.1; 95% CI, 4.01–25.10) based on 38 cases

and 12 controls who reported consumption of artificial sweetener. [The Working Group noted that the description of the study methodology was very limited, and it appeared that it was not analysed according to the epidemiological standards for case–control studies, i.e. the covariates used in the analysis were not specified and controls were not matched.]

Yin et al. (2022) reported a meta-analysis of studies evaluating ASBs (without restriction to time during which aspartame was used) and risk of glioma or cancers of the endometrium, ovary, and thyroid. [The Working Group determined that this meta-analysis did not contribute useful information, since its aim was to study the association between ASBs and cancer, without specific consideration of aspartame. Also, only one study on glioma and ovarian and thyroid cancers was included, and only two studies on endometrial cancer (Inoue-Choi et al., 2013; Hodge et al., 2018).]

2.5 Cancers of lymphatic and haematopoietic tissues

See Table 2.5.

A total of four original prospective cohort studies conducted in the USA (Lim et al., 2006; Schernhammer et al., 2012; McCullough et al., 2014, 2022), one case–control study in Spain (Palomar-Cros et al., 2023), and four meta-analyses (Toews et al., 2019; WHO et al., 2022; Yin et al., 2022; Pan et al., 2023) reported results on the association between proxies of aspartame exposure and risk of lymphatic and haematopoietic cancers.

The NIH-AARP Diet and Health Study cohort included 285 079 men and 188 905 women in the USA (Lim et al., 2006). During follow-up (1995–2000), 1888 haematopoietic cancers were ascertained by cancer registries. Estimated daily aspartame intake was derived from responses to a limited baseline self-administered FFQ on

Table 2.5 Epidemiological studies on consumption of aspartame and cancers of lymphatic and haematopoietic tissues

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Lim et al. (2006) USA Enrolment, 1995–1996/ follow-up, through 2000 Cohort	473 984 (285 079 men and 188 905 women); NIH-AARP Diet and Health Study: AARP members aged 50– 71 yr residing in the study area (California, Florida, Pennsylvania, New Jersey, North Carolina, Louisiana, Atlanta, Detroit) excluding those with prevalent cancer, invalid BMI data, outliers on reported energy intake, and proxy responders Exposure assessment method: FFQ at baseline, 5 yr of follow- up; consumption frequency of three types of soft drinks (soda, fruit drinks, and iced tea) + frequency of consumption of diet vs regular versions + three portion sizes; use of tabletop sweetener packets; standard doses of aspartame assigned to beverages and packets	All haematopoietic cancers, incidence	Aspartame intake (RR):			Age at entry, sex, ethnicity, BMI, history of diabetes	*Exposure assessment critique*: A key strength was the prospective and quantitative assessment of aspartame exposure from various ASBs and tabletop packets in a relevant period for aspartame (1995–1996 in the USA). Key limitations were the sequential assessment of first the frequency of consumption of soft drinks and then of diet/sugar-free versions with imprecise frequencies inducing inaccuracies; other sources of aspartame were not considered (although these were more limited). *Other strengths*: large cohort; case ascertainment based on cancer registries with high (> 90%) completeness; large numbers of cases. *Other limitations*: potential for bias from non-differential exposure misclassification due to older age at enrolment and a lack of data before study entry (aspartame in beverages introduced in the USA 10 yr before baseline).
			None	869	1		
			> 0, < 100 mg/day	432	0.91 (0.81–1.03)		
			≥ 100, < 200 mg/day	280	1.10 (0.96–1.26)		
			≥ 200, < 400 mg/day	137	1.01 (0.84–1.21)		
			≥ 400, < 600 mg/day	104	1.05 (0.85–1.29)		
			≥ 600 mg/day	66	0.98 (0.76–1.27)		
			Trend-test *P* value: 0.56				
		All haematopoietic cancer, incidence	Aspartame intake, men (RR):			Age at entry, ethnicity, BMI, history of diabetes	
			None	615	1		
			> 0, < 100 mg/day	294	0.95 (0.83–1.10)		
			≥ 100, < 200 mg/day	187	1.09 (0.92–1.29)		
			≥ 200, < 400 mg/day	95	1.03 (0.83–1.29)		
			≥ 400, < 600 mg/day	76	1.07 (0.84–1.36)		
			≥ 600 mg/day	51	1.06 (0.79–1.42)		
			Trend-test *P* value: 0.42				

Table 2.5   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Lim et al. (2006) (cont.)		All haematopoietic cancers, incidence	Aspartame intake, women (RR):			Age at entry, ethnicity, BMI, history of diabetes	
			None	254	1		
			> 0, < 100 mg/day	138	0.83 (0.67–1.02)		
			≥ 100, < 200 mg/day	93	1.11 (0.87–1.41)		
			≥ 200, < 400 mg/day	42	0.95 (0.68–1.32)		
			≥ 400, < 600 mg/day	28	1.02 (0.69–1.51)		
			≥ 600 mg/day	15	0.80 (0.47–1.35)		
			Trend-test P value: 0.87				
		Hodgkin lymphoma, incidence	Aspartame intake (RR):				
			None	29	1		
			> 0 mg/day	28	0.77 (0.44–1.32)		
			Trend-test P value: 0.34				
		Multiple myeloma, incidence	Aspartame intake (RR):				
			None	127	1		
			> 0, < 100 mg/day	57	0.85 (0.62–1.17)		
			≥ 100, < 200 mg/day	48	1.39 (0.99–1.96)		
			≥ 200, < 400 mg/day	20	1.13 (0.70–1.83)		
			≥ 400 mg/day	21	1.03 (0.64–1.66)		
			Trend-test P value: 0.40				

Table 2.5   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Lim et al. (2006) (cont.)		NHL, overall, incidence	Aspartame intake (RR):			Age at entry, sex, ethnicity, BMI, history of diabetes	
			None	592	1		
			> 0, < 100 mg/day	300	0.93 (0.81–1.07)		
			≥ 100, < 200 mg/day	177	1.01 (0.85–1.19)		
			≥ 200, < 400 mg/day	92	0.98 (0.78–1.22)		
			≥ 400, < 600 mg/day	73	1.06 (0.82–1.35)		
			≥ 600 mg/day	45	0.95 (0.70–1.29)		
			Trend-test P value: 0.91				
		NHL (SLL and CLL), incidence	Aspartame intake (RR):				
			None	109	1		
			> 0, < 100 mg/day	60	1.03 (0.75–1.41)		
			≥ 100, < 200 mg/day	36	1.16 (0.79–1.71)		
			≥ 200 mg/day	36	1.02 (0.69–1.52)		
			Trend-test P value: 0.84				
		NHL (immunoblastic lymphoma and lymphoblastic lymphoma/ leukaemia), incidence	Aspartame intake (RR):				
			None	23	1		
			> 0 mg/day	22	0.77 (0.42–1.42)		
			Trend-test P value: 0.40				

Table 2.5 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Lim et al. (2006) (cont.)		Non-lymphoid leukaemia), incidence	Aspartame intake (RR):			Age at entry, sex, ethnicity, BMI, history of diabetes	
			None	121	1		
			> 0, < 100 mg/day	62	0.93 (0.68–1.27)		
			≥ 100, < 200 mg/day	47	1.31 (0.93–1.85)		
			≥ 200, < 400 mg/day	20	1.05 (0.65–1.70)		
			≥ 400 mg/day	29	1.25 (0.82–1.91)		
			Trend-test P value: 0.19				
Schernhammer et al. (2012) USA Enrolment, 1976 (NHS), 1986 (HPFS)/ follow-up, 1984–2006 (NHS), 1986–2006 (HPFS Cohort	125 028 (77 218 women and 47 810 men). 1324 NHL, 285 multiple myeloma, and 339 leukaemia; Women of the NHS were included in 1976: registered nurses, aged 30–55 yr; men of the HPFS were included in 1986: dentists, veterinarians, pharmacists, optometrists, podiatrists, and osteopaths, aged 40–75 yr; participants reporting any previous diagnosis of cancer were excluded	NHL, incidence	Frequency of diet soda consumption, men (RR):			Age, questionnaire cycle, sugar-sweetened soda consumption, fruit and vegetable consumption, multivitamin use, intakes of alcohol, saturated fat, animal protein, and total energy; race, BMI, height, discretionary physical activity, smoking history	*Exposure assessment critique*: A key strength was the prospective quantitative assessment of aspartame exposure as a cumulative average from repeated diet assessments every 4 yr; aspartame was assessed from diet sodas and aspartame packets using assigned aspartame content at a very relevant period for aspartame exposure from ASBs (the USA between 1984 and 2006) potentially capturing lifetime exposure to aspartame. A key limitation was that other sources of aspartame including non-soda ASBs were not considered (although these were more limited).
			None	172	1		
			< 1 serving/wk	122	1.12 (0.88–1.43)		
			1–3.9 servings/ wk	124	1.06 (0.83–1.34)		
			4–6.9 servings/ wk	53	0.96 (0.69–1.32)		
			≥ 1 serving/day	100	1.31 (1.01–1.72)		
			Trend-test P value: 0.11				

Table 2.5   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Schernhammer et al. (2012) (cont.)	Exposure assessment method: assessment of aspartame exposure from diet soda and sachets of aspartame through repeated FFQ (every 4 yr); assigned values of aspartame for packets and sodas (weighted average of representative sodas)	NHL, incidence	Frequency of diet soda consumption, women (RR):			Age, questionnaire cycle, sugar-sweetened soda consumption, fruit and vegetable consumption, multivitamin use, intakes of alcohol, saturated fat, animal protein, and total energy; race, BMI, height, discretionary physical activity, smoking history, menopausal status and use of HRT (women only)	*Other strengths*: length of follow-up; large number of haematopoietic cancer cases overall. *Other limitations*: potential for bias from non-differential exposure misclassification.
			None	189	1		
			< 1 serving/wk	167	0.98 (0.79–1.22)		
			1–3.9 servings/ wk	173	0.90 (0.72–1.11)		
			4–6.9 servings/ wk	87	0.85 (0.65–1.10)		
			≥ 1 serving/day	137	1.00 (0.78–1.26)		
			Trend-test *P* value: 0.999				
		NHL, incidence	Frequency of diet soda consumption (RR):				
			None	361	1		
			< 1 serving/wk	289	1.04 (0.89–1.22)		
			1–3.9 servings/ wk	297	0.96 (0.82–1.13)		
			4–6.9 servings/ wk	140	0.89 (0.72–1.09)		
			≥ 1 serving/day	237	1.13 (0.94–1.34)		
			Trend-test *P* value: 0.28				

Table 2.5   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Schernhammer et al. (2012) (cont.)		NHL, incidence	Aspartame intake, men, follow-up 1994–2006 (RR):			Age, questionnaire cycle, sugar-sweetened soda consumption, fruit and vegetable consumption, multivitamin use, intakes of alcohol, saturated fat, animal protein, and total energy, race, BMI; height; discretionary physical activity, smoking history	
			None	95	1		
			< 19 mg/day	55	0.92 (0.65–1.29)		
			19–59 mg/day	65	1.13 (0.82–1.57)		
			60–142 mg/day	49	0.98 (0.68–1.40)		
			≥ 143 mg/day	69	1.64 (1.17–2.29)		
			Trend-test *P* value: 0.002				

Table 2.5 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Schernhammer et al. (2012) (cont.)		NHL, incidence	Aspartame intake, women, follow-up 1994–2006 (RR):			Age, questionnaire cycle, total sugar intake, fruit and vegetable consumption, multivitamin use, intakes of alcohol, saturated fat, animal protein, and total energy, race, BMI, height, discretionary physical activity, smoking history, menopausal status and use of HRT (women only)	
			None	172	1		
			< 19 mg/day	114	0.94 (0.74–1.20)		
			19–55 mg/day	110	0.96 (0.75–1.22)		
			56–128 mg/day	91	0.83 (0.64–1.08)		
			≥ 129 mg/day	86	0.91 (0.69–1.20)		
			Trend-test *P* value: 0.48				
		NHL, incidence	Aspartame intake, follow-up 1994–2006 (RR):				
			None	267	1		
			< 19 mg/day	169	0.93 (0.76–1.13)		
			19–59 mg/day (men), 19–55 mg/day (women)	175	1.02 (0.83–1.24)		
			60–142 mg/day (men), 56–128 mg/day (women)	140	0.88 (0.71–1.09)		
			≥ 143 mg/day (men), ≥ 129 mg/day (women)	155	1.16 (0.93–1.43)		
			Trend-test *P* value: 0.12				

Table 2.5   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Schernhammer et al. (2012) (cont.)		NHL, incidence	Frequency of diet soda consumption, men, low alcohol intake (< 6 g/day) (RR):			Age, questionnaire cycle, sugar-sweetened soda consumption, fruit and vegetable consumption, multivitamin use, intakes of alcohol, saturated fat, animal protein and total energy, race, BMI, height, discretional physical activity, and smoking history	
			None	82	1		
			< 1 serving/wk	51	0.99 (0.69–1.43)		
			1–3.9 servings/ wk	66	1.27 (0.90–1.79)		
			4–6.9 servings/ wk	22	0.85 (0.52–1.39)		
			1–1.9 servings/ day	27	1.02 (0.64–1.61)		
			≥ 2 servings/day	29	2.34 (1.46–3.76)		
			Trend-test P value: 0.004				
		NHL, incidence	Frequency of diet soda consumption, men, high alcohol intake (≥ 6 g/day) (RR):				
			None	90	1		
			< 1 serving/wk	71	1.20 (0.86–1.67)		
			1–3.9 servings/ wk	58	0.84 (0.59–1.2)		
			4–6.9 serving/ wk	31	0.99 (0.64–1.52)		
			1–1.9 servings/ day	34	1.19 (0.77–1.82)		
			≥ 2 serving/day	10	0.96 (0.48–1.90)		
			Trend-test P value: 0.98				

Table 2.5 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Schernhammer et al. (2012) (cont.)		Multiple myeloma, incidence	Frequency of diet soda consumption, men (RR):			Age, questionnaire cycle, sugar-sweetened soda consumption, fruit and vegetable consumption, multivitamin use, intakes of alcohol, saturated fat, animal protein, and total energy, race, BMI, height, discretionary physical activity, smoking history	
			None	40	1		
			< 1 serving/wk	27	1.17 (0.70–1.96)		
			1–3.9 servings/wk	23	1.04 (0.61–1.78)		
			4–6.9 servings/wk	12	1.08 (0.55–2.12)		
			≥ 1 serving/day	29	2.02 (1.20–3.40)		
			Trend-test P value: 0.01				

Table 2.5   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Schernhammer et al. (2012) (cont.)		Multiple myeloma, incidence	Frequency of diet soda consumption, women (RR):			Age, questionnaire cycle, sugar-sweetened soda consumption, fruit and vegetable consumption, multivitamin use, intakes of alcohol, saturated fat, animal protein, and total energy, race, BMI, height, discretionary physical activity, smoking history, menopausal status and use of HRT (women only)	
			None	39	1		
			< 1 serving/wk	28	0.71 (0.43–1.17)		
			1–3.9 servings/ wk	40	0.86 (0.54–1.37)		
			4–6.9 servings/ wk	23	0.95 (0.55–1.63)		
			≥ 1 serving/day	24	0.79 (0.45–1.36)		
			Trend-test *P* value: 0.79				
		Multiple myeloma, incidence	Frequency of diet soda consumption (RR):				
			None	79	1		
			< 1 serving/wk	55	0.91 (0.63–1.30)		
			1–3.9 servings/ wk	63	0.94 (0.66–1.33)		
			4–6.9 servings/ wk	35	1.00 (0.65–1.52)		
			≥ 1 serving/day	53	1.29 (0.89–1.89)		
			Trend-test *P* value: 0.10				

Table 2.5   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Schernhammer et al. (2012) (cont.)		Multiple myeloma, incidence	Aspartame intake, men, follow-up 1994–2006: (RR):			Age, questionnaire cycle, sugar-sweetened soda consumption, fruit and vegetable consumption, multivitamin use, intakes of alcohol, saturated fat, animal protein, and total energy, race, BMI, height, discretionary physical activity, smoking history	
			None	10	1		
			< 19 mg/day	17	3.33 (1.48–7.49)		
			19–59 mg/day	11	1.70 (0.68–4.23)		
			60–142 mg/day	14	2.96 (1.25–6.96)		
			≥ 143 mg/day	13	3.36 (1.38–8.19)		
			Trend-test *P* value: 0.05				

Table 2.5   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
[Schernhammer et al. (2012)](#) (cont.)		Multiple myeloma, incidence	Aspartame intake, women, follow-up 1994–2006 (RR):			Age, questionnaire cycle, total sugar intake, fruit and vegetable consumption, multivitamin use, intakes of alcohol, saturated fat, animal protein, and total energy, race, BMI, height, discretionary physical activity, smoking history, menopausal status and use of HRT (women only)	
			None	45	1		
			< 19 mg/day	14	0.40 (0.22–0.74)		
			19–55 mg/day	25	0.76 (0.46–1.27)		
			56–128 mg/day	25	0.83 (0.50–1.39)		
			≥ 129 mg/day	15	0.59 (0.32–1.09)		
			Trend-test *P* value: 0.48				
		Multiple myeloma, incidence	Aspartame intake, follow-up 1994–2006 (RR):				
			None	55	1		
			< 19 mg/day	31	0.86 (0.53–1.41)		
			19–59 mg/day (men), 19–55 mg/day (women)	36	0.92 (0.59–1.44)		
			60–142 mg/day (men), 56–128 mg/day (women)	39	1.16 (0.75–1.81)		
			≥ 143 mg/day (men), ≥ 129 mg/day (women)	28	1.03 (0.62–1.72)		
			Trend-test *P* value: 0.44				

Table 2.5 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Schernhammer et al. (2012) (cont.)		Multiple myeloma, incidence	Frequency of diet soda consumption, men, low alcohol intake (< 6 g/day) (RR):			Age, questionnaire cycle, sugar-sweetened soda consumption, fruit and vegetable consumption, multivitamin use, intakes of alcohol, saturated fat, animal protein and total energy, race, BMI, height, discretional physical activity, and smoking history	
			None	17	1		
			< 1 servings/wk	17	1.98 (0.95–4.11)		
			1–3.9 servings/ wk	10	1.38 (0.60–3.17)		
			4–6.9 servings/ wk	6	1.51 (0.56–4.08)		
			≥ 1 serving/day	19	3.79 (1.80–8.00)		
			Trend-test *P* value: 0.002				
		Multiple myeloma, incidence	Frequency of diet soda consumption, men, high alcohol intake (≥ 6 g/day) (RR):				
			None	23	1		
			< 1 serving/wk	10	0.63 (0.27–1.48)		
			1–3.9 servings/ wk	13	0.86 (0.41–1.79)		
			4–6.9 servings/ wk	6	0.73 (0.28–1.89)		
			≥ 1 serving/day	10	0.98 (0.43–2.23)		
			Trend-test *P* value: 0.85				
		Leukaemia, incidence	Frequency of diet soda consumption, men (RR):				
			None	52	1		
			< 1 serving/wk	33	1.07 (0.68–1.68)		
			1–3.9 servings/ wk	49	1.51 (1.00–2.28)		
			4–6.9 servings/ wk	19	1.29 (0.75–2.24)		
			≥ 1 serving/day	33	1.47 (0.92–2.35)		
			Trend-test *P* value: 0.13				

Table 2.5   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Schernhammer et al. (2012) (cont.)		Leukaemia, incidence	Frequency of diet soda consumption, women (RR):			Age, questionnaire cycle, sugar-sweetened soda consumption, fruit and vegetable consumption, multivitamin use, intakes of alcohol, saturated fat, animal protein, and total energy, race, BMI, height, discretionary physical activity, smoking history, menopausal status, and use of HRT (women only)	
			None	33	1		
			< 1 serving/wk	31	1.04 (0.63–1.73)		
			1–3.9 servings/wk	37	1.05 (0.64–1.72)		
			4–6.9 servings/wk	21	1.21 (0.68–2.17)		
			≥ 1 serving/day	31	1.36 (0.80–2.31)		
			Trend-test *P* value: 0.20				
		Leukaemia, incidence	Frequency of diet soda consumption (RR):				
			None	85	1		
			< 1 serving/wk	64	1.06 (0.75–1.48)		
			1–3.9 servings/wk	86	1.30 (0.95–1.78)		
			4–6.9 servings/wk	40	1.26 (0.84–1.87)		
			≥ 1 serving/day	64	1.42 (1.00–2.02)		
			Trend-test *P* value: 0.05				

Table 2.5 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Schernhammer et al. (2012) (cont.)		Leukaemia, incidence	Aspartame intake, men, follow-up 1994–2006 (RR):			Age, questionnaire cycle, sugar-sweetened soda consumption, fruit and vegetable consumption, multivitamin use, intakes of alcohol, saturated fat, animal protein, and total energy, race, BMI, height, discretionary physical activity, smoking history	
			None	23	1		
			< 19 mg/day	14	0.89 (0.45–1.77)		
			19–59 mg/day	23	1.69 (0.91–3.12)		
			60–142 mg/day	19	1.55 (0.81–2.94)		
			≥ 143 mg/day	18	1.56 (0.79–3.06)		
			Trend-test P value: 0.17				

Table 2.5   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Schernhammer et al. (2012) (cont.)		Leukaemia, incidence	Aspartame intake, women, follow-up 1994–2006 (RR):			Age, questionnaire cycle, total sugar intake, fruit and vegetable consumption, multivitamin use, intakes of alcohol, saturated fat, animal protein, and total energy, race, BMI, height, discretionary physical activity, smoking history, menopausal status and use of HRT (women only)	
			None	34	1		
			< 19 mg/day	21	0.85 (0.48–1.58)		
			19–55 mg/day	32	1.34 (0.81–2.21)		
			56–128 mg/day	21	0.95 (0.54–1.66)		
			≥ 129 mg/day	21	1.04 (0.58–1.85)		
			Trend-test P value: 0.94				
		Leukaemia, incidence	Aspartame intake, follow-up 1994–2006 (RR):				
			None	57	1		
			< 19 mg/day	35	0.86 (0.56–1.33)		
			19–59 mg/day (men), 19–55 mg/day (women)	55	1.47 (1.00–2.17)		
			60–142 mg/day (men), 56–128 mg/day (women)	40	1.17 (0.77–1.79)		
			≥ 143 mg/day (men), ≥ 129 mg/day (women)	39	1.23 (0.80–1.91)		
			Trend-test P value: 0.31				

Table 2.5 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Schernhammer et al. (2012) (cont.)		Leukaemia, incidence	Frequency of diet soda consumption, men, low alcohol intake (< 6 g/day) (RR):			Age, questionnaire cycle, sugar-sweetened soda consumption, fruit and vegetable consumption, multivitamin use, intakes of alcohol, saturated fat, animal protein and total energy, race, BMI, height, discretional physical activity, and smoking history	
			None	20	1		
			< 1 serving/wk	21	1.76 (0.91–3.37)		
			1–3.9 servings/ wk	25	2.14 (1.13–4.07)		
			4–6.9 servings/ wk	6	1.09 (0.41–2.88)		
			≥ 1 serving/day	20	1.83 (0.91–3.67)		
			Trend-test *P* value: 0.38				
		Leukaemia, incidence	Frequency of diet soda consumption, men, high alcohol intake (≥ 6 g/day) (RR):				
			None	32	1		
			< 1 serving/wk	12	0.58 (0.29–1.17)		
			1–3.9 servings/ wk	24	1.11 (0.63–1.97)		
			4–6.9 servings/ wk	13	1.44 (0.72–1.90)		
			≥ 1 serving/day	13	1.05 (0.52–2.10)		
			Trend-test *P* value: 0.40				

Table 2.5   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2014) USA Enrolment, 1999/follow-up, until 30 June 2009 Cohort	100 442 men and women; participants were adults (aged 47–95 yr) from the CPS-II nutrition cohort who returned the 1999 FFQ Exposure assessment method: aspartame exposure assessed quantitatively through an FFQ in 1999 and updated in 2003 for consumption of artificially sweetened carbonated beverage and aspartame packet; aspartame content values assigned by type for all carbonated beverages	NHL, all (including multiple myeloma), incidence	Aspartame intake quintile (RR):			Age at baseline, sex, history of diabetes, BMI, smoking status, energy intake, sugar-sweetened carbonated beverage intake	*Exposure assessment critique*: Key strengths were the prospective quantitative assessment of aspartame intake from carbonated ASBs (aspartame values assigned by type of ASB) and aspartame packets; and that exposure was assessed twice at a very relevant period for aspartame exposure through ASBs (the USA in 1999 and 2003). A key limitation was that other sources were not considered (although these were more limited). *Other strengths*: large number of NHL cases. *Other limitations*: potential for bias from non-differential exposure misclassification.
			1st quintile (median, 0 mg/day)	230	1		
			2nd quintile (median, 3.6 mg/day)	266	1.29 (1.08–1.54)		
			3rd quintile (median, 12.6 mg/day)	260	1.27 (1.06–1.52)		
			4th quintile (median, 35.8 mg/day)	234	1.10 (0.91–1.32)		
			5th quintile (median, 145 mg/day)	206	1.02 (0.84–1.24)		
			Continuous (per 50 mg/day)	1196	0.99 (0.95–1.03)		
			Trend-test *P* value: 0.69				
		NHL, all (including multiple myeloma), incidence	Aspartame intake quintile, men (RR):			Age at baseline, history of diabetes, BMI, smoking status, energy intake, sugar-sweetened carbonated beverage intake	
			1st quintile (median, 0 mg/day)	114	1		
			2nd quintile (median, 4.9 mg/day)	141	1.36 (1.05–1.74)		
			3rd quintile (median, 14.4 mg/day)	136	1.43 (1.11–1.84)		
			4th quintile (median, 47.6 mg/day)	122	1.17 (0.90–1.52)		

Table 2.5 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2014) (cont.)			5th quintile (median, 155 mg/day)	120	1.23 (0.94–1.61)		
			Continuous (per 50 mg/day)	633	1.01 (0.97–1.06)		
			Trend-test *P* value: 0.38				
		NHL, all (including multiple myeloma), incidence	Aspartame intake quintile, women (RR):			Age at baseline, history of diabetes, BMI, smoking status, energy intake, sugar-sweetened carbonated beverage intake	
			1st quintile (median, 0 mg/day)	116	1		
			2nd quintile (median, 3.6 mg/day)	125	1.21 (0.93–1.56)		
			3rd quintile (median, 9.8 mg/day)	124	1.11 (0.86–1.44)		
			4th quintile (median, 31.9 mg/day)	112	1.02 (0.78–1.33)		
			5th quintile (median, 127 mg/day)	86	0.82 (0.61–1.10)		
			Continuous (per 50 mg/day)	563	0.94 (0.88–1.01)		
			Trend-test *P* value: 0.12				

Table 2.5 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2014) (cont.)		NHL, all (including multiple myeloma), incidence	Artificially sweetened carbonated beverage intake (RR):			Age at baseline, sex, history of diabetes, BMI, smoking status, energy intake, sugar-sweetened carbonated beverage intake	
			Nondrinkers	331	1		
			> 0–3 cans/mo	424	1.14 (0.98–1.32)		
			1–4 cans/wk	303	0.97 (0.82–1.14)		
			5–6 cans/wk	40	0.77 (0.56–1.08)		
			≥ 1 can/day	98	0.92 (0.73–1.17)		
			Continuous (per 1 can/day)	1196	1.00 (0.98–1.01)		
			Trend-test P value: 0.14				
		NHL, incidence	Long-term artificially sweetened carbonated beverage intake, men (RR):			Age at baseline, sex, smoking status, BMI, weight change from 1982 to 1999, history of diabetes, energy, long-term intake of sugar-sweetened carbonated beverage	
			No past & no current	61	1		
			Low past & low current	135	1.08 (0.79–1.48)		
			High past & low current	56	1.25 (0.84–1.87)		
			Low past & high current	25	0.87 (0.54–1.41)		
			High past & high current	37	1.17 (0.74–1.83)		

Table 2.5 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2014) (cont.)		NHL, incidence	Long-term artificially sweetened carbonated beverage intake, women (RR):			Age at baseline, sex, smoking status, BMI, weight change from 1982 to 1999, history of diabetes, energy, long-term intake of sugar-sweetened carbonated beverage	
			No past & no current	68	1		
			Low past & low current	141	0.82 (0.61–1.11)		
			High past & low current	90	0.94 (0.67–1.33)		
			Low past & high current	21	0.77 (0.46–1.27)		
			High past & high current	24	0.53 (0.32–0.87)		
		NHL, excluding multiple myeloma, incidence	Aspartame intake quintile (RR):			Age at baseline, sex, history of diabetes, BMI, smoking status, energy intake, sugar-sweetened carbonated beverage intake	
			1st quintile (median, 0 mg/day)	187	1		
			2nd quintile (median, 3.6 mg/day)	221	1.31 (1.08–1.60)		
			3rd quintile (median, 12.6 mg/day)	215	1.29 (1.06–1.58)		
			4th quintile (median, 35.8 mg/day)	203	1.18 (0.96–1.45)		
			5th quintile (median, 145 mg/day)	172	1.07 (0.86–1.33)		
			Continuous (per 50 mg/day)	998	1.00 (0.96–1.04)		
			Trend-test P value: 0.83				

Table 2.5 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2014) (cont.)		NHL, excluding multiple myeloma, incidence	Artificially sweetened carbonated beverage intake (RR):			Age at baseline, sex, history of diabetes, BMI, smoking status, energy intake, sugar-sweetened carbonated beverage intake	
			Nondrinkers	272	1		
			> 0–3 cans/mo	349	1.13 (0.96–1.33)		
			1–4 cans/wk	261	1.02 (0.86–1.22)		
			5–6 cans/wk	33	0.79 (0.55–1.14)		
			≥ 1 can/day	83	0.97 (0.75–1.26)		
			Continuous (per 1 can/day)	998	1.00 (0.98–1.02)		
			Trend-test *P* value: 0.45				
		Multiple myeloma, incidence	Aspartame intake quintile (RR):				
			1st quintile (median, 0 mg/day)	43	1		
			2nd quintile (median, 3.6 mg/day)	45	1.18 (0.77–1.81)		
			3rd quintile (median, 12.6 mg/day)	45	1.18 (0.77–1.81)		
			4th quintile (median, 35.8 mg/day)	31	0.74 (0.46–1.20)		
			5th quintile (median, 145 mg/day)	34	0.83 (0.51–1.33)		
			Continuous (per 50 mg/day)	198	0.93 (0.84–1.04)		
			Trend-test *P* value: 0.14				

Table 2.5   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2014) (cont.)		Multiple myeloma, incidence	Artificially sweetened carbonated beverage intake (RR):			Age at baseline, sex, history of diabetes, BMI, smoking status, energy intake, sugar-sweetened carbonated beverage intake	
			Nondrinkers	59	1		
			> 0–3 cans/mo	75	1.15 (0.81–1.63)		
			1–4 cans/wk	42	0.71 (0.47–1.07)		
			≥ 5 cans/wk	22	0.70 (0.42–1.17)		
			Continuous (per 1 can/day)	198	0.97 (0.92–1.01)		
			Trend-test *P* value: 0.05				
		NHL (DLBCL), incidence	Aspartame intake quintile (RR):				
			1st quintile (median, 0 mg/day)	40	1		
			2nd quintile (median, 3.6 mg/day)	63	1.82 (1.22–2.72)		
			3rd quintile (median, 12.6 mg/day)	56	1.62 (1.07–2.45)		
			4th quintile (median, 35.8 mg/day)	50	1.38 (0.90–2.11)		
			5th quintile (median, 145 mg/day)	49	1.39 (0.90–2.16)		
			Continuous (per 50 mg/day)	258	1.02 (0.94–1.10)		
			Trend-test *P* value: 0.51				

Table 2.5   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2014) (cont.)		NHL (DLBCL), incidence	Artificially sweetened carbonated beverage intake (RR):			Age at baseline, sex, history of diabetes, BMI, smoking status, energy intake, sugar-sweetened carbonated beverage intake	
			Nondrinkers	67	1		
			> 0–3 cans/mo	90	1.23 (0.89–1.70)		
			1–4 cans/wk	71	1.12 (0.80–1.59)		
			≥ 5 cans/wk	30	0.92 (0.59–1.45)		
			Continuous (per 1 can/day)	258	1.01 (0.98–1.04)		
			Trend-test P value: 0.89				
		NHL (CLL and SLL), incidence	Aspartame intake quintile (RR):				
			1st quintile (median, 0 mg/day)	59	1		
			2nd quintile (median, 3.6 mg/day)	58	1.05 (0.73–1.52)		
			3rd quintile (median, 12.6 mg/day)	55	1.02 (0.70–1.48)		
			4th quintile (median, 35.8 mg/day)	52	0.95 (0.65–1.39)		
			5th quintile (median, 145 mg/day)	43	0.85 (0.56–1.29)		
			Continuous (per 50 mg/day)	267	0.96 (0.88–1.05)		
			Trend-test P value: 0.40				

Table 2.5   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2014) (cont.)		NHL (CLL and SLL), incidence	Artificially sweetened carbonated beverage intake (RR):			Age at baseline, sex, history of diabetes, BMI, smoking status, energy intake, sugar-sweetened carbonated beverage intake	
			Nondrinkers	80	1		
			> 0–3 cans/mo	92	0.97 (0.72–1.32)		
			1–4 cans/wk	68	0.89 (0.64–1.23)		
			≥ 5 cans/wk	27	0.71 (0.45–1.12)		
			Continuous (per 1 can/day)	267	0.99 (0.95–1.02)		
			Trend-test P value: 0.15				
		NHL (follicular lymphoma), incidence	Aspartame intake quintile (RR):				
			1st quintile (median, 0 mg/day)	27	1		
			2nd quintile (median, 3.6 mg/day)	31	1.41 (0.84–2.39)		
			3rd quintile (median, 12.6 mg/day)	27	1.22 (0.71–2.10)		
			4th quintile (median, 35.8 mg/day)	29	1.21 (0.71–2.09)		
			5th quintile (median, 145 mg/day)	28	1.20 (0.69–2.11)		
			Continuous (per 50 mg/day)	142	1.03 (0.94–1.13)		
			Trend-test P value: 0.72				

Table 2.5   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2014) (cont.)		NHL (follicular lymphoma), incidence	Artificially sweetened carbonated beverage intake (RR):			Age at baseline, sex, history of diabetes, BMI, smoking status, energy intake, sugar-sweetened carbonated beverage intake	
			Nondrinkers	41	1		
			> 0–3 cans/mo	46	1.08 (0.70–1.66)		
			1–4 cans/wk	36	0.97 (0.61–1.54)		
			≥ 5 cans/wk	19	0.98 (0.55–1.74)		
			Continuous (per 1 can/day)	142	1.00 (0.96–1.04)		
			Trend-test *P* value: 0.85				
		NHL (other B-cell lymphoma), incidence	Aspartame intake quintile (RR):				
			1st quintile (0 mg/day)	35	1		
			2nd quintile (median, 3.6 mg/day)	37	1.18 (0.74–1.89)		
			3rd quintile (median, 12.6 mg/day)	48	1.58 (1.01–2.46)		
			4th quintile (median, 35.8 mg/day)	43	1.39 (0.88–2.20)		
			5th quintile (median, 145 mg/day)	29	1.01 (0.60–1.68)		
			Continuous (per 50 mg/day)	192	0.94 (0.85–1.05)		
			Trend-test *P* value: 0.63				

Table 2.5   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2014) (cont.)		NHL (other B-cell lymphoma), incidence	Artificially sweetened carbonated beverage intake (RR):			Age at baseline, sex, history of diabetes, BMI, smoking status, energy intake, sugar-sweetened carbonated beverage intake	
			Nondrinkers	46	1		
			> 0–3 cans/mo	72	1.41 (0.97–2.06)		
			1–4 cans/wk	51	1.24 (0.82–1.88)		
			≥ 5 cans/wk	23	1.13 (0.67–1.91)		
			Continuous (per 1 can/day)	192	1.00 (0.96–1.04)		
			Trend-test *P* value: 0.59				
McCullough et al. (2022) USA Enrolment, 1982/follow-up, through 2016 (median, 27.7 yr) Cohort	934 777 (416 313 men, 518 464 women); CPS-II prospective cohort; adults aged ≥ 28 yr; excluded participants with personal history at baseline of diabetes or cancer other than nonmelanoma skin cancer, men aged >90 yr or women aged >95 yr at enrolment, and those reporting only prior but not current consumption of either SSBs or ASBs	NHL, mortality	ASB consumption (HR):			Age, sex, race/ ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	*Exposure assessment critique*: A key strength was the prospective assessment of ASB consumption. Key limitations were that there was only one dietary assessment at baseline in 1982 that was before the use of aspartame in ASB, hence the relevance to aspartame exposure depends on the stability of ASB consumption over up to 34 yr of follow-up, but such information was not directly available; no other sources were considered (although these were more limited); and uncertainty regarding aspartame content in ASBs after the mid-2000s. Other information: exclusion of participants who reported only prior but not current consumption of either SSBs or ASBs at baseline.
			Never	NR	1		
			< 1 drink/day	NR	0.97 (0.89–1.05)		
			1 drink/day	NR	0.97 (0.88–1.07)		
			≥ 2 drinks/day	NR	1.01 (0.92–1.12)		
			Continuous (per drink/day)	6600	1.00 (0.98–1.03)		
			Trend-test *P* value: 0.870				
		NHL, mortality	ASB consumption, men (HR):				
			Never	NR	1		
			< 1 drink/day	NR	1.01 (0.89–1.15)		
			1 drink/day	NR	0.99 (0.85–1.14)		
			≥ 2 drinks/day	NR	0.97 (0.83–1.13)		
			Continuous (per drink/day)	3409	1.00 (0.95–1.04)		
			Trend-test *P* value: 0.714				

Table 2.5   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2022) (cont.)	Exposure assessment method: questionnaire; exposure to ASBs assessed in 1982 through a question about the number of drinks/day of diet soda or ice teas (one pooled item) and potential changes over the past 10 yr; no specific assessment of aspartame content in ASB	NHL, mortality	ASB consumption, women (HR):			Age, race/ ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	*Other strengths*: large cohort with long follow-up; ability to examine multiple cancer types, stratify by sex or BMI, and limit to never-smokers; comprehensive adjustment for confounders, including SSB consumption. *Other limitations*: potential for bias from non-differential exposure misclassification.
			Never	NR	1		
			< 1 drink/day	NR	0.94 (0.85–1.04)		
			1 drink/day	NR	0.95 (0.84–1.08)		
			≥ 2 drinks/day	NR	1.04 (0.91–1.18)		
			Continuous (per drink/day)	3191	1.01 (0.97–1.05)		
			Trend-test *P* value: 0.916				
		NHL, mortality	ASB consumption, BMI, 18.5 to < 25 kg/m² (HR):				
			Never	NR	1		
			< 1 drink/day	NR	0.96 (0.85–1.08)		
			1 drink/day	NR	0.84 (0.71–0.98)		
			≥ 2 drinks/day	NR	0.89 (0.75–1.06)		
			Continuous (per drink/day)	2970	0.96 (0.91–1.01)		
			Trend-test *P* value: 0.035				
		NHL, mortality	ASB consumption, BMI, 25 to < 30 kg/m² (HR):			Age, sex, race/ ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR	1		
			< 1 drink/day	NR	1.00 (0.88–1.13)		
			1 drink/day	NR	1.05 (0.91–1.21)		
			≥ 2 drinks/day	NR	1.08 (0.93–1.25)		
			Continuous (per drink/day)	2689	1.02 (0.98–1.06)		
			Trend-test *P* value: 0.274				

Table 2.5   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2022) (cont.)		NHL, mortality	ASB consumption, BMI, ≥ 30 kg/m² (HR):			Age, sex, race/ ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR	1		
			< 1 drink/day	NR	0.89 (0.71–1.11)		
			1 drink/day	NR	1.06 (0.83–1.36)		
			≥ 2 drinks/day	NR	1.00 (0.79–1.27)		
			Continuous (per drink/day)	721	1.02 (0.95–1.08)		
			Trend-test *P* value: 0.896				
		Multiple myeloma, mortality	ASB consumption (HR):				
			Never	NR	1		
			< 1 drink/day	NR	1.00 (0.90–1.11)		
			1 drink/day	NR	1.01 (0.89–1.15)		
			≥ 2 drink/day	NR	1.03 (0.90–1.17)		
			Continuous (per drink/day)	3511	1.02 (0.98–1.06)		
			Trend-test *P* value: 0.707				
		Multiple myeloma, mortality	ASB consumption, men (HR):			Age, race/ ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR	1		
			< 1 drink/day	NR	1.02 (0.86–1.22)		
			1 drink/day	NR	0.98 (0.80–1.20)		
			≥ 2 drinks/day	NR	1.04 (0.84–1.27)		
			Continuous (per drink/day)	1791	1.02 (0.96–1.07)		
			Trend-test *P* value: 0.802				

Table 2.5   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2022) (cont.)		Multiple myeloma, mortality	ASB consumption, women (HR):			Age, race/ ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR	1		
			< 1 drink/day	NR	0.98 (0.85–1.12)		
			1 drink/day	NR	1.03 (0.87–1.21)		
			≥ 2 drinks/day	NR	1.01 (0.85–1.21)		
			Continuous (per drink/day)	1720	1.02 (0.97–1.07)		
			Trend-test P value: 0.841				
		Multiple myeloma, mortality	ASB consumption, BMI, 18.5 to < 25 kg/m² (HR):			Age, sex, race/ ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR	1		
			< 1 drink/day	NR	1.12 (0.95–1.31)		
			1 drink/day	NR	1.10 (0.91–1.34)		
			≥ 2 drink/day	NR	1.16 (0.94–1.43)		
			Continuous (per drink/day)	1603	1.07 (1.01–1.13)		
			Trend-test P value: 0.079				

Table 2.5 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
[McCullough et al. (2022)](#) (cont.)		Multiple myeloma, mortality	ASB consumption, BMI, 25 to < 30 kg/m² (HR):			Age, sex, race/ ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR	1		
			< 1 drink/day	NR	0.92 (0.77–1.09)		
			1 drink/day	NR	0.95 (0.77–1.16)		
			≥ 2 drinks/day	NR	1.04 (0.85–1.27)		
			Continuous (per drink/day)	1402	1.00 (0.95–1.06)		
			Trend-test P value: 0.997				
		Multiple myeloma, mortality	ASB consumption, BMI, ≥ 30 kg/m² (HR):				
			Never	NR	1		
			< 1 drink/day	NR	0.93 (0.70–1.26)		
			1 drink/day	NR	1.01 (0.72–1.41)		
			≥ 2 drinks/day	NR	0.76 (0.53–1.09)		
			Continuous (per drink/day)	391	0.94 (0.85–1.04)		
			Trend-test P value: 0.181				
		Leukaemia, mortality	ASB consumption (HR):				
			Never	NR	1		
			< 1 drink/day	NR	0.95 (0.88–1.04)		
			1 drink/day	NR	0.99 (0.90–1.10)		
			≥ 2 drinks/day	NR	0.97 (0.87–1.07)		
			Continuous (per drink/day)	6319	0.99 (0.96–1.02)		
			Trend-test P value: 0.444				

Table 2.5   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2022) (cont.)		Leukaemia, mortality	ASB consumption, men (HR):			Age, race/ ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR	1		
			< 1 drink/day	NR	0.94 (0.83–1.07)		
			1 drink/day	NR	1.06 (0.92–1.21)		
			≥ 2 drink/day	NR	0.96 (0.83–1.12)		
			Continuous (per drink/day)	3551	0.99 (0.95–1.04)		
			Trend-test *P* value: 0.797				
		Leukaemia, mortality	ASB consumption, women (HR):				
			Never	NR	1		
			< 1 drink/day	NR	0.96 (0.86–1.07)		
			1 drink/day	NR	0.94 (0.82–1.08)		
			≥ 2 drinks/day	NR	0.96 (0.84–1.11)		
			Continuous (per drink/day)	2768	0.98 (0.94–1.03)		
			Trend-test *P* value: 0.417				
		Leukaemia, mortality	ASB consumption, BMI, 18.5 to < 25 kg/m^2 (HR):			Age, sex, race/ ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR	1		
			< 1 drink/day	NR	1.05 (0.93–1.19)		
			1 drink/day	NR	0.99 (0.85–1.16)		
			≥ 2 drinks/day	NR	0.90 (0.75–1.08)		
			Continuous (per drink/day)	2769	0.96 (0.91–1.02)		
			Trend-test *P* value: 0.373				

Table 2.5   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2022) (cont.)		Leukaemia, mortality	ASB consumption, BMI, 25 to < 30 kg/m² (HR):			Age, sex, race/ ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR	1		
			< 1 drink/day	NR	0.88 (0.77–1.00)		
			1 drink/day	NR	1.01 (0.87–1.16)		
			≥ 2 drink/day	NR	1.04 (0.90–1.21)		
			Continuous (per drink/day)	2688	1.01 (0.96–1.05)		
			Trend-test *P* value: 0.783				
		Leukaemia, mortality	ASB consumption, BMI ≥ 30 kg/m²) (HR):				
			Never	NR	1		
			< 1 drink/day	NR	0.92 (0.73–1.16)		
			1 drink/day	NR	0.97 (0.74–1.26)		
			≥ 2 drinks/day	NR	0.79 (0.60–1.04)		
			Continuous (per drink/day)	656	0.96 (0.89–1.04)		
			Trend-test *P* value: 0.108				

Table 2.5   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Palomar-Cros et al. (2023) Spain 2008–2013 Case–control	Cases: 109; aged 20–85 yr with newly diagnosed histologically confirmed cancer (prevalent CLL cases were also recruited and were retained for this analysis if diagnosis was done 1 yr before the interview), resided in catchment area for at least 6 mo; no prior history of their cancer; enrolled as soon as possible after diagnosis; frequency-matched on age, sex, and region to population controls. Controls: 3629; randomly selected from administrative records of selected primary health care centres within catchment area. Exposure assessment method: self-administered, semiquantitative FFQ, 140 food items, assessing usual dietary intake during the previous year	NHL (CLL, ICD-10 C91.1), incidence	Consumption of aspartame-containing products (OR): Non-consumers Medium intake (< 3rd quartile among controls) High intake (≥ 3rd quartile among controls) Trend-test *P* value: 0.6	89 9 11	1 0.56 (0.25–1.08) 1.76 (0.84–3.41)	Age, sex, study centre, education, smoking, radiation exposure, total WCRF score continuous, total energy intake, total sugar intake, other ASs	*Exposure assessment critique*: A key strength was the categorization of intake of ASBs and tabletop sweeteners by type (aspartame vs others) using public data on ingredients in food supply, but it was unclear whether the assumption of aspartame content in products was correct. A key limitation was that beverages and tabletop sweeteners were assessed, but there was no consideration of ASs in the rest of the food supply. *Other strengths*: large sample size for some outcomes; histopathological confirmation of cancer cases; extensive assessment of confounding, including from BMI (captured in a combined score based on WCRF/AICR evidence on lifestyle factors; Romaguera et al., 2017); stratification by diabetes status to evaluate heterogeneity of associations.

Table 2.5   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
[Palomar-Cros et al. (2023)](#) (cont.)		NHL (CLL, ICD-10 C91.1), incidence	Consumption of aspartame-containing products, participants without diabetes (OR):			Age, sex, study centre, education, smoking, radiation exposure, total WCRF score continuous, total energy intake, total sugar intake, other ASs	*Other limitations*: non-prospective study design (case–control); selection bias due to low participation among controls (mean participation rate, 53%); recall bias in exposure assessment; potential for exposure measurement error and residual confounding due to other correlates of AS use among those with diabetes; relatively low exposure contrasts for aspartame-containing products; potential for chance findings due to small number in some strata.
			Non-consumers	75	1		
			Medium intake (< 3rd quartile among controls)	7	0.56 (0.23–1.18)		
			High intake (≥ 3rd quartile among controls)	9	2.15 (0.93–4.51)		
			Trend-test P value: 0.4				
		NHL (CLL, ICD-10 C91.1), incidence	Consumption of aspartame-containing products, participants with diabetes (OR):				
			Non-consumers	14	1		
			Medium intake (< 3rd quartile among controls)	2	0.55 (0.08–2.37)		
			High intake (≥ 3rd quartile among controls)	2	0.63 (0.06–3.41)		
			Trend-test P value: 0.6				

AICR, American Institute of Cancer Research; AS, artificial sweetener; ASB, artificially sweetened beverage; BMI, body mass index; CI, confidence interval; CLL, chronic lymphocytic leukaemia; CPS-II, Cancer Prevention Study-II; DLBCL, diffuse large B-cell lymphoma; FDA, Food and Drug Administration; FFQ, food frequency questionnaire; HPFS, Health Professionals Follow-up Study; HR, hazard ratio; HRT, hormone replacement therapy; ICD-10, International Classification of Diseases 10th revision; mo, month(s); NHL, non-Hodgkin lymphoma; NHS, Nurses' Health Study; NIH-AARP, National Institutes of Health-American Association of Retired Persons; NR, not reported; OR, odds ratio; RR, relative risk; SLL, small lymphocytic lymphoma; SSB, sugar-sweetened beverage; vs, versus; WCRF, World Cancer Research Fund; wk, week(s); yr, year(s).

four aspartame-containing beverages and aspartame added to hot coffee and tea during the past year. No association with aspartame intake was detected for all haematopoietic cancers, overall or in either men or women, or for Hodgkin lymphoma, multiple myeloma, non-Hodgkin lymphoma (NHL) overall or two of its subtypes (small lymphocytic lymphoma and CLL; immunoblastic lymphoma and lymphoblastic lymphoma/leukaemia) and non-lymphoid leukaemia. [This prospective cohort included a large sample of participants and a substantial number of incident cases ascertained by registries. Another strength was the prospective and quantitative assessment of aspartame exposure from various ASBs and tabletop packets between 1995 and 1996, a relevant period for aspartame use in beverages in the USA. However, the length of follow-up was relatively limited, and the age at enrolment was relatively high (average age of cohort at baseline, 62 years, with no assessment of aspartame intake from younger ages). Additional weaknesses were that the questionnaire used to estimate aspartame exposure was limited and prone to exposure misclassification, only focusing on beverage and tabletop sweetener sources (there was no information on other dietary sources of aspartame – although these would be expected to be less common); that the question on the frequency of the type of sweetener in a beverage (regular versus diet) followed the question on the frequency of consumption by type of beverage (soda, fruit drinks, or iced tea); and the minimally specific assessment of the type of sweetener ingested (the questionnaire asked about whether sweetener added to coffee or tea was "Equal or aspartame" or "Sweet'N Low or saccharin"). All these factors may have limited the ability of the authors to detect an association.]

The association between aspartame-containing soda and incidence of haematopoietic cancers was investigated in the NHS (77 218 women) and the HPFS (47 810 men) (Schernhammer et al., 2012). Over 22 years of follow-up (1984–2006 for the NHS and 1986–2006 for the HPFS), 1324 NHL, 285 multiple myeloma, and 339 leukaemia cases were ascertained. In men, ≥ 1 daily serving of diet soda was associated with increased risk of NHL (RR, 1.31; 95% CI, 1.01–1.72; P for trend, 0.11) and multiple myeloma (RR, 2.02; 95% CI, 1.20–3.40; P for trend, 0.01) compared with men who did not consume diet soda. No association was observed for women for NHL (RR, 1.00; 95% CI, 0.78–1.26; P for trend, 0.999) or for multiple myeloma (RR, 0.79; 95% CI, 0.45–1.36; P for trend, 0.79). When data from the two cohorts were combined in a pooled analysis to maximize statistical precision, diet soda intake was associated with increased risk of leukaemia (RR, 1.42; 95% CI, 1.00–2.02; P for trend, 0.05). Since aspartame became most broadly used in the USA in soda in 1992 when its patent expired and the price dropped substantially (so the aspartame measure using diet soda consumption probably became more precise after that date), and since the questionnaires started to include tabletop sweeteners after that date, a secondary analysis was conducted, starting follow-up from 1994. In that analysis, among men, an association was observed between aspartame exposure (estimated from diet soda and aspartame packets consumed) and increased risk of NHL (RR for ≥ 143 mg/day versus none, 1.64; 95% CI, 1.17–2.29; P for trend, 0.002) and multiple myeloma (RR for ≥ 143 mg/day versus none, 3.36; 95% CI, 1.38–8.19; P for trend, 0.05); these associations were not observed in women. For leukaemia, the RR for ≥ 143 mg/day versus none was 1.56 (95% CI, 0.79–3.06; P for trend, 0.17) in men. For NHL, an interaction ($P = 0.03$) with alcohol was detected among men: compared with no consumption, ≥ 2 servings of diet soda/day was associated with increased risk (RR, 2.34; 95% CI, 1.46–3.76; P for trend, 0.004) in men who consumed < 6 g alcohol/day (median intake) but not in men with a higher alcohol consumption (RR, 0.96; 95% CI, 0.48–1.90; P for trend, 0.98). Risks of multiple myeloma and leukaemia

associated with diet soda at ≥ 1 serving/day were also higher in men with a lower alcohol intake. In contrast, for women, risks associated with diet soda did not differ by alcohol consumption for any of the outcomes [data not provided], although few women in this cohort consumed high amounts of alcohol. The authors hypothesized that these sex-specific differences may have been due to the recognized higher enzymatic activity of alcohol dehydrogenase type I in men, which possibly induced higher conversion rates from methanol to the carcinogenic substrate formaldehyde. Because the concurrent ingestion of ethanol inhibits methanol metabolism (Lee et al., 2011), they conducted analyses stratified by alcohol intake. [The Working Group noted that these results supported the authors' initial hypothesis that men with lower regular alcohol consumption would have more unbound alcohol dehydrogenase activity (Frezza et al., 1990) and thus higher formaldehyde conversion rates if they consumed large amounts of diet soda and, consequently, higher cancer risk. The length of follow-up, large number of haematopoietic cancer cases, prospective design, and updating of dietary intake data every 4 years at a relevant period for aspartame exposure from ASBs in the USA (between the early to mid-1980s and mid-2000s) constituted important strengths of this study. The main limitation related to potential measurement error in aspartame exposure, which was derived only from diet soda and packets used at the table assessed by FFQ (other sources of aspartame including non-soda ASBs were not considered, although these would be expected to be less common), without brand information for products other than tabletop sweeteners, and thus no data on the specific type of sweetener ingested. The secondary analyses starting in 1994 suggested that more precise measures might strengthen the results.]

Two studies were conducted within the CPS-II prospective cohort to investigate the association between ASBs and aspartame and risk of lymphoid neoplasm incidence (McCullough et al., 2014) and mortality (McCullough et al., 2022). The first study (McCullough et al., 2014), in the CPS-II nutrition cohort (a subcohort of the parent CPS-II mortality cohort), included 100 442 men and women who completed a modified Willett FFQ in 1999 (which was re-administered in 2003). Mean consumption of artificially sweetened carbonated beverages ["1 glass, bottle, or can (355 mL)"] during the past year was queried (categories ranging from "never" to "≥ 4 per day"). Reported intake of specific artificially and sugar-sweetened carbonated beverage types listed (i.e. cola with caffeine, other carbonated beverages with or without caffeine) were summed. Participants were asked about "use of NutraSweet or Equal (1 packet) (not Sweet'N Low)" (from "never" to "≥ 6 per day"). Total aspartame intake was calculated using the following values: 180 mg/355 mL (1 serving) of low-calorie cola with caffeine, 90 mg/355 mL of other low-calorie soda with caffeine, and 70 mg/355 mL of other low-calorie soda without caffeine; and 20 mg of aspartame per packet of artificial sweetener reported. Over a 10-year follow-up, 1196 cases of NHL were ascertained and verified by medical records or by linkage with cancer registries. No association was detected between consumption of artificially sweetened carbonated beverages or aspartame and NHL risk overall or by subtype (multiple myeloma, diffuse large B-cell lymphoma, CLL/small lymphocytic lymphoma, and follicular and other B-cell lymphoma). No heterogeneity in the association was suggested by sex. A secondary analysis was conducted to examine "long-term" past consumption patterns by incorporating participant responses from a questionnaire completed 10 years before baseline (as part of the parent cohort). In 1982, participants were asked about usual consumption of artificially sweetened carbonated beverages per day as "cups, glasses, or drinks" (responses ranged from none to 10 servings/day, assuming 355 mL per serving).

Aspartame consumption was not specifically calculated for this exposure assessment. This analysis suggested an inverse association between "long-term" regular consumption of artificially sweetened carbonated beverages and NHL in women (RR versus long-term non-consumers, 0.53; 95% CI, 0.32–0.87). These inverse associations were however interpreted by the authors as potentially caused by chance or confounding by other unknown exposures. [This study benefited from the large number of haematopoietic cancer cases, the prospective design, and the quantitative assessment of aspartame intake from carbonated ASBs (aspartame values assigned by type of ASB – although not brand-specific) and aspartame packets. Exposure was assessed twice in 1999 and 2003, at a relevant period for aspartame exposure through ASBs in the USA. Limitations pertained to the level of detail in the aspartame exposure assessment and the fact that no other sources were considered (although these would be expected to be less important), thus resulting in potential misclassification. Although the direction of the potential bias cannot be definitively determined, these non-differential classification errors were more likely to bias observed associations towards the null. Regarding the "long-term" analysis, although aspartame was not quantified, the consumption of ASBs was considered to be an informative proxy for aspartame exposure, since aspartame had just been authorized in the USA in 1983, i.e. 1 year after the measurement of exposure in 1982.]

In the second investigation in the CPS-II cohort, analyses were conducted on 934 777 cancer-free men and women who provided answers to the 1982 questionnaire about their usual consumption of ASBs (McCullough et al., 2022). From 1982 through 2016 (follow-up, 34 years), 135 093 cancer deaths occurred, of which 6600 were from NHL, 6319 from leukaemia, and 3511 from multiple myeloma (ascertained by personal inquiries to participants or their relatives and verified by death certificates, and from

1989 onwards by linkage to the National Death Index). No association was detected for any of these outcomes in men, women, or sex-combined analyses. An interaction with BMI was detected for NHL (P for interaction, 0.029): the hazard ratio estimates for ≥ 2 drinks/day of ASBs versus non-consumers were 0.89 (95% CI, 0.75, 1.06) for normal weight (18.5 to < 25 kg/m^2), 1.08 (95% CI, 0.93, 1.25) for overweight (25 to < 30 kg/m^2), and 1.00 (95% CI, 0.79, 1.27) for obese (≥ 30 kg/m^2) participants. [This study was based on a very large number of cases, long follow-up, and prospective design. The main limitation lay in the evaluation of the exposure, which was based on a unique baseline frequency question, "How many cups, glasses, or drinks of diet soda or diet iced teas do you usually drink a day, and for how many years?", focused only on beverage sources, with no specific assessment of the type of sweetener ingested and no brand-specific data. As mentioned above, aspartame had only been authorized in the USA in 1983, i.e. 1 year after exposure was assessed. However, combining data from McCullough et al. (2014, 2022) indicated some stability in ASB consumption ranking over years; hence, the Working Group concluded that ASB consumption assessed in 1982 may reflect later consumption, when aspartame became the predominant sweetener in ASBs. These misclassification errors probably limited the ability to detect associations (although not systematically, so that non-differential classification errors probably biased associations towards the null).]

The MCC-Spain study conducted in 2008–2013 included 109 cases of CLL with no prior history of disease and a total of 3629 controls (1631 controls for CLL-specific analyses) (Palomar-Cros et al., 2023). Consumption of products containing artificial sweeteners was estimated for tabletop sweeteners and ASB, assessed through a self-administered validated FFQ. No association was observed between exposure to "aspartame-containing products" and CLL risk either in all participants or in analyses stratified

by diabetes status. [The main limitation of this study related to its non-prospective design (case–control). Another limitation related to the precision of the aspartame exposure estimate, which was derived only from ASBs and tabletop sweeteners assessed by FFQ, without brand information, and thus with few data on the specific type of sweetener ingested and relying on the assumption that all low- or no-calorie beverages contained aspartame, which may not be realistic considering the time period for this case–control study in the Spanish context. In addition, the numbers of cases overall and in diabetes strata were small, and results should be interpreted cautiously.]

Four meta-analyses presented results on aspartame and/or ASBs and haematopoietic cancer risk.

In 2019, Toews et al. performed a meta-analysis of randomized and non-randomized controlled trials and observational studies on the association between intake of non-sugar sweeteners and health outcomes, following a PRISMA flow chart of included studies and the Cochrane Collaboration methodology for the systematic literature review and meta-analysis (Toews et al., 2019). Of individual studies finally included in this review, two related to haematopoietic cancers (i.e. Lim et al., 2006, and McCullough et al., 2014, presented above). Results of the meta-analyses showed no association between higher consumption of aspartame and incidence of the main subtypes of lymphoid cancers, NHL subtypes, or non-lymphoid leukaemia. However, the study by Schernhammer et al. (2012) was not included in the review [no specific explanation found in the article], nor was the study by McCullough et al. (2022) (which was published later).

In 2022, WHO published a report (coordinated by Rios-Leyvraz and Montez) on the health effects of use of non-sugar sweeteners that included a systematic review and meta-analyses of studies published through July 2021 (WHO et al., 2022). Although this overall report did not

focus only on aspartame, for the specific cancer location studied here (haematopoietic cancers) the four prospective cohort studies (NIH-AARP, NHS, HPFS, CPS-II) from the three articles published at that time (i.e. all those described previously in this section except for the study by McCullough et al., 2022) were those deemed relevant for the present monograph (Lim et al., 2006; Schernhammer et al., 2012; McCullough et al., 2014). The results of these meta-analyses found little evidence of positive associations, but the number of included studies remained low: leukaemia, three cohorts, RR, 1.24; 95% CI, 0.92–1.69, I^2 = 0%; multiple myeloma, four cohorts, RR, 1.05; 95% CI, 0.70–1.59, I^2 = 70%; Hodgkin lymphoma, one cohort, RR, 0.77; 95% CI, 0.44–1.33; NHL, four cohorts, RR, 1.08; 95% CI, 0.87–1.34, I^2 = 64%.

Also in 2022, Yin et al. published a meta-analysis of prospective studies on ASB consumption and cancer risk (Yin et al., 2022). The same studies as in the WHO report (WHO et al., 2022) were those included for haematopoietic cancers (Lim et al., 2006; Schernhammer et al., 2012; McCullough et al., 2014) (although the overall publication did not focus only on aspartame and also included studies conducted before 1980). In contrast to the WHO meta-analysis, Yin et al. found that high intake of ASBs was associated with elevated risk of non-lymphoid leukaemia (for three studies, RR, 1.35; 95% CI, 1.03–1.77; P = 0.030, with evidence of low heterogeneity across studies). Dose–response analysis indicated a positive linear association between ASB intake and the risk of leukaemia (P for a linear relation, 0.027). The risk associated with daily ASB intake increased by 15% per increment of one serving (355 mL) (RR, 1.15; 95% CI, 1.02–1.30). [The Working Group noted that the three cohorts included in the WHO report (WHO et al., 2022) and in the study by Yin et al. (2022) for the analysis of leukaemia were the same, i.e. Lim et al. (2006) for NIH-AARP, and Schernhammer et al. (2012) for NHS and HPFS. However, the estimates used

for NHS and HPFS differed, since Yin et al. used estimates from models of ASB intake, whereas WHO used models of "total aspartame".] The results of Yin et al. were consistent with those of the WHO report in that no association was found between ASB intake and multiple myeloma (RR for highest versus lowest category, 1.18; 95% CI, 0.69–2.02; $P = 0.537$; $I^2 = 68.9\%$), NHL (RR for highest versus lowest category, 1.05; 95% CI, 0.91–1.21; $P = 0.506$; $I^2 = 16.3\%$), or Hodgkin lymphoma (RR for highest versus lowest category, 0.77; 95% CI, 0.45–1.33; $P = 0.351$; $I^2 = 0.0\%$).

Pan et al. (2023) published a dose–response meta-analysis of prospective cohort studies (from inception to June 2022) on ASB intake and several cancer types, including haematopoietic cancers. The publications by Schernhammer et al. (2012) and McCullough et al. (2014) were included, but that by Lim et al. (2006) was omitted. The authors reported that an increment of ASBs of 250 mL/day was associated with a 16% increase in risk of leukaemia (RR, 1.16; 95% CI, 1.00–1.35; $I^2 = 0\%$), but only the publication of Schernhammer et al. (2012) (considering two estimates, one from the US NHS cohort and one from the US HPFS cohort) was included in this meta-analysis. No association was observed for NHL or multiple myeloma, but the number of included studies was very small (Schernhammer et al., 2012; McCullough et al., 2014).

2.6 Obesity-related cancers and other groupings

See Table 2.6.

Overall, three cohort studies reported on aspartame or ASB consumption and the risk of developing or dying from obesity-related cancers. A separate analysis in one of these cohorts evaluated the association between artificially sweetened soft drinks and non-obesity-related cancers.

The MCCS included 35 593 men and women aged 40–69 years and enrolled between 1990 and 1994 (Hodge et al., 2018). The baseline diet questionnaire, with 121 drink and food items, included questions about the number of times that diet soft drinks (artificially sweetened) were consumed per day, per week or per month. During follow-up, 3283 cases of obesity-related cancers were diagnosed, including cancers of the liver, prostate (aggressive), ovary, gallbladder, kidney, colorectum, oesophagus (adenocarcinoma), breast (postmenopausal), pancreas, endometrium, and gastric cardia. Proportional hazard models were adjusted for age and other potential confounders, including socioeconomic position, alcohol intake, country of birth, Mediterranean diet index, physical activity, sex, smoking, frequency of sugar-sweetened soft drink consumption, and waist circumference. Using no consumption or consumption of < 1 time/month as the reference category, the study did not find a positive association with increasing consumption of artificial sweeteners. The hazard ratios were 0.87 (95% CI, 0.76–1.00) for consumption of 1–3 times/month, 1.04 (95% CI, 0.93–1.17) for 1–6 times/week, 0.81 (95% CI, 0.66–0.99) for 1 time/day, and 1.00 (95% CI, 0.79–1.27) for > 1 time/day. The analyses of the association using soft drink consumption expressed as a continuous variable using the median daily equivalent frequency to each of the five categories of consumption also found no positive association with the risk of developing obesity-related cancers (HR, 0.98; 95% CI, 0.90–1.07).

Data from the MCCS were also used to evaluate the associations between consumption of artificially sweetened soft drinks and non-obesity-related cancers (Bassett at al., 2020). Over 19 years of follow-up, 4789 cancers not related to obesity occurred among 35 109 eligible participants. After adjusting for age, alcohol intake, country of birth, Mediterranean diet score, physical activity, socioeconomic position, sex,

Table 2.6 Epidemiological studies on consumption of aspartame and obesity-related cancers and other groupings

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Hodge et al. (2018) Australia Enrolment, 1990–1994/ follow-up until 30 June 2013 Cohort	35 593; MCCS – a prospective cohort study of men and women aged 40–69 yr at recruitment and free of cancer, angina, heart attack, and diabetes at baseline; participants with extreme baseline energy intake were excluded; obesity-related cancers according to the WCRF/AICR (WCRF/AICR, 2018) Exposure assessment method: self-administered 121-item FFQ with separate questions on frequency of consumption in the past year of diet (artificially sweetened) soft drinks	Obesity-related cancers: liver, prostate (aggressive), ovary, gallbladder, kidney, colorectum, oesophagus (adenocarcinoma), breast (postmenopausal), pancreas, uterus/ uterine corpus (endometrium), stomach (gastric cardia), incidence	Artificially sweetened soft drink consumption (HR): Never or < 1/mo 1–3/mo 1–6/wk 1/day > 1/day Continuous (per beverage/ day) Trend-test *P* value: 0.61	2498 233 376 102 74 3283	1 0.87 (0.76–1.00) 1.04 (0.93–1.17) 0.81 (0.66–0.99) 1.00 (0.79–1.27) 0.98 (0.90–1.07)	Age, sex, socioeconomic index, country of birth, alcohol intake, smoking status, physical activity, Mediterranean diet score, sugar-sweetened soft drink consumption, waist circumference	*Exposure assessment critique*: Key strengths were that it was a prospective study, that assessment was after aspartame introduction in diet soft drinks in Australia (1987), and that first half of follow-up largely overlapped with period of aspartame use in Australia. A key limitation was the FFQ assessment with no specific estimate of aspartame exposure, ASBs as a proxy and exposure data at baseline only; small number of consumers. *Other strengths*: adjustment for key confounders, including a measure of obesity (waist circumference). *Other limitations*: likely bias from non-differential exposure misclassification given single baseline assessment and long follow-up.

Table 2.6   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Bassett et al. (2020) Australia Enrolment, 1990–1994/ follow-up, 30 June 2015 Cohort	35 109; MCCS – a prospective cohort study of men and women aged 27–76 yr at recruitment (99% aged between 40 and 70 yr) and free of cancer, heart attack, angina and diabetes at baseline; participants with extreme energy intake were excluded Exposure assessment method: self-administered 121-item FFQ with separate questions on frequency of consumption in the past year of diet (artificially sweetened) soft drinks	Non-obesity-related cancers: sites other than oesophagus (adenocarcinoma), pancreas, colorectum, breast (postmenopausal), uterus/ uterine corpus (endometrium), kidney, ovary, gallbladder, liver, stomach (gastric cardia), meningioma, thyroid, multiple myeloma, incidence	Artificially sweetened soft drink consumption (HR): Never or < 1/mo 1–3/mo 1–6/wk 1/day > 1/day Continuous (per 1 serving/ day) Trend-test *P* value: 0.006	3625 371 490 189 114 4789	1 0.96 (0.86–1.07) 0.96 (0.87–1.06) 1.23 (1.06–1.43) 1.23 (1.02–1.48) 1.10 (1.03–1.17)	Age, sex, alcohol intake, country of birth, Mediterranean diet score, physical activity, socioeconomic position, smoking status, sugar-sweetened soft drink consumption	*Exposure assessment critique*: A key strength was that it was prospective study. A key limitation was the FFQ assessment with no specific estimate of aspartame exposure, ASBs were used as a proxy, and exposure data were reported at baseline only. *Other strengths*: large cohort. *Other limitations*: heterogeneous group (combined different cancer types with different etiology, hence difficult controlling for all potential confounders); likely bias from non-differential exposure misclassification.

Table 2.6 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Debras et al. (2022b) France Enrolment, 2009–2021/ follow-up until 22 January 2021 (median, 7.8 yr) Cohort	102 865; population-based cohort NutriNet-Santé (web-based); men and women aged ≥ 18 yr Exposure assessment method: records; participants are asked every 6 mo to complete a series of three validated web-based 24 h dietary records randomly assigned over a 2-wk period (2 weekdays, 1 weekend day); at least two 24 h dietary records during the first 2 yr of follow-up considered in analyses (mean ± SD, 5.6 ±3.0)	Obesity-related cancers: colorectum, stomach, liver, mouth, pharynx, larynx, oesophagus, breast, ovary, uterus/ uterine corpus (endometrium), and prostate, incidence	Aspartame intake (HR): Non-consumers	1401	1	Age, sex, BMI, height, percentage weight gain during follow-up, physical activity, smoking status, number of smoked cigarettes in pack-years, educational level, number of 24 h dietary records, family history of cancer, prevalent diabetes, energy intake without alcohol, daily intakes of alcohol, sodium, saturated fatty acids, fibre, sugar, fruit and vegetables, whole-grain foods, and dairy products, other AS intake	*Exposure assessment critique*: A key strength was that it was a prospective study using dietary records and quantitative assessment of aspartame based on food composition data updates for food supply changes over time. A key limitation was the baseline assessment in the main analysis, but sensitivity analysis conducted using all 24 h dietary records available during follow-up. *Other strengths*: large cohort; large number of cases; sensitivity analyses excluded prevalent diabetes or used all available 24 h dietary records throughout follow-up. *Other limitations*: low aspartame use in the cohort (28%); self-selection may limit generalizability; potential for residual confounding and reverse causation.
			Lower consumers (men, < 14.45 mg/day; women, < 15.39 mg/day)	337	1.08 (0.96–1.22)		
			Higher consumers (men, ≥ 14.45 mg/day; women, ≥ 15.39 mg/day) Trend-test *P* value: 0.026	285	1.15 (1.01–1.32)		

Table 2.6   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Debras et al. (2022b) (cont.)		Obesity-related cancers colorectum, stomach, liver, mouth, pharynx, larynx, oesophagus, breast, ovary, uterus/ uterine corpus (endometrium), and prostate, incidence	Aspartame intake, participants without diabetes (HR):			Age, sex, BMI, height, percentage weight gain during follow-up, physical activity, smoking status, number of smoked cigarettes in pack-years, educational level, number of 24 h dietary records, family history of cancer, energy intake without alcohol, daily intakes of alcohol, sodium, saturated fatty acids, fibre, sugar, fruit and vegetables, whole-grain foods, and dairy products, other AS intake	
			Non-consumers	1360	1		
			Lower consumers (men, < 14.45 mg/day; women, < 15.39 mg/day)	318	1.09 (0.96–1.23)		
			Higher consumers (men, ≥ 14.45 mg/day; women, ≥ 15.39 mg/day)	260	1.16 (1.01–1.34)		
			Trend-test P value: 0.024				

Table 2.6 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Debras et al. (2022b) (cont.)		Obesity-related cancers: colorectum, stomach, liver, mouth, pharynx, larynx, oesophagus, breast, ovary, uterus/ uterine corpus (endometrium), and prostate, incidence	Aspartame intake (time-dependent) (HR):			Age, sex, BMI, height, percentage weight gain during follow-up, physical activity, smoking status, number of smoked cigarettes in pack-years, educational level, number of 24 h dietary records, family history of cancer, prevalent diabetes, energy intake without alcohol, daily intakes of alcohol, sodium, saturated fatty acids, fibre, sugar, fruit and vegetables, whole-grain foods, and dairy products, other AS intake	
			Non-consumers	1401	1		
			Lower consumers (men, < 14.45 mg/day; women, < 15.39 mg/day)	337	1.07 (0.96–1.20)		
			Higher consumers (men, ≥ 14.45 mg/day; women, ≥ 15.39 mg/day)	285	1.13 (0.99–1.30)		
			Trend-test P value: 0.046				

Table 2.6   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level		Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2022) USA Enrolment, 1982/follow-up, through 2016 (median, 27.7 yr) Cohort	934 777 (416 313 men, 518 464 women); CPS-II prospective cohort; adults 28 yr and older; excluded participants with personal history at baseline of diabetes or cancer other than nonmelanoma skin cancer, men aged > 90 yr or women aged > 95 yr at enrolment, and those reporting only prior but not current consumption of either SSBs or ASBs Exposure assessment method: questionnaire; exposure to ASBs assessed in 1982 through a question about the number of drinks/day of diet soda or ice teas (one pooled item) and potential changes over the past 10 yr; no specific assessment of aspartame content in ASB	Obesity-related cancers: oesophagus, stomach, colorectum, liver, gallbladder, pancreas, breast (postmenopausal), uterus/ uterine corpus (endometrium), ovary, kidney, and multiple myeloma, mortality	ASB consumption (HR): Never < 1 drink/day 1 drink/day ≥ 2 drinks/day Continuous (per drink/ day) Trend-test *P* value: 0.001	NR NR NR NR 50 613	1 1.02 (0.99–1.05) 1.04 (1.01–1.08) 1.05 (1.01–1.08) 1.02 (1.01–1.03)	Age, sex, race/ ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption	*Exposure assessment critique*: A key strength was the prospective assessment of ASB consumption. Key limitations were that there was only one dietary assessment at baseline in 1982 that was before the use of aspartame in ASB, hence the relevance to aspartame exposure depends on the stability of ASB consumption over up to 34 yr of follow-up, but such information was not available; no other sources were considered (although these were more limited); and uncertainty of aspartame content in ASBs after the mid-2000s. Other information: exclusion of participants who reported only prior but not current consumption of either SSBs or ASBs at baseline.
		Obesity-related cancers: oesophagus, stomach, colorectum, liver, gallbladder, pancreas, kidney, and multiple myeloma, mortality	ASB consumption, men (HR): Never < 1 drink/day 1 drink/day ≥ 2 drinks/day Continuous (per drink/ day) Trend-test *P* value: 0.002	NR NR NR NR 20 595	1 1.02 (0.97–1.07) 1.06 (1.00–1.13) 1.08 (1.02–1.15) 1.03 (1.01–1.04)	Age, race/ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption	

Table 2.6 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2022) (cont.)		Obesity-related cancers: oesophagus, stomach, colorectum, liver, gallbladder, pancreas, breast (postmenopausal), uterus/ uterine corpus (endometrium), ovary, kidney, and multiple myeloma, mortality	ASB consumption, women (HR): Never	NR	1	Age, race/ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption	*Other strengths*: large cohort with long follow-up; ability to examine multiple cancer types, stratify by sex or BMI, and limit to never-smokers; comprehensive adjustment for confounders, including SSB consumption. *Other limitations*: likely non-differential exposure misclassification.
			< 1 drink/day	NR	1.02 (0.99–1.05)		
			1 drink/day	NR	1.04 (0.99–1.08)		
			≥ 2 drinks/day	NR	1.03 (0.99–1.08)		
			Continuous (per drink/ day)	30 018	1.01 (1.00–1.03)		
			Trend-test *P* value: 0.038				
		Obesity-related cancers: oesophagus, stomach, colorectum, liver, gallbladder, pancreas, breast (postmenopausal), uterus/ uterine corpus (endometrium), ovary, kidney, and multiple myeloma, mortality	ASB consumption, BMI-adjusted (HR): Never	NR	1	Age, sex, race/ ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			< 1 drink/day	NR	0.98 (0.95–1.01)		
			1 drink/day	NR	1.00 (0.97–1.03)		
			≥ 2 drinks/day	NR	0.99 (0.95–1.02)		
			Continuous (per drink/ day)	50 613	1.00 (0.99–1.01)		
			Trend-test *P* value: 0.469				

Table 2.6 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2022) (cont.)		Obesity-related cancers: oesophagus, stomach, colorectum, liver, gallbladder, pancreas, kidney, and multiple myeloma, mortality	ASB consumption, men, BMI-adjusted (HR):			Age, race/ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
			Never	NR	1		
			< 1 drink/day	NR	0.98 (0.93–1.03)		
			1 drink/day	NR	1.01 (0.96–1.08)		
			≥ 2 drinks/day	NR	1.02 (0.96–1.08)		
			Continuous (per drink/ day)	20 595	1.01 (0.99–1.03)		
			Trend-test *P* value: 0.599				
		Obesity-related cancers: oesophagus, stomach, colorectum, liver, gallbladder, pancreas, breast (postmenopausal), uterus/ uterine corpus (endometrium), ovary, kidney, and multiple myeloma, mortality	ASB consumption, women, BMI-adjusted (HR):				
			Never	NR	1		
			< 1 drink/day	NR	0.98 (0.95–1.01)		
			1 drink/day	NR	0.99 (0.95–1.03)		
			≥ 2 drinks/day	NR	0.98 (0.94–1.02)		
			Continuous (per drink/ day)	30 018	1.00 (0.98–1.01)		
			Trend-test *P* value: 0.213				

AICR, American Institute of Cancer Research; AS, artificial sweetener; ASB, artificially sweetened beverage; BMI, body mass index; CI, confidence interval; CPS-II, Cancer Prevention Study-II; FDA, Food and Drug Administration; FFQ, food frequency questionnaire; HR, hazard ratio; MCCS, Melbourne Collaborative Cohort Study; mo, month(s); NR, not reported; RR, relative risk; standard deviation; SSB, sugar-sweetened beverage; WCRF, World Cancer Research Fund; wk, week(s); yr, year(s).

smoking status, and sugar-sweetened soft drink consumption, intake of > 1 serving/day of artificially sweetened soft drinks relative to none or < 1 time/month was associated with an increased risk of non-obesity-related cancers (HR, 1.23; 95% CI, 1.02–1.48; *P* for trend, 0.006), with a hazard ratio of 1.10 (95% CI, 1.03–1.17) for each extra serving per day.

[The Working Group noted that the study provided no specific estimate of aspartame exposure, using only ASBs as a proxy, and information only on consumption frequency; therefore, non-differential misclassification of exposure to aspartame was likely.]

The NutriNet-Santé cohort study investigated the association between aspartame and risk of developing obesity-related cancers (Debras et al., 2022b). The cohort was followed up from 2009 until 22 January 2021, and included 102 865 adults, among whom 2023 incident obesity-related cancers were diagnosed. In this cohort study, individual diet was measured every 6 months by three non-consecutive web-based 24-hour dietary records, randomly assigned over 15 days (2 weekdays and 1 weekend day). Baseline dietary intakes were evaluated by averaging all 24-hour dietary records provided during the first 2 years of follow-up, with a minimum of 2 and up to 15 dietary records per study participant. Detailed, brand-specific consumption of a wide variety of individual artificial sweeteners was assessed. Obesity-related cancers were selected on the basis of the report by WCRF/AICR in 2018 (WCRF/AICR, 2018), in which the scientific evidence supporting a link between obesity and cancer risk was classified as "probable" or "convincing". [The Working Group noted that, according to the WCRF, "probable" is the level of evidence that should trigger public health action (WCRF/AICR, 2018).] The statistical model adjusted for all potential confounders considered in this study (age, sex, BMI, height, percentage weight gain during follow-up, physical activity, smoking status, number of smoked cigarettes in

pack-years, educational level, number of 24-hour dietary records, family history of cancer, history of diabetes at baseline, energy intake without alcohol, daily intakes of alcohol, sodium, saturated fatty acids, fibre, sugar, fruit and vegetables, whole-grain foods, and dairy products, and intake of other artificial sweeteners), used "non-consumers" as the reference category, and estimated that the relative risk (hazard ratio) of developing one of the obesity-related cancers considered in their analyses was 1.08 (95% CI, 0.96–1.22) for lower-consumers of aspartame and 1.15 (95% CI, 1.01–1.32) for higher-consumers (i.e. above the sex-specific median of exposure among aspartame users), with a test for trend over the three exposure categories of *P* = 0.026. Similar results were observed in sensitivity analyses that excluded participants with a history of diabetes at baseline or accounted for all available dietary records completed during follow-up. No interaction between aspartame and BMI was detected for obesity-related cancer (*P* for interaction, 0.925). [The Working Group noted that a major strength of this study was the assessment and separation of the consumption of foods and drinks specifically containing aspartame and the careful incorporation of this information into the epidemiological analysis.]

The CPS-II study included 934 777 men and women aged ≥ 28 years at baseline (McCullough et al., 2022). The study started in 1982, and follow-up was extended through 2016, with a median follow-up of 27.7 years. The baseline questionnaire included a grid that asked how many cups, glasses, or drinks of ASBs were usually consumed a day and for how many years, with write-in reporting by frequency and duration. Diet soda and diet iced teas were considered as ASBs, whereas "non-diet colas" and "other non-diet soft drinks" were considered as sugar-sweetened beverages. The CPS-II study used cancer mortality as the end-point. All Cox proportional hazards models used age as the timescale and adjusted for several

potential confounders that included sex, race/ethnicity, smoking, marital status, education, red and processed meat intake, fruit and vegetable intake, alcohol, and sugar-sweetened beverage intake. The authors presented the analyses of the association between ASB consumption and obesity-related cancers risk separately with non-BMI-adjusted and BMI-adjusted statistical models. When not adjusted for BMI, the estimated trends for increasing levels of consumption of ASBs reached statistical significance. Considering non-consumers as the reference category, the hazard ratios were 1.02 (95% CI, 0.99–1.05) for consumption of < 1 drink/day; 1.04 (95% CI, 1.01–1.08) for 1 drink/day; and 1.05 (95% CI, 1.01–1.08) for ≥ 2 drinks/day; with a *P* for trend across groups of 0.001, for men and women combined. In the statistical analyses additionally adjusted for BMI, the hazard ratio for the same increasing levels of exposure were all close to null (HR for < 1 drink/day, 0.98; 95% CI, 0.95–1.01; HR for 1 drink/day, 1.00; 95% CI, 0.97–1.03; and HR for ≥ 2 drinks/day, 0.99; 95% CI, 0.95–1.02). In analyses stratified by sex, the BMI-adjusted hazard ratio for consumption of ≥ 2 drinks/day versus none was 1.02 (95% CI, 0.96–1.08; *P* for trend, 0.599) for men and 0.98 (95% CI, 0.94–1.02; *P* for trend, 0.213) for women (*P* for interaction, 0.425). [The Working Group noted that, from a quantitative point of view, the hazard ratios reported by the CPS-II study were rather modest, in fact just slightly higher than 1, and they were statistically precise because of the extremely large sample size of the CPS-II study, which has more than 900 000 participants. The adjustment for BMI had a negligible impact on the point estimate and only slightly increased the confidence interval width.]

[The Working Group noted that these three cohort studies (Hodge et al., 2018; Debras et al., 2022b; McCullough et al., 2022) shared the aim of investigating the association between ASBs, more specifically aspartame in one study (Debras et al., 2022b), and the risk of obesity-related cancers; however, the cancer sites included in each study's case definition had some overlap but also some differences. Table 2.7 summarizes and compares the cancer end-points included in the obesity-related group in each study.]

A single meta-analysis reported on cancers related to obesity and on cancers not related to obesity, finding no association for either (Yin et al., 2022). [The Working Group noted that only the studies of Hodge et al. (2018) and Bassett et al. (2020) specifically looked at these cancer groupings, so the results included in the summary estimates came from studies with results for various individual cancer sites but were not reported as a group of "obesity-related cancers". Furthermore, the study by Debras et al. (2022b) was included in the non-obesity-related cancer meta-analysis, despite not reporting results for this cancer group. Additionally, some of the studies involved exposure to other artificial sweeteners and were not specific for aspartame.]

2.7 Cancer of all sites combined

See Table 2.8.

Eight studies including seven cohorts evaluated overall cancer incidence (NutriNet-Santé, Chazelas et al., 2019 and Debras et al., 2022b; and PLCO, You et al., 2022) or mortality (EPIC, Mullee et al., 2019; NHS, HPFS, Malik et al., 2019; National Health and Nutrition Examination Survey, NHANES, Zhang et al., 2021 and Fulgoni and Drewnowski, 2022; and CPS-II, McCullough et al., 2022) in relation to different estimates of aspartame consumption. Most studies used artificially sweetened soft drink intake or frequency as the exposure, but an analysis from NutriNet-Santé (Debras et al., 2022b) investigated specific estimates of aspartame intake derived from different sources using detailed information from multiple dietary records. [The Working Group noted that this study also included longer follow-up of the NutriNet-Santé cohort; hence, the study by Chazelas et al. (2019) was not further

Table 2.7 Cancers included in the "obesity-related" category used in the three cohort studies reported in Section 2.6

Cancer	Cohort study		
	NutriNet-Santé (Debras et al., 2022b)[a]	MCCS (Hodge et al., 2018)[a]	CPS-II mortality cohort (McCullough et al., 2022)[b]
Colorectum	✓	✓	✓
Stomach	✓	Gastric cardia	✓
Liver	✓	✓	✓
Mouth	✓	–	–
Pharynx	✓	–	–
Larynx	✓	–	–
Oesophagus	✓	Adenocarcinoma	✓
Breast, postmenopausal	✓	✓	✓
Breast, premenopausal	✓	–	–
Ovary	✓	✓	✓
Endometrium	✓	✓	✓
Prostate	✓	Aggressive	–
Pancreas	–	✓	✓
Kidney	–	✓	✓
Gallbladder	–	✓	✓
Multiple myeloma	–	–	✓
Meningioma	–	–	–

✓, Included; –, not included; CPS-II, Cancer Prevention Study-II; MCCS, Melbourne Collaborative Cohort Study.

[a] Based on the definition in WCRF/AICR (2018).

[b] This study evaluated mortality. No reference was provided regarding the definition of the obesity-related cancer grouping.

considered.] A ninth study (Liu et al., 2022b) examined consumption of coffee with artificial sweetener in the United Kingdom Biobank. [The Working Group considered this study to be uninformative since the exposure was not an adequate proxy for aspartame intake. An additional limitation of this study was that, in the United Kingdom Biobank and similarly in the results on coffee from the AARP-NIH and EPIC studies, coffee was associated with reduced risk of deaths both from all causes and from cancer, which would confound any association between artificial sweetener use and cancer.]

A prospective study of 102 865 adults followed for a median of 7.8 years in the French population-based NutriNet-Santé cohort examined aspartame consumption in relation to the incidence of all cancers combined (Debras et al., 2022b). The study observed 3358 cancers that were verified using medical and anatomopathological reports. In this analysis, aspartame intake from all possible dietary sources was specifically assessed. Intake estimates were based on all records available (at least two or more) during the first 2 years of follow-up. Individuals classified as having a higher intake of aspartame (i.e. above the sex-specific median among aspartame consumers, from an average of 5.6 dietary records per participant) had an elevated cancer risk compared with non-consumers (HR for higher-consumers, 1.15; 95% CI, 1.03–1.28; P for trend, 0.002). Similar results were observed in sensitivity analyses that excluded participants with a history of diabetes at baseline or accounted for all available dietary records completed during follow-up. No interaction between aspartame and BMI was detected for overall cancer (P = 0.893). [An important strength of this study

Table 2.8 Epidemiological studies on consumption of aspartame and cancer of all sites combined

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Malik et al. (2019) USA Enrolment, 1976 (NHS), 1986 (HPFS/follow-up, 1980–2014 (NHS), 1986–2014 (HPFS) Cohort	37 716 men; 80 647 women; female registered nurses aged 30–55 yr in the NHS and male health professionals aged 40–75 yr in the HPFS; excluding those with history of diabetes, cardiovascular disease, or cancer at baseline, or with implausible dietary intake Exposure assessment method: questionnaire; prospective assessment of ASB consumption through repeated FFQs between 1980–1986 and 2010	All cancers combined, mortality	ASB intake (HR):			Age, race, smoking, alcohol intake, postmenopausal hormone use (NHS), physical activity, family history of diabetes; family history of myocardial infarction, family history of cancer, multivitamin use, aspirin use, baseline history of hypertension and hypercholesterolaemia, intake of whole grains, fruit, vegetables, or red and processed meat, total energy, BMI, SSB intake	*Exposure assessment critique*: A key strength was the prospective assessment of ASB consumption as a cumulative average from repeated, validated diet assessments every 4 yr, the majority at a very relevant period for aspartame exposure from ASBs (the USA between the 1980s and 2010) potentially capturing lifetime exposure to aspartame. A key limitation was that other sources of aspartame were not considered (although these were more limited); and uncertainty regarding aspartame content in ASBs after the mid-2000s. *Other strengths*: large cohort with long follow-up.
			< 1 serving/mo	6272	1		
			1–4 servings/mo	1803	1.01 (0.96–1.07)		
			2–6 servings/wk	2516	0.99 (0.94–1.04)		
			1 to < 2 servings/day	1013	1.00 (0.93–1.07)		
			≥ 2 servings/day	776	1.04 (0.96–1.12)		
			Continuous (per serving/day)	12 380	1.01 (0.98–1.03)		
			Trend-test *P* value: 0.58				
		All cancers combined, mortality	ASB intake estimated using cumulative average intake (HR):				
			< 1 serving/mo	NR	1		
			1–4 servings/mo	NR	0.90 (0.85–0.95)		
			2–6 servings/wk	NR	0.88 (0.84–0.92)		
			1 to < 2 servings/day	NR	0.89 (0.83–0.95)		
			≥ 2 servings/day	NR	0.91 (0.84–1.00)		
			Continuous (per serving/day)	NR	0.98 (0.96–1.01)		
			Trend-test *P* value: 0.03				

Table 2.8   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Malik et al. (2019) (cont.)		All cancers combined, mortality	ASB intake, 8-yr lag (HR):			Age, race, smoking, alcohol intake, postmenopausal hormone use (NHS), physical activity, family history of diabetes; family history of myocardial infarction, family history of cancer, multivitamin use, aspirin use, baseline history of hypertension and hypercholesterolaemia, intake of whole grains, fruit, vegetables, or red and processed meat, total energy, BMI, SSB intake	*Other limitations*: likely bias from non-differential misclassifications of exposure to aspartame; stratified numbers of deaths not provided for specific cancer sites.
			< 1 serving/mo	NR	1		
			1–4 servings/mo	NR	0.96 (0.90–1.02)		
			2–6 servings/wk	NR	0.93 (0.88–0.98)		
			1 to < 2 servings/day	NR	0.91 (0.84–0.98)		
			≥ 2 servings/ day	NR	1.01 (0.93–1.10)		
			Continuous (per serving/day)	NR	1.01 (0.99–1.04)		
			Trend-test *P* value: 0.83				
		All cancers combined, mortality	ASB intake, men (HPFS) (HR):			Age, race, smoking, alcohol intake, physical activity, family history of diabetes; family history of myocardial infarction, family history of cancer, multivitamin use, aspirin use, baseline history of hypertension and hypercholesterolaemia, intake of whole grains, fruit, vegetables, or red and processed meat, total energy, BMI, SSB intake	
			< 1 serving/mo	2118	1		
			1–4 servings/mo	469	1.01 (0.92–1.12)		
			2–6 servings/wk	996	1.03 (0.95–1.11)		
			1 to < 2 servings/day	283	0.99 (0.87–1.13)		
			≥ 2 servings/day	196	0.95 (0.82–1.11)		
			Continuous (per serving/ day)	4062	0.99 (0.95–1.03)		
			Trend-test *P* value: 0.51				

Table 2.8 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Malik et al. (2019) (cont.)		All cancers combined, mortality	ASB intake, women (NHS) (HR):			Age, race, smoking, alcohol intake, postmenopausal hormone use (NHS), physical activity, family history of diabetes; family history of myocardial infarction, family history of cancer, multivitamin use, aspirin use, baseline history of hypertension and hypercholesterolaemia, intake of whole grains, fruit, vegetables, or red and processed meat, total energy, BMI, SSB intake	
			< 1 serving/mo	4154	1		
			1–4 servings/mo	1334	1.00 (0.94–1.07)		
			2–6 servings/wk	1520	0.95 (0.90–1.01)		
			1 to < 2 servings/day	730	0.98 (0.91–1.06)		
			≥ 2 servings/day	580	1.05 (0.96–1.15)		
			Continuous (per serving/ day)	8318	1.01 (0.98–1.04)		
			Trend-test *P* value: 0.50				

Table 2.8   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Mullee et al. (2019) Europe Enrolment, 1992–2000/ follow-up, through 2009–2013 (depending on study centre; mean, 16.4 yr) Cohort	451 743; EPIC cohort study participants from 10 European countries (Denmark, France, Greece, Germany, Italy, Netherlands, Norway, Spain, Sweden, and the United Kingdom); men and women excluding those with prevalent cancer, heart disease, stroke, or diabetes or with implausible dietary intake; for artificially sweetened soft drinks, participants from Italy, Spain, and Sweden were not included. Exposure assessment method: questionnaire; exposure to artificially sweetened soft drinks overall assessed once at baseline through country-specific validated tools (mainly FFQ) covering the usual diet over the past year; no specific assessment of aspartame or AS content of the artificially sweetened soft drinks	All cancers combined: mortality	Consumption of artificially sweetened soft drink (glass, 250 mL) (HR): < 1 glass/mo 1 to 4 glasses/mo > 1 to 6 glasses/ wk 1 to < 2 glasses/ day ≥ 2 glasses/day Trend-test *P* value: 0.23	9359 1246 1251 72 303	1 0.96 (0.90–1.02) 1.00 (0.94–1.06) 0.92 (0.73–1.16) 1.10 (0.97–1.23)	Age, centre, sex, BMI, physical activity index, educational status, alcohol consumption, smoking status, smoking intensity, smoking duration, ever use of contraceptive pill, menopausal status, ever use of menopausal hormone therapy, intakes of total energy, red and processed meat, fruits and vegetables, coffee, fruit and vegetable juice, sugar-sweetened soft drinks	*Exposure assessment critique*: A key strength was the prospective assessment of ASB consumption (several types of beverage) in several western Europe countries at a period relevant for aspartame exposure (between 1991 and 2000). Key limitations were that no other sources of aspartame were considered (although these were more limited); uncertainty regarding the aspartame content in ASBs in every country; and that there was only one assessment at baseline.

Table 2.8 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Mullee et al. (2019) (cont.)		All cancers combined, mortality	Consumption of artificially sweetened soft drink (glass, 250 mL), men (HR):			Age, centre, BMI, physical activity index, educational status, alcohol consumption, smoking status, smoking intensity, smoking duration, intakes of total energy, red and processed meat, fruits and vegetables, coffee, fruit and vegetable juice, sugar-sweetened soft drinks	Other information: context of low ASB consumption in middle-aged adults. *Other strengths*: large population-based cohort spanning multiple countries with different behaviours; large number of cases; results adjusted for appropriate potential confounders, including BMI.
			< 1 glass/mo	NR	1		
			1 to 4 glasses/mo	NR	0.99 (0.89–1.09)		
			> 1 to 6 glasses/ wk	NR	1.10 (0.99–1.22)		
			1 to < 2 glasses/ day	NR	1.13 (0.76–1.66)		
			≥ 2 glasses/day	NR	1.14 (0.95–1.37)		
			Trend-test *P* value: 0.06				
		All cancers combined, mortality	Consumption of artificially sweetened soft drink (glass, 250 mL), women (HR):			Age, centre, BMI, physical activity index, educational status, alcohol consumption, smoking status, smoking intensity, smoking duration, ever use of contraceptive pill, menopausal status, ever use of menopausal hormone therapy, intakes of total energy, red and processed meat, fruits and vegetables, coffee, fruit and vegetable juice, sugar-sweetened soft drinks	
			< 1 glass/mo	NR	1		
			1 to 4 glasses/mo	NR	0.95 (0.87–1.02)		
			> 1 to 6 glasses/ wk	NR	0.95 (0.88–1.02)		
			1 to < 2 glasses/ day	NR	0.83 (0.62–1.11)		
			≥ 2 glasses/day	NR	1.06 (0.91–1.24)		
			Trend-test *P* value: 0.98				

Table 2.8 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Zhang et al. (2021) USA Enrolment, 1999–2014/ follow-up, through 2015 Cohort	31 402; NHANES participants, nationally representative sample of adult men and women aged ≥ 20 yr; participants excluded for current pregnancy or missing data Exposure assessment method: exposure to ASBs (sugar-free soft drinks and carbonated water) assessed at baseline with one or two 24 h dietary recalls	All cancers combined, mortality	Daily intake of ASBs (HR): None > 0 to < 1 serving/day 1 to < 2 servings/day ≥ 2 servings/day Continuous (per 1 serving/ day)	727 68 64 24 883	1 0.91 (0.64–1.29) 1.18 (0.84–1.66) 0.61 (0.35–1.04) 0.93 (0.83–1.06)	Age, sex, family income to poverty ratio, race, cigarette smoking, alcohol consumption, marital status, leisure time physical activity, BMI, prevalent high cholesterol, hypertension and diabetes, history of CVD and cancer, Healthy Eating Index-2015, total energy intake, SSB intake, complex sample design of NHANES	*Exposure assessment critique*: A key strength was the prospective assessment of ASB consumption through dietary recalls, potentially capturing all types of ASB. Key limitations were the small number of dietary recalls (only one or two); no other sources were considered; only one assessment at baseline; and uncertainty regarding the aspartame content in ASBs in the USA after the mid-2000s (i.e. for the most part of the study assessment period, 1999–2014). *Other strengths*: prospective design; representative sample; consideration of nonlinear associations using cubic splines analysis. *Other limitations*: few cases among consumers of ASBs; likely non-differential exposure misclassification.

Table 2.8 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
[Debras et al. (2022b)](#) France Enrolment, 2009–2021/ follow-up until 22 January 2021 (median, 7.8 yr) Cohort	102 865; population-based cohort NutriNet-Santé (web-based); men and women aged ≥ 18 yr Exposure assessment method: records; participants are asked every 6 mo to complete a series of three validated web-based 24 h dietary records randomly assigned over a 2-wk period (2 weekdays, 1 weekend day); at least two 24 h dietary records during the first 2 yr of follow-up considered in analyses (mean ± SD, 5.6 ± 3.0)	All cancers combined, incidence	Aspartame intake (HR):			Age, sex, BMI, height, percentage weight gain during follow-up, physical activity, smoking status, number of smoked cigarettes in pack-years, educational level, number of 24 h dietary records, family history of cancer, prevalent diabetes, energy intake without alcohol, daily intakes of alcohol, sodium, saturated fatty acids, fibre, sugar, fruit and vegetables, whole-grain foods, and dairy products, other AS intake	*Exposure assessment critique*: A key strength was that it was a prospective study using dietary records and quantitative assessment of aspartame based on food composition data updates for food supply changes over time. A key limitation was the baseline assessment in the main analysis but the sensitivity analysis was conducted using all 24 h dietary records available during follow-up. There was low aspartame use in the cohort (28%). *Other strengths*: large prospective cohort; large number of cases; sensitivity analyses excluded prevalent diabetes or used all available 24 h dietary records throughout follow-up.
			Non-consumers	2309	1		
			Lower consumers (men, < 14.45 mg/day; women, < 15.39 mg/day)	572	1.12 (1.02–1.23)		
			Higher consumers (men, ≥ 14.45 mg/day; women, ≥ 15.39 mg/day)	477	1.15 (1.03–1.28)		
			Trend-test *P* value: 0.002				
		All cancers combined, incidence	Aspartame intake, participants without diabetes (HR):			Age, sex, BMI, height, percentage weight gain during follow-up, physical activity, smoking status, number of smoked cigarettes in pack-years, educational level, number of 24 h dietary records, family history of cancer, energy intake without alcohol, daily intakes of alcohol, sodium, saturated fatty acids, fibre, sugar, fruit and vegetables, whole-grain foods, and dairy products, other AS intake	
			Non-consumers	2238	1		
			Lower consumers (men, < 14.45 mg/day; women, < 15.39 mg/day)	542	1.13 (1.03–1.24)		
			Higher consumers (men, ≥ 14.45 mg/day; women, ≥ 15.39 mg/day)	439	1.16 (1.04–1.29)		
			Trend-test *P* value: 0.002				

Table 2.8   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
[Debras et al. (2022b)](#) (cont.)		All cancers combined, incidence	Aspartame intake (time-dependent) (HR):			Age, sex, BMI, height, percentage weight gain during follow-up, physical activity, smoking status, number of smoked cigarettes in pack-years, educational level, number of 24 h dietary records, family history of cancer, prevalent diabetes, energy intake without alcohol, daily intakes of alcohol, sodium, saturated fatty acids, fibre, sugar, fruit and vegetables, whole-grain foods, and dairy products, other AS intake	*Other limitations*: self-selection may limit generalizability; potential for residual confounding and reverse causation.
			Non-consumers	2309	1		
			Lower consumers (men, < 14.45 mg/day; women, < 15.39 mg/day)	572	1.14 (1.05–1.25)		
			Higher consumers (men, ≥ 14.45 mg/day; women, ≥ 15.39 mg/day)	477	1.13 (1.02–1.25)		
			Trend-test *P* value: 0.003				

Table 2.8 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Fulgoni and Drewnowski (2022) USA Enrolment, 1988–1994/ follow-up, through 2019 Cohort	12 438; NHANES participants, nationally representative sample of adult men and women aged > 19 yrs; participants excluded for current pregnancy, history of myocardial infarction, chronic heart failure, stroke, cancer, or missing data; 978 cancer deaths overall (546 men, 432 women) Exposure assessment method: exposure to low-calorie sweeteners/ aspartame assessed through one or maximum two dietary recalls in several cycles of NHANES; aspartame specifically considered in NHANES 1988–1994 (beverages and added sweeteners); limited reporting of methodology	All cancers combined, mortality	Tertile of aspartame intake (HR): Non-consumers Tertile 1 Tertile 2 Tertile 3 Trend-test *P* value: 0.9755	NR NR NR NR	1 0.70 (0.49–0.99) 0.72 (0.49–1.06) 1.32 (0.94–1.85)	Age, sex, race/ethnicity, education, current smoking status (yes/no), alcohol consumption, physical activity level, and BMI (continuous) as covariates; NHANES 1988–1994 exam weights	*Exposure assessment critique*: A key strength was the prospective assessment of aspartame exposure through 24 h recalls considering the most relevant source at the time of dietary assessment (1988–1994). Key limitations were the low number of dietary recalls (one recall for analysis with aspartame intake); and assessment at baseline only with up to 31 yr of follow-up and changes in the use of aspartame during that period. *Other strengths*: large representative cohort, large number of deaths. *Other limitations*: lacking detail on the mortality data used; likely bias from non-differential exposure misclassification.
		All cancers combined, mortality	Tertile of aspartame intake, men (HR): Non-consumers Tertile 1 Tertile 2 Tertile 3 Trend-test *P* value: 0.8547	NR NR NR NR	1 0.61 (0.37–1.02) 0.55 (0.32–0.94) 1.50 (0.74–3.05)	Age, race/ethnicity, education, current smoking status (yes/no), alcohol consumption, physical activity level, and BMI (continuous) as covariates. NHANES 1988–1994 exam weights	
		All cancers combined, mortality	Tertile of aspartame intake, women (HR): Non-consumers Tertile 1 Tertile 2 Tertile 3 Trend-test *P* value: 0.9777	NR NR NR NR	1 0.83 (0.52–1.34) 0.81 (0.52–1.26) 1.15 (0.81–1.65)		

Table 2.8   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
[Fulgoni and Drewnowski (2022)](#) (cont.)		All cancers combined, mortality	Tertile of aspartame intake, age 19–50 yr (HR):			Age, sex, race/ethnicity, education, current smoking status (yes/no), alcohol consumption, physical activity level, and BMI (continuous) as covariates. NHANES 1988–1994 exam weights	
			Non-consumers	NR	1		
			Tertile 1	NR	0.38 (0.18–0.82)		
			Tertile 2	NR	0.57 (0.27–1.23)		
			Tertile 3	NR	1.54 (0.91–2.62)		
			Trend-test *P* value: 0.7825				
		All cancers combined, mortality	Tertile of aspartame intake, age 19–50 yr, men (HR):			Age, race/ethnicity, education, current smoking status (yes/no), alcohol consumption, physical activity level, and BMI (continuous) as covariates. NHANES 1988–1994 exam weights	
			Non-consumers	NR	1		
			Tertile 1	NR	0.44 (0.17–1.14)		
			Tertile 2	NR	1.19 (0.51–2.77)		
			Tertile 3	NR	1.46 (0.55–3.89)		
			Trend-test *P* value: 0.5494				
		All cancers combined, mortality	Tertile of aspartame intake, age 19–50 yr, women (HR):				
			Non-consumers	NR	1		
			Tertile 1	NR	0.35 (0.11–1.16)		
			Tertile 2	NR	0.46 (0.18–1.17)		
			Tertile 3	NR	1.28 (0.65–2.50)		
			Trend-test *P* value: 0.7151				
		All cancers combined, mortality	Tertile of aspartame intake, age 51+ yr (HR):			Age, sex, race/ethnicity, education, current smoking status (yes/no), alcohol consumption, physical activity level, and BMI (continuous) as covariates. NHANES 1988–1994 exam weights	
			Non-consumers	NR	1		
			Tertile 1	NR	0.86 (0.61–1.23)		
			Tertile 2	NR	0.65 (0.37–1.14)		
			Tertile 3	NR	0.99 (0.68–1.44)		
			Trend-test *P* value: 0.4396				

Table 2.8   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
[Fulgoni and Drewnowski (2022)](#) (cont.)		All cancers combined, mortality	Tertile of aspartame intake, age 51+ yr, men (HR):			Age, race/ethnicity, education, current smoking status (yes/no), alcohol consumption, physical activity level, and BMI (continuous) as covariates. NHANES 1988–1994 exam weights	
			Non-consumers	NR	1		
			Tertile 1	NR	0.72 (0.38–1.36)		
			Tertile 2	NR	0.28 (0.13–0.56)		
			Tertile 3	NR	1.01 (0.56–1.81)		
			Trend-test *P* value: 0.0884				
		All cancers combined, mortality	Tertile of aspartame intake, age 51+ yr, women (HR):				
			Non-consumers	NR	1		
			Tertile 1	NR	1.06 (0.69–1.63)		
			Tertile 2	NR	0.95 (0.48–1.88)		
			Tertile 3	NR	1.24 (0.78–1.97)		
			Trend-test *P* value: 0.4677				

Table 2.8 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2022) USA Enrolment, 1982/follow-up, through 2016 (median, 27.7 yr) Cohort	934 777 (416 313 men, 518 464 women); CPS-II prospective cohort; adults aged ≥ 28 yr; excluded participants with personal history at baseline of diabetes or cancer other than nonmelanoma skin cancer, men aged > 90 yr or women aged > 95 yr at enrolment, and those reporting only prior but not current consumption of either SSBs or ASBs Exposure assessment method: questionnaire; exposure to ASBs assessed in 1982 through a question about the number of drinks/day of diet soda or ice teas (one pooled item) and potential changes over the past 10 yr; no specific assessment of aspartame content in ASBs	All cancers combined, mortality	ASB consumption (HR): Never < 1 drink/day 1 drink/day ≥ 2 drinks/day Continuous (per drink/day) Trend-test P value: 0.227	NR NR NR NR 135 093	1 0.98 (0.96–0.99) 0.99 (0.97–1.01) 0.99 (0.97–1.02) 1.00 (0.99–1.00)	Age, sex, race/ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption	*Exposure assessment critique*: A key strength was the prospective assessment of ASB consumption. Key limitations were that only one dietary assessment was carried out at baseline in 1982 that was before the use of aspartame in ASB, hence the relevance to aspartame exposure depends on the stability of ASB consumption over up to 34 yr of follow-up, but such information was not available; no other sources considered (although more limited); uncertainty of aspartame content in ASBs after the mid-2000s. Other information: exclusion of participants who reported only prior but not current consumption of either SSBs or ASBs at baseline.
		All cancers combined, mortality	ASB consumption, BMI-adjusted (HR): Never < 1 drink/day 1 drink/day ≥ 2 drinks/day Continuous (per drink/day) Trend-test P value: 0.001	NR NR NR NR 135 093	1 0.96 (0.95–0.98) 0.98 (0.96–1.00) 0.97 (0.95–0.99) 0.99 (0.99–1.00)	Age, sex, race/ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	
		All cancers combined, mortality	ASB consumption, men, BMI-adjusted (HR): Never < 1 drink/day 1 drink/day ≥ 2 drinks/day Continuous (per drink/day) Trend-test P value: 0.033	NR NR NR NR 70 834	1 0.97 (0.94–1.00) 0.98 (0.94–1.01) 0.98 (0.94–1.01) 1.00 (0.99–1.00)	Age, race/ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	

Table 2.8   (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McCullough et al. (2022) (cont.)		All cancers combined, mortality	ASB consumption, women, BMI-adjusted (HR):			Age, race/ethnicity, smoking, marital status, education, red and processed meat consumption, fruit and vegetable consumption, alcohol consumption, SSB consumption, BMI	*Other strengths*: large cohort with long follow-up; ability to examine multiple cancer types, stratify by sex or BMI, and limit to never-smokers; comprehensive adjustment for confounders, including SSB consumption. *Other limitations*: likely bias from non-differential exposure misclassification.
			Never	NR	1		
			< 1 drink/day	NR	0.95 (0.93–0.97)		
			1 drink/day	NR	0.96 (0.93–0.99)		
			≥ 2 drinks/day	NR	0.95 (0.92–0.98)		
			Continuous (per drink/day)	64 259	0.98 (0.98–0.99)		
			Trend-test *P* value: < 0.0001				

Table 2.8 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
You et al. (2022) USA Enrolment, 1993–2001/ follow-up through 2009 (median, 11.3 yr) Cohort	92 997; PLCO cancer screening trial participants, men and women aged 55–74 yr in 10 study centres; participants with history of cancer or diabetes were excluded Exposure assessment method: diet history questionnaire: FFQ with 156 items	All cancers combined, incidence	Type of soft drink consumption (HR):			Age, sex, race, study centre, arm, total energy intake, alcohol consumption, smoking status, BMI categories (baseline), physical activity, education, red meat intake, amounts of fruits and vegetables, coffee, family history of lung cancer	*Exposure assessment critique*: A key strength was the timing of exposure, which was consistent for aspartame being the major AS in beverage; ASBs were the major source of aspartame in this country and time frame. Key limitations were that there was no specific estimate of aspartame exposure, ASBs were used as a proxy; and only consumption vs non-consumption was considered (not dose); exposure data were reported at baseline only. *Other strengths*: prospective analysis. *Other limitations*: likely bias from non-differential exposure misclassification.
			No soft drink consumption	1089	1		
			Regular only	5202	1.03 (0.97–1.11)		
			Diet only	4557	1.06 (0.99–1.13)		
			Both	2078	0.99 (0.92–1.07)		
		All cancers combined, incidence	Type of soft drink consumption, men (HR):			Age, race, study centre, arm, total energy intake, alcohol consumption, smoking status, BMI categories (baseline), physical activity, education, red meat intake, amounts of fruits and vegetables, coffee, family history of lung cancer	
			No soft drink consumption	539	1		
			Regular only	3532	1.02 (0.93–1.12)		
			Diet only	2221	1.04 (0.94–1.14)		
			Both	1368	0.98 (0.89–1.09)		
		All cancers combined, incidence	Type of soft drink consumption, women (HR):			Age, race, study centre, arm, total energy intake, alcohol consumption, smoking status, BMI categories (baseline), physical activity, education, red meat intake, amounts of fruits and vegetables, coffee, family history of lung cancer, estrogen use	
			No soft drink consumption	[550]	1		
			Regular only	[1670]	1.03 (0.93–1.14)		
			Diet only	[2336]	1.07 (0.98–1.18)		
			Both	[710]	1.00 (0.89–1.12)		

Table 2.8 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
You et al. (2022) (cont.)		All cancers combined, incidence	Type of soft drink consumption, never-smokers (HR):			Age, sex, race, study centre, arm, total energy intake, alcohol consumption, BMI categories (baseline), physical activity, education, red meat intake, amounts of fruits and vegetables, coffee, family history of lung cancer	
			No soft drink consumption	446	1		
			Regular only	2183	1.04 (0.94–1.15)		
			Diet only	1858	1.09 (0.98–1.21)		
			Both	953	1.03 (0.92–1.16)		
		All cancers combined, incidence	Type of soft drink consumption, ever/current smokers (HR):				
			No soft drink consumption	643	1		
			Regular only	3017	1.04 (0.95–1.14)		
			Diet only	2699	1.02 (0.93–1.11)		
			Both	1124	0.95 (0.86–1.05)		

AICR, American Institute of Cancer Research; AS, artificial sweetener; ASB, artificially sweetened beverage; BMI, body mass index; CI, confidence interval; CPS-II, Cancer Prevention Study-II; CVD, cardiovascular disease; EPIC, European Prospective Investigation into Cancer and Nutrition; FDA, Food and Drug Administration; FFQ, food frequency questionnaire; HPFS, Health Professionals Follow-up Study; HR, hazard ratio; mo, month(s); NHANES, National Health and Nutrition Examination Survey; NHS, Nurses' Health Study; NR, not reported; PLCO, Prostate, Lung, Colorectal and Ovarian Cancer screening trial; RR, relative risk; SD, standard deviation; SSB, sugar-sweetened beverage; WCRF, World Cancer Research Fund; wk, week(s); yr, year(s).

was its estimation of aspartame intake from all dietary sources using brand-specific and time-matched composition data, which no other study provided; limitations included the relatively small proportion of aspartame users, meaning that the study could not evaluate finer classifications of amount consumed.]

In a prospective analysis of 80 647 women from the NHS cohort and 37 716 men from the HPFS cohort (Malik et al., 2019), 12 380 deaths from cancer occurred during a 34-year follow-up. ASB consumption was assessed 7–8 times during follow-up from 1980 in the NHS and 1986 in the HPFS, both studies ending follow-up in 2014. No association with all cancer mortality was observed (HR for ASB consumption of ≥ 2/day versus < 1/month, 1.04; 95% CI, 0.96–1.12). [An important strength of this analysis was that ASB consumption was assessed on multiple occasions, and the data were updated each time to account for changes in intakes. However, the results from this analysis were similar to those using the cumulative average of intake (HR for ASB consumption of ≥ 2/day versus < 1/month, 0.91; 95% CI, 0.84–1.00). As with other studies, the main limitations related to the lack of information on other sources of aspartame and the contribution of aspartame to artificial sweeteners used in beverages over time, although follow-up included the period when aspartame was the main artificial sweetener and soft drinks were the main source of aspartame in the USA.]

In the EPIC study, which included cohorts from seven countries (ASB data were not collected in all centres in Italy, Spain, or Sweden; therefore, those countries were excluded from this analysis) and more than 12 000 cancer deaths, the hazard ratio for ≥ 2 glasses/day of artificially sweetened soft drinks versus < 1 glass/month was 1.10 (95% CI, 0.97–1.23) (Mullee et al., 2019). [EPIC is a large prospective study with many cases, and the results were adjusted for appropriate potential confounders. The Working Group considered results adjusted for BMI to be the most informative. Although data were collected during a relevant time period for aspartame use in Europe, the collection of intake data at a single time point and the variation in products across countries and over time may lead to exposure misclassification and bias towards the null.]

Zhang et al. (2021) analysed NHANES data from 1999–2014 in relation to cancer mortality. Intake data were based on two 24-hour dietary recalls since 2003 ($n = 22 348$) or a single 24-hour dietary recall before 2003 ($n = 9054$). After a mean follow-up of 7.9 years, 3878 deaths, including 883 cancer deaths, were identified. A hazard ratio of 0.61 (95% CI, 0.35–1.04) was found for consumption of ≥ 2 servings/day of ASBs relative to no consumption. [The Working Group noted the wide confidence interval for this estimate. Notable strengths of the study included consideration of nonlinear associations using cubic splines analysis and use of a representative sample. The major weakness, as in most other studies, was the lack of specific data on aspartame intakes, and also that ASB intake may not be a good proxy for aspartame intake after 2003, the relatively small number of cancer deaths, and use of one or two dietary recalls that may not represent usual intake.]

Using 24-hour recall data from 12 438 participants aged > 19 years in the 1988–1994 NHANES survey, Fulgoni and Drewnowski (2022) evaluated aspartame intake in relation to all-cancer mortality. Results were reported overall and stratified by both sex and age (19–50 years, ≥ 51 years). For all ages combined, the hazard ratio was 1.32 (95% CI, 0.94–1.85; P for trend, 0.9755) for the top tertile of intake relative to non-consumers (Fulgoni and Drewnowski, 2022). [The Working Group acknowledged the wide confidence interval and the lack of a trend across increasing intakes. Strengths were the nationally representative sample and calculation of aspartame intake from diet beverages and tabletop sweeteners, which were the main sources of aspartame at the time; weaknesses included

that specific aspartame information was limited to the 1988–1994 cycle (NHANES III), so that only a smaller group with 978 deaths could be included in this analysis. Intake estimates were based on a single recall so could not be assumed to be representative of usual intake. No information was provided on mortality data, so it was not clear how cancer mortality was defined. Despite the potential strength of using specific aspartame intake in this analysis, the unclear outcome definition meant that the earlier analysis of NHANES data was considered to be more informative, although both analyses were consistent in not finding strong evidence linking aspartame or ASB intake with cancer mortality.]

During a median follow-up of 27.7 years, 135 093 CPS-II participants died from cancer (25.9% of all deaths) (McCullough et al., 2022). The cancer mortality hazard ratio for ≥ 2 drinks of ASBs relative to none was 0.99 (95% CI, 0.97–1.02) without adjustment for BMI and 0.97 (95% CI, 0.95–0.99) with BMI adjustment. The study used only a single assessment of ASB intake at baseline, which was before the approval and use of aspartame for soft drinks in the USA in 1983. [The Working Group considered that the results adjusted for BMI were the most informative. The strengths of the CPS-II study were its large size and prospective design. The Working Group noted that the relevance of this study with regard to aspartame exposure depended on the stability of ASB consumption over time since the introduction of aspartame into ASBs in 1983 in the USA. Although no information was available regarding potential variations in ASB intake over time during the (up to) 34 years of follow-up, some data from McCullough et al. (2014) in the CPS-II cohort suggested a stable ranking of participants with respect to ASB consumption between 1982 and 1999 for the subsample providing data at both time points. The Working Group still noted uncertainty regarding aspartame content in ASBs from the mid-2000s. Non-differential misclassification was therefore likely, probably leading to bias towards the null.] The only study reporting on the interaction between sex and ASB consumption (McCullough et al., 2022) did not find evidence of an effect of sex on the association (*P* for interaction, 0.145).

You et al. (2022) analysed prospective data from the PLCO Cancer Screening Trial, which followed more than 90 000 men and women for a median of 11.3 years. Hazard ratios slightly greater than 1 were found for use of diet soft drinks only, relative to no soft drink consumption, for several subgroups: overall HR, 1.06; 95% CI, 0.99–1.13; HR for women, 1.07; 95% CI, 0.98–1.18; HR for never-smokers, 1.09; 95% CI, 0.98–1.21. [A major limitation of this study was that it assessed soft drink intake in only four categories: none, regular only, diet only, and both regular and diet soft drinks, with no quantification or assessment of aspartame.]

Five meta-analyses were identified (Zhang et al., 2021; Yin et al., 2022; Pan et al., 2022, 2023; and Yan et al., 2022), none of which found evidence of an association between exposure and overall cancer mortality or incidence. [The Working Group determined that three of these studies did not contribute useful information, since too few studies were included. For example, only two studies were included in Zhang et al., with no overall risk estimate provided, and Pan et al. (cancer mortality in Pan et al., 2022; and cancer incidence in Pan et al., 2023) included four and two cohorts, respectively.] Yan et al. (2022) and Yin et al. (2022) identified more studies, including the study by Debras et al. (2022b) that specifically evaluated aspartame. [A limitation of the meta-analyses by Yin et al. (2022) and Yan et al. (2022) when estimating risk estimates for "overall cancer" pertained to their combining of individual reported cancer sites to produce "all cancer" estimates.]

2.8 Evidence synthesis for cancer in humans

This section provides a synthesis of studies of consumption of aspartame and ASBs and/or artificial sweeteners containing aspartame in relation to the risk of human cancer at various organ sites. A detailed definition of aspartame, as the agent of investigation in the present monograph, has been provided in Section 1.1. It is important to note that the body of evidence regarding the carcinogenicity of aspartame in humans was for the most part derived from several studies that assessed consumption of ASBs and only a few studies that more specifically focused on aspartame. In several cases, no clear distinction could be made between consumption of drinks and foods containing aspartame versus other artificial sweeteners, in which case the evidence was considered relevant only if the timing of the exposure assessment could be aligned with the time when aspartame was the prevailing artificial sweetener used in the respective country or region. Only the NutriNet-Santé prospective cohort provided information on exposure to aspartame across the entire spectrum of sources (beverages, dairy, tabletop sweeteners, and other sources, together with brand-specific information) with repeated 24-hour diet record assessments during the follow-up period. Two prospective cohort studies from the USA (NHS and HPFS) assessed aspartame intake from the two main sources (diet soft drinks and tabletop sweeteners, representing more than 90% of all aspartame-containing products on the market in the USA between the mid-1980s and mid-2000s) repeatedly (every 4 years) over two decades that specifically covered the time period when aspartame was approved for use and when it represented by far the most prevalent artificial sweetener in the USA (Rimm et al., 1992; Feskanich et al., 1993). The EPIC study measured diet with diet history questionnaires, including assessment of several hundreds of simple foods,

mixed foods, and recipes and drinks that were adapted to the local diet. Diet questionnaires were complemented by 24-hour diet records collected in about 10% of the cohort (Riboli et al., 2002). In most of the participating centres, information on consumption of ASBs was collected (Navarrete-Muñoz et al., 2016; Stepien et al., 2016; Mullee et al., 2019; Heath et al., 2021; Zamora-Ros et al., 2022).

2.8.1 Studies evaluated

The association between aspartame or ASB consumption and different cancers was investigated in 12 prospective cohort studies and 13 retrospective case–control studies. Cancers that were studied more extensively were those of the digestive tract, including the colon and rectum (seven cohort and three case–control studies), liver (three studies including four cohorts), and pancreas (five cohort and four case–control studies); breast (six cohort and three case–control studies); urinary tract (four cohort and three case–control studies); prostate (five cohort and two case–control studies); and lymphatic and haematopoietic tissues (four cohort studies including five cohorts and one case–control study). Fewer studies were available for other cancer types such as brain (two cohort and two case–control studies), uterus (three cohort studies and one case–control study), thyroid (one cohort and one case–control study), larynx (one case–control study), ovary (two cohort studies, one case–control study), stomach (two cohort studies, three case–control studies), oesophagus (one cohort study, one case–control study), or lung (three cohort studies including four cohorts). With few exceptions (Australia, Argentina), these studies were conducted in the USA and Europe. In all these countries, the use of artificially sweetened products, containing aspartame as the main or one of the main artificial sweeteners, has been common over the past few decades; several detailed nutritional cohort

studies have been conducted, allowing for assessment of the association between aspartame use (primarily as its main dietary source, ASBs) and cancer. Although the studies were conducted in a limited geographical area, the results can probably be generalized to other populations. The studies were conducted in various regions of the USA and countries within Europe, with substantial diversity in dietary and lifestyle habits, as well as different cancer prevalence rates and average amounts of aspartame consumption.

In assessing the carcinogenicity of aspartame use, substantial weight was given to the results from the NutriNet-Santé study (Hercberg et al., 2010; Debras et al., 2022b), a prospective web-based cohort study of 102 865 individuals who had a median follow-up of 7.8 years (2009–2021). The NutriNet-Santé study collected detailed information on consumption of ASBs and other dietary sources of artificial sweeteners, by type, via multiple 24-hour dietary records collected every 6 months. Baseline diet was the average of all records over the first 2 years of follow-up, and all participants had at least two individual diet records over this period (92.3% had three or more records, Chazelas et al., 2019). In sensitivity analyses, a model with a time-dependent exposure variable used yearly average aspartame intake, thereby taking advantage of all available measurements conducted during the follow-up. The NutriNet-Santé study was the only one that applied a robust methodology for estimating individual food additives and was able to specifically single out exposure to aspartame, versus exposure to other sweeteners, by building a brand- and year-specific food composition table including specific data on the artificial sweetener content of drinks and foods. Although the NutriNet-Santé study results offered high-quality data, the sample sizes for incident cancer cases during the follow-up period tended to be large enough to examine individually only a few cancer sites (e.g. prostate and breast). Another characteristic of the NutriNet-Santé study was

that, compared with other studies, a small proportion (28%) of cohort members were aspartame users, so the study could not look at finer stratification of the amount consumed. In addition, the potential for self-selection of women who were older, more health-conscious, and better educated may limit the generalizability of the results of the study, although the adjustment for many confounders minimized concerns about biases in risk estimates.

Several other cohort studies provided results on aspartame or ASB exposure and the risk of cancer. In particular, the NHS and HPFS (Schernhammer et al., 2005, 2012) used cumulatively updated diet assessments from the 1980s for up to two decades (based on repeated FFQs), potentially capturing very long-term exposure to aspartame from sodas and tabletop sweeteners in these populations. The studies controlled for BMI at baseline and updated diabetes diagnoses during follow-up. As noted above, other informative prospective cohort studies for the evaluation included the EPIC cohort and the pooled PLCO-NIH-AARP cohort.

In addition to the eleven cohort studies from the USA and Europe, one cohort study in Australia also provided results on several different cancer end-points: The MCCS used an FFQ to quantify the frequency of consumption of diet soft drinks at baseline in 1990–1994, which was after the approval of aspartame use in foods and drinks in Australia in 1986 (Hodge et al., 2018).

Of the total 13 case–control studies, only 11 were considered informative, although the degree to which each study was informative varied substantially. These studies were exclusively conducted in the USA and in Europe, except for a case–control study from Argentina (Andreatta et al., 2008). As generally is the case, the case–control studies were at greater risk of selection and information bias, and they tended to have relatively small numbers of cases. Some case–control studies were adjusted for multiple confounders, presented exposure–response anal-

yses, provided results stratified by BMI, and incorporated exposure only up to a certain period before diagnosis, avoiding reverse causation; however, other case–control studies were less informative because of a lack of information in one or more of the areas discussed above.

Case series, cross-sectional studies, and ecological studies were reviewed and considered by the Working Group but ultimately not included in this review because they were uninformative for the assessment of the association between aspartame consumption and cancer. In some cohort study publications, the outcome was cancer mortality, rather than incidence (NHS, HPFS: Malik et al., 2019; EPIC: Mullee et al., 2019, Heath et al., 2021; CPS-II: McCullough et al., 2022); however, other reports from the same cohorts presented data on cancer incidence for certain cancer types (NHS, HPFS: Schernhammer et al., 2005, 2012; EPIC: Stepien et al., 2016, Navarrete-Muñoz et al., 2016, Heath et al., 2021; with the latter reporting on both incidence and mortality; CPS-II: McCullough et al., 2014). The results of the reports on cancer incidence were qualitatively and quantitatively similar to those of the reports focused on cancer mortality. [The Working Group noted that, for cancer sites with medium to high lethality rates, mortality may be an acceptable proxy for incidence.]

2.8.2 Exposure assessment and misclassification of exposure

The quality of the exposure assessment was a limitation of all except one of the studies evaluated. Most of the existing studies of cancer in humans adopted different types of dietary assessment instruments, including FFQs, food consumption history questionnaires, and repeated 24-hour dietary recalls or records to estimate consumption of ASBs and use of tabletop sweetener packets. The EPIC study used more elaborate questionnaires on habitual diet across hundreds of country- and region-specific foods, separate assessment of usual portions and frequency of consumption, and consumption of foods and drinks during different seasons of the year. Other studies considered artificial sweeteners overall or ASB, and only five cohort studies (including six cohorts) (Lim et al., 2006; Schernhammer et al., 2012; McCullough et al., 2014; Debras et al., 2022b; Fulgoni and Drewnowski, 2022) specifically considered aspartame as the exposure. Four of these studies (Lim et al., 2006; Schernhammer et al., 2012; McCullough et al., 2014; Fulgoni and Drewnowski, 2022) were based in the USA and derived aspartame exposure from the consumption of ASBs and the use of aspartame tabletop sweetener packets, assessed through FFQs. The more recent cohort study, the French NutriNet-Santé study (Debras et al., 2022b), was the only one that derived aspartame exposure from all aspartame-containing foods and beverages assessed through repeated 24-hour dietary records, considering variations between products or brands and over time and with repeated exposure assessments. [The Working Group noted that the USA-based studies, even though they only considered the two main sources of aspartame (ASBs and tabletop packets), were likely to have captured more than 90% of all aspartame intake because of the time period during which they were conducted and the high prevalence of aspartame as the main or sole artificial sweetener used in the USA at that time (Rimm et al., 1992; Feskanich et al., 1993).]

A main limitation of many of the other cohort studies was that exposure was assessed only at a single time point, at baseline. Misclassification of exposure is inevitable when there is a single assessment and follow-up over longer time periods; however, because information on aspartame exposure was collected before the occurrence of disease in the prospective cohort studies, the resulting non-differential (with respect to cancer outcome) misclassification would tend to

bias results towards the null. Exposure assessments in the case–control studies were similarly incomplete (i.e. based on the assessment of consumption of ASBs or tabletop sweetener packets containing aspartame only) and additionally subject to recall bias, which is inherent in the design of retrospective case–control studies. In fact, recall bias (a potential limitation of all the included case–control studies) may bias estimates in either direction if cases were more likely than controls to recall artificial sweetener consumption (or vice versa). Exposure misclassification and the potential for selective exposure in relation to past medical history (e.g. selective consumption of ASBs among those newly diagnosed with diabetes) may occur to a various degree in both the cases and the controls; on the other hand, both may contribute to the potential for a bias towards the null.

Variation in aspartame consumption is driven by the geographical context or period and resulting differences in the type of artificial sweetener and amount of aspartame used in ASB, as well as an increase over time in the range of products (beyond ASBs and tabletop sweetener packets) containing aspartame, including the emergence of "diet" versions of yogurt, ice cream, or breakfast cereals. Mixed exposure to a variety of artificial sweeteners is also likely to have introduced the potential for exposure misclassification both in the exposed and reference groups, which would probably be non-differential with respect to cancer outcome, and to cause bias towards the null.

The NutriNet-Santé study conducted repeated assessments collecting detailed data on aspartame consumption, which included duration, frequency, and amount of use. Given the detailed and frequent repeat assessments of diet and the use of food composition databases to derive aspartame content for each single dietary item consumed, the data provided by NutriNet-Santé (Debras et al., 2022b) were the most detailed and therefore the quality of the exposure assessment was high. The Working Group considered differences between the results for this very detailed cohort study and those of the other available cohort studies and concluded that the accuracy of the NutriNet-Santé study exposure data may have provided a relatively more specific and precise exposure assessment, but that the three USA-based cohort studies (describing four cohorts) (Lim et al., 2006; Schernhammer et al., 2012; McCullough et al., 2014) were also of particular interest because they probably captured most of the aspartame consumed in these populations.

2.8.3 Confounding and selection bias

In its assessment of causality for consumption of aspartame or artificial sweeteners and risk of various cancers, the Working Group used directed acyclic graphs (DAGs) to determine the major confounding factors that should be adjusted for when estimating the effect of aspartame on cancer risk. The Working Group concluded that age, sex, BMI, socioeconomic status, diabetes, and sugar and/or sugar-sweetened beverages represented the minimal sufficient adjustment sets for estimating the effect of aspartame on certain cancers. Additionally, for specific cancer types (e.g. breast, liver), relevant cancer-specific confounders were also considered. The DAG drawn for liver cancer assessment is reported in Fig. S2.1 (see Annex 3, Supplementary material for Section 2, Cancer in Humans, also available from: https://publications.iarc.who.int/627). The consumption of sugar-sweetened beverages and sugar intake more generally was included because it may affect cancer risk either directly (e.g. Hur et al., 2021; Laguna et al., 2021; Yuan et al., 2022) or indirectly through its influence on BMI (Faruque et al., 2019). Aspartame use or the consumption of artificial sweeteners, although not entirely consistently, tended to be higher in individuals who have higher BMI (e.g. Bleich et al., 2014), diabetes (Mackenzie et al., 2006),

lower socioeconomic status, and a generally unhealthier lifestyle. All these attributes are also associated with increased risk of several cancers (e.g. Giovannucci et al., 2010). Most studies either adjusted for or matched on sex and age and adjusted for BMI. Likewise, many studies adjusted for lifestyle variables or stratified their results by BMI and diabetes. The results from the NutriNet-Santé study (Debras et al., 2022b), which were based on the most specific aspartame exposure assessment, were also thoroughly adjusted for the major confounders, including age, sex, lifestyle, and BMI. In sensitivity analyses, cases in participants with a history of diabetes at baseline were excluded.

Case–control studies varied in their adjustment for confounders; most adjusted for age, sex, and BMI; however, selection bias remained a concern in these studies. With the exception of one case–control study in Italy (Gallus et al., 2007; Bosetti et al., 2009), some of the population-based (e.g. Mayne et al., 2006, as reported in Gammon et al., 1997; Chan et al., 2009; Palomar-Cros et al., 2023, as reported in Castaño-Vinyals et al., 2015) and hospital-based (e.g. Davis et al., 2023) case–control studies may have been subject to selection bias because of low participation rates, if those who chose to participate varied from the source population in terms of sweetener use.

2.8.4 Reverse causation

It should be considered whether the association between aspartame consumption and cancer may be subject to reverse causation. In patients with a cancer diagnosis, early symptoms could potentially have led to changes in diet soda consumption habits, although the direction of any such changes remains speculative. Major reverse causation bias entirely explaining the observed associations is unlikely in prospective cohorts, since the participants did not have cancer at baseline. Some of the studies (e.g. Bao et al., 2008; Navarrete-Muñoz et al., 2016; Malik et al., 2019; Debras et al., 2022b) also applied lag-time analyses, excluding cases diagnosed during various periods of follow-up, and the results tended to remain unchanged.

2.8.5 Cancer of the liver

Three publications reporting on four cohorts (Stepien et al., 2016; Jones et al., 2022; McCullough et al., 2022) examined the association between ASB consumption and liver cancer risk. No other studies investigated associations with liver cancer. The Working Group carefully considered potential confounders at baseline of an association between aspartame and liver cancer (Fig. S2.1, Annex 3, Supplementary material for Section 2, Cancer in Humans, also available from: https://publications.iarc.who.int/627). The study in the EPIC cohort (Stepien et al., 2016) found that each increment of one 330 mL serving of artificially sweetened soft drinks per week was associated with an increased risk of hepatocellular cancer (HR, 1.06; 95% CI, 1.03–1.09; 151 cases). [The Working Group noted that this study was well adjusted for confounders for liver cancer, including BMI (height and weight measured at baseline by trained investigators), baseline diabetes, alcohol consumption, and smoking, and the investigators evaluated potential confounding by coffee and sugar-sweetened beverage intake. Although HBV or HCV infection is a strong risk factor for liver cancer, it is unlikely that there is an association between aspartame consumption and HBV or HCV infection, making it unlikely that HBV and HCV are confounders of the association between aspartame consumption and liver cancer. An association between aspartame and HBV or HCV via diabetes (Cacoub and Saadoun, 2021) is plausible but was addressed by adjustment for diabetes at baseline. Moreover, the prevalence of HBV or HCV infection in the EPIC cohort was very low (about 3%) (Trichopoulos et al., 2011).]

A pooled analysis of the NIH-AARP and PLCO cohorts (Jones et al., 2022) found a positive association with risk of liver cancer during the first 12-year follow-up interval among participants with diabetes at baseline (but not among those without diabetes). This interval corresponds to a time period when aspartame was the main artificial sweetener used in ASBs in the USA (see Section 1.6.1(c)). The hazard ratios for units of consumption per day were 1.13 (95% CI, 1.02–1.25) for ASBs overall; 1.13 (95% CI, 1.01–1.27) for artificially sweetened soda; and 1.17 (95% CI, 0.92–1.48) for artificially sweetened fruit punch. All findings were null for the second follow-up period beyond 12 years since baseline. These findings indicated that diabetes could be an effect modifier of the association, possibly due to greater ASB exposure; however, it was unclear whether biological mechanisms specific to individuals with diabetes could play a role in explaining these findings.

The study in the CPS-II cohort (McCullough et al., 2022) reported no association with liver cancer risk overall. There was a weak indication that, in analyses restricted to male never-smokers, the association between ≥ 2 drinks/day of artificially sweetened drinks (compared with 0 drinks/day) and liver cancer was positive (HR, 1.44; 95% CI, 0.99–2.08; P for trend, 0.040), although the hazard ratio was 1.21 after adjustment for BMI (P for trend, 0.335). Therefore, this study did not refute the findings of a positive association reported in the other studies. [The Working Group noted that a unique feature of this cohort was that its large size allowed restriction to never-smokers and stratification by sex. Additionally, participants with a history of diabetes at baseline were excluded in all analyses.]

A recent study by Debras et al. (2023) in the NutriNet-Santé cohort showed a positive association between aspartame consumption and diabetes incidence; therefore, it is possible that diabetes may be on a causal pathway between aspartame consumption and liver cancer risk.

All these cohort studies controlled for baseline diabetes, by adjustment (Stepien et al., 2016), stratification (Jones et al., 2022), or exclusion (McCullough et al., 2022). These cohort studies did not control for incident diabetes during cohort follow-up, thereby allowing insulin resistance or diabetes to contribute to observed positive associations between aspartame and liver cancer. Other mechanisms may also play a role, since associations with ASB consumption were observed among diabetic patients.

The Working Group concluded that the three studies, which included four cohorts, examining the association between ASB consumption and cancer of the liver were informative. Other studies, including the highly informative NutriNet-Santé study, did not investigate liver cancer separately. Although some level of non-differential misclassification may exist in these studies, which would probably bias associations towards the null, the positive and statistically precise associations between aspartame use and liver cancer identified by these studies were considered to be important evidence. Although bias could not be ruled out with reasonable confidence, the findings demonstrated consistency, and the studies that were reviewed provided credible evidence for a positive association with liver cancer.

2.8.6 Cancer of the breast

The Working Group evaluated six cohort studies (NutriNet-Santé, NHS, NHS-II, CPS-II, EPIC, MCCS) and three case–control studies on consumption of aspartame, or ASBs or tabletop sweetener packets containing aspartame, and the risk of breast cancer.

The NutriNet-Santé study (Debras et al., 2022b) found a significantly increased risk of breast cancer overall (HR, 1.22; 95% CI, 1.01–1.48; P for trend, 0.036) for women with higher aspartame consumption, i.e. above the median for female aspartame users, compared with

non-users. The two NHS cohorts, NHS and NHS-II (Romanos-Nanclares et al., 2021), also had repeated diet exposure updates throughout follow-up. The studies found no increased risk of breast cancer associated with ASB consumption. Similarly, consumption of ASBs was not associated with increased breast cancer mortality (Malik et al., 2019) in the NHS cohort. The CPS-II cohort study (McCullough et al., 2022) adjusted in detail for confounding, including for BMI, and reported no association between consumption of ASBs and postmenopausal breast cancer risk and mortality. The EPIC cohort study (Mullee et al., 2019) reported no positive association between artificially sweetened soft drinks and breast cancer mortality, although a lower breast cancer mortality was observed among women consuming 1–4 glasses/month (HR, 0.79; 95% CI, 0.63–0.98) versus < 1 glass/month. Also, the study in the MCCS cohort (Hodge et al., 2018) found no association between consumption of artificially sweetened soft drinks and breast cancer risk.

Of the three case–control studies, one (Ewertz and Gill, 1990) was considered uninformative because the assessment of artificial sweetener consumption preceded approval for aspartame in the study country (Denmark). Another case–control study (Gallus et al., 2007) was conducted in Italy and reported an inverse association between breast cancer and consumption of artificial sweeteners other than saccharin. The large multicase–control study in Spain (MCC-Spain) (Palomar-Cros et al., 2023), investigated associations between use of artificial sweeteners and low- or no-calorie sweetened beverages and breast cancer risk (1510 cases). Overall, no increased risk of breast cancer was observed among women with high aspartame consumption (defined as third quartile or above). Among participants with diabetes, there was an inverse association when comparing high consumers with non-consumers (OR, 0.28; 95% CI, 0.08–0.83; *P* for trend, 0.03).

In sum, with the exception of one study in which aspartame was measured specifically and that suggested an elevated risk of breast cancer, the available studies on ASB consumption and breast cancer did not show positive associations. Although the high-quality NutriNet-Santé study (Debras et al., 2022b) found that intake of aspartame was associated with higher risk of breast cancer, the Working Group considered that, because of the potential for misclassification and inconsistent findings, the available studies were of insufficient informativeness about aspartame exposure and insufficient consistency to permit a conclusion to be drawn on the presence or absence of a causal association between aspartame consumption and breast cancer risk.

2.8.7 Cancers of lymphatic and haematopoietic tissues

The association between proxies of aspartame exposure and lymphatic and haematopoietic cancer risk was assessed by four cohort studies (NIH-AARP, NHS, HPFS, CPS-II). One case–control study (MCC-Spain) also contributed to this evaluation.

A study in the NHS and HPFS cohorts (Schernhammer et al., 2012) evaluated associations between aspartame-containing soda and incidence of haematopoietic cancers. With 22 years of follow-up and repeated exposure assessments covering the relevant period of aspartame use in ASBs in the USA, this study was particularly informative, comprising 1324 cases of NHL, 285 cases of multiple myeloma, and 339 cases of leukaemia. Compared with men who did not consume diet soda, men who consumed ≥ 1 daily servings of diet soda had an increased risk of NHL (RR, 1.31; 95% CI, 1.01–1.72; *P* for trend, 0.11) and multiple myeloma (RR, 2.02; 95% CI, 1.20–3.40; *P* for trend, 0.01, respectively). No association was observed for women (RR for NHL, 1.00; 95% CI, 0.78–1.26; *P* for trend, 0.999; and RR for multiple myeloma, 0.79; 95% CI, 0.45–1.36; *P*

for trend, 0.79). When data from the two cohorts were combined in a pooled analysis to maximize statistical precision, diet soda intake was associated with an increased risk of leukaemia (RR, 1.42; 95% CI, 1.00–2.02; *P* for trend, 0.05). Starting follow-up in 1994, when aspartame had become more widely used after the expiry of the patent in 1992, observed associations were confirmed among men, and an interaction with alcohol consumption was reported. Specifically, with follow-up from 1994, in the analysis among men, an association was observed between aspartame exposure (estimated from diet soda and aspartame packets consumed) and increased risk of NHL (RR for ≥ 143 mg/day versus none, 1.64; 95% CI, 1.17–2.29; *P* for trend, 0.002) and multiple myeloma (RR for ≥ 143 mg/day versus none, 3.36; 95% CI, 1.38–8.19; *P* for trend, 0.05); these associations were not observed in women. For leukaemia, the RR for ≥ 143 mg/day versus none was 1.56 (95% CI, 0.79–3.06; *P* for trend, 0.17) in men.

Two analyses from CPS-II reported on the association between ASB intake and lymphoid neoplasms. McCullough et al. (2022) examined ASB consumption and mortality from lymphoid neoplasms in the full CPS-II mortality cohort (at baseline in 1982, there were almost 935 000 participants), and McCullough et al. (2014) examined ASB consumption and estimated aspartame consumption in relation to NHL risk among 100 000 men and women in the CPS-II nutrition cohort (baseline, 1999), a subset of CPS-II participants followed for cancer incidence. Over 10 years of follow-up, no association was detected between consumption of artificially sweetened carbonated beverages or aspartame and NHL incident risk overall or by subtypes (multiple myeloma, diffuse large B-cell lymphoma, CLL/ small lymphocytic lymphoma, and follicular and other B-cell lymphomas). Positive associations for estimated aspartame exposure from ASBs plus aspartame packets were observed for total NHL, diffuse large B-cell lymphoma, follicular,

and other B-cell lymphomas in quintiles 2 and/or 3, but the associations were not linear across the range of intakes. For mortality in the parent CPS-II cohort (McCullough et al., 2022), no association was detected for NHL, multiple myeloma, or leukaemia in men, women, or sex-combined analyses. An interaction with BMI was detected for NHL: an inverse association for NHL was seen among people with a BMI of 18.5 to < 25 kg/m^2 (*P* for trend, 0.035; *P* for interaction, 0.029), whereas there was no association in the groups of people with a BMI of 25–30 kg/m^2 (*P* = 0.274) or BMI of > 30 kg/m^2 (*P* = 0.896).

The NIH-AARP study (Lim et al., 2006) reported no association between aspartame intake and all haematopoietic cancers (overall or in either men or women), Hodgkin lymphoma, multiple myeloma, NHL (or in two of its subtypes, small lymphocytic lymphoma and CLL; immunoblastic lymphoma and lymphoblastic lymphoma/leukaemia) and non-lymphoid leukaemia. The study was limited by a single baseline exposure assessment, relatively short follow-up (maximum, 5 years), and high age at study entry (mean, 62 years at baseline).

The MCC-Spain study (Palomar-Cros et al., 2023) found no association between high consumption of aspartame-containing products (low- or no-calorie soft drinks and tabletop sweeteners other than saccharin) and CLL risk overall.

The Working Group concluded that the body of evidence from four cohort studies, which did not include the NutriNet-Santé cohort, was small and did not consistently indicate a positive association between aspartame intake and cancers of lymphatic and haematopoietic tissues. Given the small number of studies and the diversity of their sizes, end-point definitions, and exposure definitions (each conveying some uncertainty), and despite the fact that some studies suggested an increased risk, no conclusion could be drawn on the presence or absence of a causal association

between aspartame consumption and risk of cancers of lymphatic and haematopoietic tissues.

2.8.8 Cancer of the pancreas

Four cohort studies, which included five cohorts, (Schernhammer et al., 2005; Bao et al., 2008; Navarrete-Muñoz et al., 2016; McCullough et al., 2022) and four case–control studies (Norell et al., 1986; Bosetti et al., 2009; Chan, et. al., 2009; Davis et al., 2023) examined the association between artificial sweetener exposure and pancreatic cancer risk. The results from the cohort studies were mixed: some findings were null (Schernhammer et al., 2005; Bao et al., 2008) but others were positive, including in one study overall (McCullough et al., 2022) and in another study among men but not women (Navarrete-Muñoz et al., 2016). In the CPS-II cohort, McCullough et al. (2022) reported an 11% increase in risk of pancreatic cancer in people consuming artificially sweetened drinks at a frequency of ≥ 2 drinks/day versus never (HR per ≥ 2 drinks/day, 1.11; 95% CI, 1.02–1.20) after adjusting for BMI in the multivariable-adjusted model; the risk among never-smokers was also elevated (HR per ≥ 2 drinks/day, 1.19; 95% CI, 1.05–1.34). Using data from seven participating countries in the EPIC cohort, the association between artificially sweetened soft drink consumption and adenocarcinoma of the exocrine pancreas was studied (Navarrete-Muñoz et al., 2016). Although no association between artificially sweetened soft drink consumption of > 92.2 g/day versus 0.1–2.0 g/day and pancreatic cancer risk was reported in models for men and women combined, a 25% increase in risk was observed in continuous models (per 336 g/day) among men but not women (P for interaction by sex, 0.004). In the NHS and HPFS cohort studies (Schernhammer et al., 2005), the investigators observed no association between frequency of diet soft drink consumption and pancreatic cancer risk. The study used repeated assessment of ASB,

finely controlled for potential confounders, and stratified by BMI. The fact that overall consumption of ASBs was low in these cohorts was one of the limitations of this otherwise informative study. The NIH-AARP study (Bao et al., 2008) also found no association between diet soft drink consumption and pancreatic cancer risk. In sensitivity analyses, the first 2 years of follow-up were excluded to rule out an effect of subclinical pancreatic cancer on added sugar intake, again finding no association (P for trend, 0.19).

Among the four case–control studies, no association was observed between artificial sweetener consumption and pancreatic cancer in a small case–control study in Sweden (Norell et al., 1986) or between diet cola consumption and pancreatic cancer risk in a case–control study in the USA (Davis et al., 2023). Another US population-based case–control study (Chan et al., 2009) examined sugar-free carbonated beverage consumption and adenocarcinoma of the exocrine pancreas, finding a higher risk of pancreatic cancer with some indication of potential effect modification by diabetes: risk was elevated among participants without diabetes (OR for ≥ 1 serving/day versus 0, 1.6; 95% CI, 1.1–2.3), but not among those with diabetes (OR, 0.9; 95% CI, 0.3–2.6). No associations were found between consumption of low-calorie sweeteners other than saccharin and pancreatic cancer in the case–control study in Italy (Bosetti et al., 2009).

In sum, the available studies on the association between artificial sweetener exposure and risk of pancreatic cancer lacked sufficient consistency, quality, and precision to permit a conclusion to be drawn on the absence or presence of a causal association.

2.8.9 Cancer of the stomach

Two cohort studies (Hodge et al., 2018; McCullough et al., 2022) and three case–control study (Mayne et al., 2006; Bosetti et al., 2009; Palomar-Cros et al., 2023) examined the

association between artificial sweetener exposure and stomach cancer risk. The Australian MCCS (Hodge et al., 2018) and the study in the CPS-II cohort (McCullough et al., 2022) found no association between ASB consumption and cancers of the gastric cardia or stomach, respectively. Among the three case–control studies, the results were inconsistent. In the multicase–control study in Spain (MCC-Spain) (Palomar-Cros et al., 2023), the overall results were null for stomach cancer, but a positive association was observed for aspartame-containing products (no- or low-calorie soft drinks and tabletop sweeteners other than saccharin) for high versus no consumption among participants with diabetes (OR, 2.04; 95% CI, 0.70–5.40; *P* for trend, 0.05). The small body of available studies lacked sufficient informativeness, precision, and consistency to permit a conclusion to be drawn on the presence or absence of a causal association between artificial sweetener exposure and stomach cancer risk.

2.8.10 Cancer of the oesophagus

One cohort study (McCullough et al., 2022) and two case–control studies (Mayne et al., 2006; Gallus et al., 2007) examined the association between artificial sweetener exposure and oesophageal cancer risk. The study in the CPS-II cohort (McCullough et al., 2022) reported no association between ASB consumption and oesophageal cancer. No consistent associations were observed between diet carbonated beverage consumption and oesophageal adenocarcinoma or oesophageal squamous cell carcinoma in the case–control study in the USA (Mayne et al., 2006) or between diet carbonated beverage consumption and oesophageal cancer in the case–control study in Italy (Gallus et al., 2007). In sum, the small body of available studies were of insufficient quality, lacked consistency, and did not provide support for a positive association between aspartame consumption and oesophageal cancer.

2.8.11 Cancers of the colon and rectum

Studies in seven cohorts (NutriNet-Santé, CPS-II, EPIC, NHS, NHS-II, HPFS, and MCCS) and two case–control studies were considered informative for the assessment of the association between aspartame intake, either directly or through consumption of ASB, and colon and/or rectal cancer incidence or mortality.

The NutriNet-Santé study (Chazelas et al., 2019) found no association between consumption of ASBs and the risk of colorectal cancer (sub-distribution hazard ratio for top quartile versus bottom, sHR, 0.80; 95% CI, 0.44 to 1.46). These results were adjusted in great detail for major confounders. None of the other five cohort studies reported a positive association between consumption of artificially sweetened soft drinks and colorectal cancer risk (Hodge et al., 2018; Malik et al., 2019; Mullee et al., 2019; Hur et al., 2021; McCullough et al., 2022). Four of the five cohort studies examined mortality (Malik et al., 2019; Mullee et al., 2019; Hur et al., 2021; McCullough et al., 2022). Two of these cohort studies were considered less informative because of the collection of intake data at a single time point, together with variation in products across countries over time (Mullee et al., 2019), or with collection of data 1 year before the approval of aspartame use in ASBs (McCullough et al., 2022), which would lead to non-differential exposure misclassification.

There were also two informative case–control studies from Italy (Gallus et al., 2007) and Spain (Palomar-Cros et al., 2023). The Italian case–control study found no association between artificial sweetener consumption and cancer of the colon or rectum. The MCC-Spain study considered aspartame-containing products (low- or no-calorie soft drinks and tabletop sweeteners other than saccharin) and found no association between high aspartame consumption and colorectal cancer risk either overall or among participants with diabetes.

The Working Group concluded that the available studies that were considered informative were consistent with there being no positive association between aspartame consumption and cancer of the colon or rectum. Although bias could not be ruled out (considering the generally weak exposure assessments), given the consistency of null findings, the studies that were reviewed did not provide support for a positive association with cancers of the colon or rectum.

2.8.12 Cancer of the prostate

The Working Group evaluated five cohort studies (HPFS, CPS-II, NutriNet-Santé, MCCS, EPIC) and two case–control studies on aspartame consumption, or consumption of ASBs or tabletop sweetener packets containing aspartame, and the risk of prostate cancer.

The NutriNet-Santé study (Debras et al., 2022b), although of limited power, found no association between higher aspartame consumption (i.e. above the median among male aspartame users) and prostate cancer (HR versus non-users, multivariable-adjusted, 1.28; 95% CI, 0.91–1.79; *P* for trend, 0.28). The prospective study in the HPFS cohort in the USA (Malik et al., 2019) found no association between consumption of ASBs and prostate cancer mortality (HR for ≥ 2 servings/day versus < 1 serving/month, 1.01; 95% CI, 0.67–1.52). The study in the large CPS-II cohort (McCullough et al., 2022) found no increased risk of prostate cancer mortality with ASB consumption overall, and a suggestive interaction with BMI (*P* for interaction, 0.013); there was a positive association only among participants categorized as obese. The study in the EPIC cohort (Mullee et al., 2019) reported null findings for prostate cancer mortality (HR for artificially sweetened soft drinks consumption of ≥ 1 glass/day versus < 1 glass/month, 1.05; 95% CI, 0.64–1.75; *P* for trend, 0.53), although higher mortality from prostate cancer was observed among those consuming > 1–6 glasses per week

(HR, 1.36; 95% CI, 1.05–1.78). The MCCS cohort found no association between artificially sweetened soft drinks and risk of aggressive prostate cancer (HR for ≥ 1 time per day versus < 1 time per month, 0.81; 95% CI, 0.49–1.33) (Hodge et al., 2018).

The case–control study in Italy (Gallus et al., 2007), which included 1294 prostate cancer cases and 1451 controls, reported that the overall association with consumption of sachets of artificial sweeteners other than saccharin was null. The MCC-Spain study (Palomar-Cros et al., 2023) investigated associations between consumption of artificial sweeteners and risk of prostate cancer (972 cases). Overall, no increased risk of prostate cancer was observed among those with high aspartame consumption (defined as third quartile or above) versus non-consumers, although the odds ratio among participants with diabetes was 1.91 (95% CI, 0.87–4.20; *P* for trend, 0.3).

In sum, of all the available studies, none reported an overall positive association between aspartame consumption and prostate cancer incidence or mortality, and two studies reported a positive association with mortality or incidence of prostate cancer in subgroups (among obese men and among those with diabetes). The available studies were of insufficient quality and consistency to permit a conclusion to be drawn on the presence or absence of a causal association.

2.8.13 Cancers of the urinary tract

Of the informative studies investigating the role of consumption of artificial sweeteners, particularly aspartame, and cancers of the urinary tract (kidney, bladder, and lower urinary tract combined), four were cohort studies (CPS-II, WHI-OS, MCCS, EPIC) (Hodge et al., 2018; Heath et al., 2021; McCullough et al., 2022; Ringel et al., 2023) and three were case–control studies (Nomura et al., 1991; Gallus et al., 2007; Andreatta et al., 2008).

The study in the CPS-II cohort (McCullough et al., 2022) investigated the association between ASB consumption and kidney or bladder cancer mortality, finding no association. The USA-based WHI-OS (Ringel et al., 2023) found no increased risk of urinary bladder cancer or kidney cancer among participants consuming ≥ 1 serving/day compared with those never consuming or consuming < 1 serving/week. In the MCCS cohort, there was no significant association observed between artificially sweetened soft drink consumption and kidney cancer (Hodge et al., 2018). In the EPIC cohort, Heath et al. (2021) found that the risk of either being diagnosed with or dying from renal cell carcinoma was not associated with artificially sweetened soft drink intake, assessed at baseline.

The case–control study in Italy by Gallus et al. (2007) found no association between exposure to artificial sweeteners other than saccharin and renal cell carcinoma. Two additional case–control studies examined the association between several proxies of aspartame use and (lower) urinary tract tumours. In the first, a case–control study in Argentina (Andreatta et al., 2008), long-term (≥ 10 years) consumption of artificial sweeteners, exclusively when used as an additive in infusions (tea, coffee, mate), without consideration of consumption from other sources such as soft drinks or dietetic foods, was associated with a significantly increased risk of urinary tract tumours, compared with non-use (OR, 2.18; 95% CI, 1.22–3.89). [The Working Group noted that there were very small numbers of cases and controls exposed and probably exposure misclassification.] A case–control study in Hawaii, USA (Nomura et al., 1991) evaluated consumption of artificial sweeteners on the basis of the use of saccharin and ingestion of diet beverages; no association was observed between lower urinary tract cancer and either ever consumption or cumulative consumption of diet beverages.

In sum, given the weak exposure definitions and potential for misclassification, bias could not be ruled out. The available studies were of insufficient quality and statistical precision to permit a conclusion to be drawn on the presence or absence of a causal association between aspartame consumption and risk of cancers of the urinary tract.

2.8.14 Cancer of the brain

In total, two cohort studies reported on proxies of aspartame intake and ASB consumption and brain cancer (NIH-AARP, CPS-II) (Lim et al., 2006; McCullough et al., 2022). In addition, two case–control studies reported on aspartame and artificial sweeteners and cancers of the brain (Gurney et al., 1997; Cabaniols et al., 2011). The CPS-II cohort study (McCullough et al., 2022) found no association between ASB intake and the risk of dying from cancer of the brain. The study in the NIH-AARP cohort (Lim et al., 2006) found that aspartame was not positively associated with glioma overall or with glioblastoma (the largest subtype of brain tumours) when non-users of aspartame was set as the reference category.

One USA-based case–control study (Gurney et al., 1997) investigated the risk of brain tumours in childhood in relation to the consumption of aspartame during infancy and childhood. Results indicated no association between aspartame consumption and brain tumour risk in childhood. A case–control study in France (Cabaniols et al., 2011) reported no association between regular (≥ 1 intake/week) aspartame consumption (no further definition of the aspartame assessment was provided) and brain cancer.

The Working Group concluded that all the studies found no association between aspartame intake and cancers of the brain. Given the insufficient informativeness of these studies and the lack of consistency, the evidence did not permit a conclusion to be drawn on the presence or absence of a causal association between

aspartame consumption and risk of cancers of the brain.

2.8.15 Other cancers

One cohort study also examined the association between artificial sweetener exposure and cancers of the small intestine, gall bladder, larynx, lip, oral cavity, and pharynx. In the CPS-II cohort, McCullough et al. (2022) reported an association between ASB consumption and small intestine cancer mortality that appeared to be elevated (HR per drink/day, 1.11; 95% CI, 1.00–1.22; *P* for trend, 0.244; after adjustment for BMI). Furthermore, among men, the association with oral, pharyngeal, and laryngeal cancer mortality combined per drink/day was 1.07 (95% CI, 1.00–1.14; *P* for trend, 0.034; after adjustment for BMI), but several limitations affected the interpretability of this finding.

Additional cohort studies reported on cancers of the thyroid (Zamora-Ros et al., 2022), lung (Malik et al., 2019; McCullough et al., 2022; You et al., 2022), and ovary or endometrium (Inoue-Choi et al., 2013; Hodge et al., 2018; McCullough et al., 2022). Four case–control studies reported on cancers of the uterus (Bosetti et al., 2009), thyroid (Singh et al., 2020), and larynx and ovary (Gallus et al., 2007). There were no findings of increased risk for ovary, endometrial, laryngeal, or lung cancer, and the evidence was too sparse and of insufficient informativeness to permit conclusions to be drawn on the presence or absence of causal associations between aspartame consumption and risk of cancer at any of these sites.

2.8.16 Obesity-related cancers and other groupings

Overall, three cohort studies reported on aspartame or ASB consumption and the risk of developing or dying from obesity-related cancers. A separate analysis from one of these cohorts examined the association between artificially sweetened soft drinks and non-obesity-related cancers.

The NutriNet-Santé study (Debras et al., 2022b) found that, compared with non-consumers, the hazard ratio (adjusted for BMI) of developing one of the obesity-related cancers considered in their analyses was 1.08 (95% CI, 0.96–1.22) for lower-level aspartame consumers and 1.15 (95% CI, 1.01–1.32) for higher-level consumers (i.e. above the sex-specific median of exposure) (*P* for trend, 0.026). [The Working Group noted that "absence of excess body fatness" has been found to be cancer-preventive by the *IARC Handbooks* programme, with *sufficient* or *limited* evidence for several cancer sites (IARC, 2018c).]

In the CPS-II cohort, McCullough et al. (2022) evaluated the association between ASB consumption (soft drinks and iced tea) and obesity-related cancers. In multivariable-adjusted statistical analyses (additionally adjusted for BMI), the hazard ratio for consumption of ≥ 2 drinks/day versus none was 1.02 (95% CI, 0.96–1.08; *P* for trend, 0.599) for men and 0.98 (95% CI, 0.94–1.02; *P* for trend, 0.213) for women.

The MCCS cohort (Hodge et al., 2018) included cancers of the liver, prostate (aggressive), ovary, gallbladder, kidney, colorectum, oesophagus (adenocarcinoma), breast (postmenopausal), pancreas, endometrium, and gastric cardia as being obesity-related. The study found no association between ASBs consumption and obesity-related cancers. In the MCCS cohort (Bassett at al., 2020), intake of > 1 serving/day of artificially sweetened soft drinks versus none or < 1/ month was also associated with an increased risk of non-obesity-related cancers: the hazard ratio was 1.23 (95% CI, 1.02–1.48; *P* for trend, 0.006), or 1.10 (95% CI, 1.03–1.17) for each extra serving per day.

The three cohort studies reporting on obesity-related cancers each included a slightly different selection of cancer sites. In sum, given

the small body of evidence based on three cohort studies with mixed findings as well as diversity in size, end-point definitions, and exposure definitions (each having substantial uncertainty), no conclusion could be drawn on the presence or absence of a causal association between aspartame consumption and risk of obesity-related cancers as a group.

2.8.17 Cancer of all sites combined

Studies in seven cohorts looked at overall cancer incidence (NutriNet-Santé, PLCO), or mortality (EPIC, NHS, HPFS, NHANES, CPS-II) in relation to different estimates of aspartame consumption and were considered to be informative.

In the NutriNet-Santé study (Debras et al., 2022b), individuals classified as having a lower or higher intake of aspartame (from an average of 5.6 dietary records per participant) had an elevated cancer risk compared with non-consumers (HR for higher-consumers, 1.15; 95% CI, 1.03–1.28; P for trend, 0.002). In the NHS and HPFS cohorts (Malik et al., 2019), no association was observed between ASB consumption and cancer mortality. In the CPS-II cohort, McCullough et al. (2022) reported a hazard ratio for cancer deaths of 0.99 (95% CI, 0.97–1.02) for ASB consumption of ≥ 2 drinks/day relative to none without adjustment for BMI, and 0.97 (95% CI, 0.95–0.99) after adjustment for BMI. In the PLCO cohort, You et al. (2022) found no evidence that consumption of diet soft drinks only, relative to no soft drink consumption, was associated with all cancer risk. Similarly, the EPIC study (Mullee et al., 2019) found no association between artificially sweetened soft drink consumption and cancer death. In NHANES, two independent studies (Zhang et al., 2021; Fulgoni and Drewnowski, 2022) found that consumption of ≥ 2 servings/day of ASBs relative to no consumption (Zhang et al., 2021) or increasing tertile of aspartame intake

(Fulgoni and Drewnowski, 2022) were not associated with overall cancer mortality.

The available evidence, based on seven cohort studies with diverse size, exposure definitions (most with substantial uncertainty) and findings, did not permit a conclusion to be drawn on the presence or absence of a causal association between aspartame consumption and risk of all cancers combined.

2.8.18 Results stratified by sex and other attributes of the study participants

Where data were provided, mostly null associations between aspartame and ASB consumption and cancer risk were seen for both men and women. Direct comparisons between two cohorts – NHS (women only) and HPFS (men only) (Schernhammer et al., 2012) – suggested differences by sex, with higher risks of NHL and multiple myeloma associated with artificial sweetener consumption in men.

A study reporting on the interaction between sex and ASB consumption was conducted in the context of all cancer mortality (McCullough et al., 2022) and did not find evidence of an effect of sex on the association (P for interaction, 0.145). In the EPIC cohort, the association between ASB consumption and adenocarcinoma of the exocrine pancreas was stratified by sex (Navarrete-Muñoz et al., 2016); a 25% increase in risk was observed in continuous models (per 336 g/day) among men, but not women (P for interaction by sex, 0.004). Overall, there was no pattern suggesting consistent effect modification by sex.

Several studies examined effect modification by BMI. For example, in the CPS-II cohort McCullough et al. (2022) found no overall increase in risk of prostate cancer death, and a suggestive interaction with BMI (P for interaction, 0.013) with a positive association only among obese participants. An interaction with BMI was also detected for NHL in the same cohort,

showing an inverse association for NHL among participants with a BMI of 18.5 to < 25 kg/m², and no association among participants with a BMI of 25–30 kg/m² or > 30 kg/m². On the other hand, the NutriNet-Santé study (Debras et al., 2022b) found no indication of BMI interaction, regardless of cancer end-point. Overall, no consistent patterns emerged regarding effect modification by BMI.

A combined analysis of the NIH-AARP and PLCO cohorts (Jones et al., 2022) found positive associations between risk of liver cancer and several exposure types among participants with diabetes (but not among those without diabetes). In the MCC-Spain study (Palomar-Cros et al., 2023), although the results were negative overall, there was a positive association between aspartame-containing products and cancers of the stomach (OR, 2.04; 95% CI, 0.70–5.40; *P* for trend, 0.05) and prostate (OR, 1.91; 95% CI, 0.87–4.20; *P* for trend, 0.3) for high consumption versus no consumption, among participants with diabetes. In contrast, in the same study, there was an inverse association between aspartame consumption and breast cancer (OR, 0.28; 95% CI, 0.08–0.83; *P* for trend, 0.03) for aspartame consumers compared with non-consumers, among participants with diabetes. For CLL, the risk of which was null overall and among participants with diabetes, a positive association (OR, 2.15; 95% CI, 0.93–4.51; *P* for trend, 0.4) was found between high consumption of aspartame and CLL among participants without diabetes. A case–control study in the USA (Chan et al., 2009) examined ASB consumption and adenocarcinoma of the exocrine pancreas, reporting some indication of potential effect modification by diabetes: risk was elevated among those without diabetes but not among those with diabetes.

In sum, although there was some suggestion of effect modification by BMI and diabetes, the direction of this modification remained unclear given the inconsistency in the results of the available studies, which did not allow any conclusions to be drawn.

3. Cancer in Experimental Animals

3.1 Mouse

See Table 3.1.

3.1.1 Oral administration (drinking-water)

In a study conducted in a genetically modified C57BL/6 Ela1-Tag mouse model (which expresses the simian virus SV40 large T antigen under the control of the elastase 1 acinar cell promoter, giving spontaneous formation of pancreatic cancers of acinar origin), 25 male mice (previously treated with aspartame in utero) were treated with drinking-water containing aspartame (purity not reported) at a concentration of 0% (13 mice) or 0.035% (12 mice) from birth until age 21 weeks. Survival rates were unaffected in all treated groups compared with controls. No data on body weight or water consumption were available (Dooley et al., 2017).

There were no effects on the cumulative incidence of pancreatic acinar carcinoma, the age of tumour onset, tumour growth rate, or tumour-induced mortality. [The Working Group noted that this study was limited by the use of a single low dose and a single sex, the lack of information on aspartame purity, and the limited number of organs examined without detailed histopathological information being reported. Therefore, the Working Group judged this study to be inadequate for the evaluation of the carcinogenicity of aspartame in experimental animals.]

3.1.2 Oral administration (feed)

In a study of carcinogenicity conducted in 1974 (EFSA_E75, 2011), 182 male and 178 female HAM-ICR Swiss mice (age, 28 days) were

Table 3.1 Studies of carcinogenicity with aspartame in mice

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, HAM-ICR Swiss (M) 28 days 104 wk EFSA_E75 (2011)	Oral administration (feed) Purity, NR (conversion product, diketopiperazine, SC-19192, 0.8–1.2%) Feed 0, 1, 2, 4 g/kg bw per day Dose adjusted by body-weight change 72, 36, 37, 37 17, 9, 10, 6	No significant increase in tumour incidence in treated animals		*Principal strengths*: long-term study (> 2 yr); multiple-dose study; use of males and females. *Principal limitations*: study from pre-GLP era (1974); uncertainty regarding purity; < 50 animals per treated group; lack of measurement of stability and homogeneity of aspartame in the diet. *Other comments*: original document was the report submitted to Searle Laboratories in 1974.
Full carcinogenicity Mouse, HAM-ICR Swiss (F) 28 days 104 wk EFSA_E75 (2011)	Oral administration (feed) Purity, NR (conversion product, diketopiperazine, SC-19192, 0.8–1.2%) Feed 0, 1, 2, 4 g/kg bw per day Dose adjusted by body-weight change 72, 36, 35, 35 26, 11, 11, 10	No significant increase in tumour incidence in treated animals		*Principal strengths*: long-term study (> 2 yr); multiple-dose study; use of males and females. *Principal limitations*: number of animals in treated group was 35–36; study from pre-GLP era (1974); uncertainty regarding purity. *Other comments*: original document was the report submitted to Searle Laboratories in 1974.

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse (transgenic), FVB/N-TgN(v-Ha-ras)Led (Tg.AC) Hemizygous (M) 6 wk 40 wk NTP (2005)	Oral administration (feed) Purity, > 98% Feed 0, 3125, 6250, 12 500, 25 000, 50 000 ppm (approx. 0, 490, 980, 1960, 3960, and 7660 mg/kg bw per day) 15, 15, 15, 15, 15, 15 9, 12, 8, 12, 11, 10	*Lung* Bronchioloalveolar adenoma 0/14, 0/15, 2/14 (14%), 1/15 (7%), 2/14 (14%), 2/14 (14%) *Salivary gland* Duct, carcinoma 0/15, 0/15, 2/15 (13%), 0/15, 1/15 (7%), 1/15 (7%)	NS NS	*Principal strengths*: GLP study; multiple-dose study; use of males and females. *Principal limitations*: low sensitivity of this transgenic mouse model under the reported study design. *Other comments*: there were no statistically significant differences in feed consumption, body weight, and survival.
Full carcinogenicity Mouse (transgenic), FVB/N-TgN(v-Ha-ras)Led (Tg.AC) homozygous (F) 6 wk 40 wk NTP (2005)	Oral administration (feed) Purity, > 98% Feed 0, 3125, 6250, 12 500, 25 000, 50 000 ppm (approx. 0, 550, 1100, 2260, 4420, and 8180 mg/kg bw per day) 15, 15, 15, 15, 15, 15 11, 10, 9, 9, 11, 8	*Salivary gland* Duct, carcinoma 0/15, 0/15, 0/15, 1/15 (7%), 0/15, 1/15 (7%)	NS	*Principal strengths*: GLP study; multiple-dose study; use of males and females. *Principal limitations*: low sensitivity of this transgenic mouse model under the reported study design. *Other comments*: body weight in group at 50 000 ppm was greater than in control group; there were no statistically significant differences in feed consumption and survival.
Full carcinogenicity Mouse (transgenic), B6.129-*Trp*53[tm1Brd] (N5) haploinsufficient (M) 7 wk 40 wk NTP (2005)	Oral administration (feed) Purity, > 98% Feed 0, 3125, 6250, 12 500, 25 000, 50 000 ppm (approx. 0, 490, 970, 1860, 3800, and 7280 mg/kg bw per day) 15, 15, 15, 15, 15, 15 14, 15, 13, 15, 14, 14	No significant increase in tumour incidence in treated animals		*Principal strengths*: GLP study; multiple-dose study; use of males and females. *Principal limitations*: low sensitivity of this transgenic mouse model under the reported study design. *Other comments*: mean body weights in groups at 6250, 12 500, 25 000, and 50 000 ppm were less than those in the control group for several weeks near the end of the study; there were no statistically significant differences in feed consumption and survival.

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse (transgenic), B6.129-*Trp53*[tm1Brd] (N5) haploinsufficient (F) 7 wk 40 wk NTP (2005)	Oral administration (feed) Purity, > 98% Feed 0, 3125, 6250, 12 500, 25 000, 50 000 ppm (approx. 0, 630, 1210, 2490, 5020, and 9620 mg/kg bw per day) 15, 15, 15, 15, 15, 15 14, 14, 14, 15, 15, 15	No significant increase in tumour incidence in treated animals		*Principal strengths*: GLP study; multiple-dose study; use of males and females. *Principal limitations*: low sensitivity of this transgenic mouse model under the reported study design. *Other comments*: there were no statistically significant differences in feed consumption, body weight, and survival.
Full carcinogenicity Mouse (transgenic), B6.129-*Cdkn2a*[tm1Rdp] (N2) deficient (M) 7–9 wk 40 wk NTP (2005)	Oral administration (feed) Purity, > 98% Feed 0, 3125, 6250, 12 500, 25 000, 50 000 ppm (approx. 0, 490, 960, 1900, 3700, and 7400 mg/kg bw per day) 15, 15, 15, 15, 15, 15 14, 14, 15, 14, 14, 15	No significant increase in tumour incidence in treated animals		*Principal strengths*: GLP study; multiple-dose study; use of males and females. *Principal limitations*: low sensitivity of this transgenic mouse model under the current study design. *Other comments*: mean body weights in the group at 3125 ppm after week 29 and at 6250 ppm after week 16 were less than those in the control group; there were no statistically significant differences in feed consumption and survival.
Full carcinogenicity Mouse (transgenic), B6.129-*Cdkn2a*[tm1Rdp] (N2) deficient (F) 7–9 wk 40 wk NTP (2005)	Oral administration (feed) Purity, > 98% Feed 0, 3125, 6250, 12 500, 25 000, 50 000 ppm (approx. 0, 610, 1200, 2300, 4850, and 9560 mg/kg bw per day) 15, 15, 15, 15, 15, 15 13, 15, 13, 15, 15, 14	*All organs* Haemangiosarcoma 0/15, 0/15, 2/15 (13%), 0/15, 0/15, 1/15 (7%)	NS	*Principal strengths*: GLP study; multiple-dose study; use of males and females. *Principal limitations*: low sensitivity of this transgenic mouse model under the reported study design. *Other comments*: there were no statistically significant differences in feed consumption, body weight, and survival.

Table 3.1   (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, Swiss (M) Day 12 of gestation 130 wk Soffritti et al. (2010)	Oral administration (feed) Purity, 98.7% (impurities, diketopiperazine, 0.2%, and L-phenylalanine, 0.1%) Feed 0, 2000, 8000, 16 000, 32 000 ppm (approx. 0, 247, 987, 1919, and 3909 mg/kg bw per day) 117, 103, 62, 64, 83 NR, NR, NR, NR, NR	*Liver* Hepatocellular adenoma 9/117 (7.7%), 10/103 (9.7%), 4/62 (6.5%), 6/64 (9.4%), 2/83 (2.4%) Hepatocellular carcinoma 6/117 (5.1%), 12/103 (11.7%), 9/62 (14.5%)*, 10/64 (15.6%)**, 15/83 (18.1%)*** Hepatocellular adenoma or carcinoma (combined) 15/117 (12.8%), 22/103 (21.4%), 13/62 (21.0%), 16/64 (25.0%)*, 17/83 (20.5%) *Lung* Bronchioloalveolar adenoma 8/117 (6.8%), 9/103 (8.7%), 7/62 (11.3%), 7/64 (10.9%), 6/82 (7.2%)	NS $P < 0.01$, trend test [test not specified] *[$P = 0.0331$, Fisher exact test]; **$P < 0.05$, Cox proportional hazard model [$P = 0.0196$, Fisher exact test]; ***$P < 0.01$, Cox proportional hazard model [$P = 0.0035$, Fisher exact test] *$P < 0.05$, logistic analysis [$P = 0.0321$, Fisher exact test] NS	*Principal strengths*: long-term study (> 2 yr); multiple-dose study; use of males and females; adequate number of animals per group; adequate duration of exposure and observation. *Principal limitations*: uncertainties regarding litter effects; conditions of animal husbandry not clear; see text for details. *Other comments*: no statistically significant differences in feed consumption, body weight, and survival; all organs and tissues were preserved in 70% alcohol, apart from bone tissues, which were preserved in 10% formalin. Historical controls (1047 male Swiss mice): hepatocellular carcinoma 3.2%, (range, 0–26.3%); bronchioloalveolar carcinoma 1.45%, (range, 0–14.3%). A statistical reanalysis by Gnudi et al. (2023) showed a significant positive trend for leukaemia (all types), with the incidence being significantly increased at all doses; a significant increase in the incidence of lymphoblastic leukaemia at all doses, of monocytic leukaemia, and of the total myeloid tumours at 16 000 ppm, as well as a significant increase in the incidence of lymphoblastic lymphoma or lymphoblastic leukaemia (combined) at 8000 and 32 000 ppm were also reported (see text for more details).

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, Swiss (M) Day 12 of gestation 130 wk Soffritti et al. (2010) (cont.)		Bronchioloalveolar carcinoma 7/117 (6.0%), 6/103 (5.8%), 7/62 (11.3%), 8/64 (12.5%), 11/83 (13.3%)* Bronchioloalveolar adenoma or carcinoma (combined) 15/117 (12.9%), 15/103 (14.6%), 14/62 (22.6%), 15/64 (23.4%), 17/83 (20.5%)*	*P* < 0.05, trend test [test not specified] *P* < 0.01, Cox proportional hazard model [NS, Fisher exact test] *P* < 0.05, logistic analysis trend test *P* < 0.05, Cox proportional hazard model [NS, Fisher exact test]	
Full carcinogenicity Mouse, Swiss (F) Day 12 of gestation 130 wk Soffritti et al. (2010)	Oral administration (feed) Purity, 98.7% (impurities, diketopiperazine, 0.2%, and ʟ-phenylalanine, 0.1%) Feed 0, 2000, 8000, 16 000, 32 000 ppm (approx. 0, 247, 987, 1919, and 3909 mg/kg bw per day) 102, 122, 73, 64, 62 NR, NR, NR, NR, NR	*Liver* Hepatocellular adenoma 1/102 (1.0%), 6/122 (4.9%), 2/73 (2.7%), 0/64, 0/62 Hepatocellular carcinoma 0/102, 2/122 (1.6%), 0/73, 2/64 (3.1%), 0/62 Hepatocellular adenoma or carcinoma (combined) 1/102 (1%), 8/122 (6.5%), 2/73 (2.7%), 2/64 (3.1%), 0/62	NS NS NS	*Principal strengths*: long-term study (> 2 yr); multiple-dose study; use of males and females. *Principal limitations*: study not conducted under GLP. *Other comments*: no statistically significant differences in feed consumption, body weight, and survival; all organs and tissues were preserved in 70% alcohol, apart from bone tissues, which were preserved in 10% formalin. In the historical controls (999 female Swiss mice), the overall incidence of hepatocellular carcinoma was 0.2% (range, 0–2.1%) A statistical reanalysis by Gnudi et al. (2023) showed a significant increase in the incidence of leukaemia (all types) at the lowest dose (see text for more details).

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, Swiss (F) Day 12 of gestation 130 wk Soffritti et al. (2010) (cont.)		*Lung* Bronchioloalveolar adenoma 4/102 (3.9%), 9/122 (7.4%), 3/73 (4.1%), 2/64 (3.1%), 3/62 (4.8%) Bronchioloalveolar carcinoma 7/102 (6.9%), 10/122 (8.2%), 6/73 (8.2%), 7/64 (10.9%), 2/62 (3.2%) Bronchioloalveolar adenoma or carcinoma (combined) 11/102 (10.8%), 19/122 (15.6%), 9/73 (19.2%), 9/64 (23.4%), 5/62 (8.06%)	NS NS NS	

approx., approximately; bw, body weight; F, female; GLP, Good Laboratory Practice; M, male; NR, not reported; NS, not significant; ppm, parts per million; wk, week(s); yr, year(s).

treated with feed containing aspartame (purity not reported; impurities, concentrations of the conversion product, diketopiperazine (SC-19192), ranged from 0.8% to 1.2%) at a dose of 0 (control), 1, 2, or 4 g/kg body weight (bw) per day, adjusted according to body-weight change, for up to 104 weeks. The feed and drinking-water were available ad libitum. Survival rates were unaffected in all treated groups of males and females compared with controls. At study termination, survival was 17/72, 9/36, 10/37, and 6/37 in males, and 26/72, 11/36, 11/35, and 10/35 in females, for the groups at 0 (control), 1, 2, or 4 g/kg bw per day, respectively. Body-weight gains for males at the three treatment doses during the first year were statistically lower than those for the male controls, with actual group mean differences not exceeding 3% at week 52; mean terminal body weights in the second year were similar to those of male controls. Body weights for the females were generally similar to those of the female controls throughout the study. Total food consumption during the first year was significantly lower for males in the groups at 1 g/kg bw and 4 g/kg bw than for the controls, slightly but not significantly lower for males in the group at 2 g/kg bw per day and females in the groups at 2 and 4 g/kg bw per day than for the respective controls, and similar to that for controls for females in the group at 1 g/kg bw per day. During the second year, mean group food consumption for males and females tended to be slightly below that for the respective controls. At each treatment dose for each sex, mean daily consumption of aspartame for the entire 104-week test period was within ± 4% of the planned dosage levels (EFSA_E75, 2011).

Histopathological examination was performed on all gross lesions from all animals at each treatment dose, and 20–27 grossly unremarkable organs from all mice in the control group and in the group at the highest dose, and from about two thirds and one third of the mice in the groups at 2 and 1 g/kg bw per day, respectively. Dietary administration of aspartame did not cause a significant increase in the incidence of any type of malignant neoplasm in either sex.

Regarding non-neoplastic lesions, no consistent alterations were detected in the tissues evaluated that could be attributed to the administration of aspartame. [The Working Group noted that this long-term study used multiple dose groups and both sexes, and that the duration covered most of the mouse lifespan. However, it was limited by selective histopathology, the lack of measurement of the stability and homogeneity of aspartame in the diet, the lack of information on aspartame purity, and the use of fewer than 50 animals in each dosed group (35–37 mice per group).]

In a study that complied with Good Laboratory Practice (GLP) and used a genetically modified FVB/N-TgN(v-Ha-*ras*)Led (Tg.AC) hemizygous mouse model [this model introduced the v-Ha-*ras* coding sequence with point mutations in codons 12 and 59 and an SV40 polyadenylation sequence under the control of the promoter of the mouse embryonic *zeta*-globin gene, giving a reporter phenotype (skin papilloma) in response to genotoxic or nongenotoxic carcinogens], groups of 15 male and 15 female transgenic mice (age, 6 weeks) were treated with feed containing aspartame (purity, > 98%) at a concentration of 0 (control), 3125, 6250, 12 500, 25 000, or 50 000 ppm (approximately equivalent to doses of 0, 490, 980, 1960, 3960, and 7660 mg/kg bw per day in males and 0, 550, 1100, 2260, 4420, and 8180 mg/kg bw per day in females) for up to 40 weeks. Basal diet and drinking-water were available ad libitum. Survival rates were unaffected in all treated groups of males and females compared with controls. Body weights in the exposed groups were similar to those in the control group for males and greater than those in the control group for females at 50 000 ppm (statistical data were not shown). Food consumption was unaffected in all treated groups of males and females compared with controls (NTP, 2005).

Histopathological examination showed no evidence of an increased incidence of neoplastic or non-neoplastic lesions in males or females in all treated groups. [The Working Group noted that this was a well-conducted study that followed the National Toxicology Program (NTP) guidelines for transgenic mouse studies and that complied with GLP and used multiple doses and both sexes. The Working Group concurred with the study authors' conclusion that this transgenic mouse model using the study design reported may not have sufficient sensitivity to detect a carcinogenic effect of aspartame.]

In a study that complied with GLP and that used a genetically modified B6.129-*Trp53*^{tm1Brd} (N5) haploinsufficient mouse model [this model introduced a null mutation into one *p53* allele by insertion of a neo cassette, thus deleting a 450-base pair gene fragment, and giving a phenotype that is susceptible to mostly genotoxic carcinogens], groups of 15 male and 15 female transgenic mice (age, 7 weeks) were treated with feed containing aspartame (purity, > 98%) at a concentration of 0 (control), 3125, 6250, 12 500, 25 000, or 50 000 ppm (approximately equivalent to doses of 0, 490, 970, 1860, 3800, and 7280 mg/kg bw per day in males and 0, 630, 1210, 2490, 5020, and 9620 mg/kg bw per day in females) for up to 40 weeks. Basal diet and drinking-water were available ad libitum. Survival rates were unaffected in all treated groups of males and females compared with controls. Body weights in all exposed groups of males and females were similar to those in the control groups. Food consumption was unaffected in all treated groups of males and females compared with controls ([NTP, 2005](#)).

Histopathological examination showed no evidence of increased neoplastic or non-neoplastic lesions in males or females in all treated groups. [The Working Group noted that this was a well-conducted study that followed the NTP guidelines for transgenic mouse studies, complied with GLP, and used multiple doses and

both sexes. The Working Group concurred with the study authors' conclusion that this transgenic mouse model using the study design reported may not have sufficient sensitivity to detect a carcinogenic effect of aspartame.]

In a study that complied with GLP and used a genetically modified B6.129-*Cdkn2a*^{tm1Rdp} (N2) deficient mouse model [this model had a targeted deletion in the *Cdkn2a* locus (*Cdkn2a* deletion), causing disruption in p16^{Ink4a} and p19^{Arf} protein function and giving a phenotype of increased cell proliferation], groups of 15 male and 15 female transgenic mice (age, 7–9 weeks) were treated with feed containing aspartame (purity, > 98%) at a concentration of 0 (control), 3125, 6250, 125 000, 25 000, or 50 000 ppm (approximately equivalent to doses of 0, 490, 960, 1900, 3700, and 7400 mg/kg bw per day in males and 0, 610, 1200, 2300, 4850, and 9560 mg/kg bw per day in females) for up to 40 weeks. Basal diet and drinking-water were available ad libitum. Survival rates were unaffected in all treated groups of males and females compared with controls. Body weights in the exposed groups were less than those in the control group for males at 3125 ppm (after 29 weeks) and at 6250 ppm (after 16 weeks), and similar to those in the control group for all treated groups of females. Food consumption was unaffected in all treated groups of males and females compared with controls ([NTP, 2005](#)).

Histopathological examination showed no evidence of an increased incidence of neoplastic lesions in males or females. Regarding non-neoplastic lesions, minimal to mild cytoplasmic vacuolization of periportal hepatocytes was found in control and exposed male mice, with an increase in incidence in the group at 6250 ppm compared with controls. [The Working Group noted that this was a well-conducted study that followed the NTP guidelines for transgenic mouse studies and that complied with GLP and used multiple doses and both sexes. The Working Group concurred with the author's statement that this transgenic mouse model using the study

design reported may not have sufficient sensitivity to detect a carcinogenic effect of aspartame.]

3.1.3 Transplacental and perinatal administration followed by oral administration (feed)

In a study of chronic toxicity and carcinogenicity, 429 male and 423 female Swiss mice were obtained by mating groups containing an equal number of males and females (three groups of 40 and two groups of 60 mice, with a total of 240 overall, no further details provided). Dams were treated with feed containing aspartame (purity, 98.7%; impurities, 0.2% diketopiperazine and 0.1% L-phenylalanine) at a concentration of 0 (control), 2000, 8000, 16 000, or 32 000 ppm (approximately equivalent to doses of 0, 247, 987, 1919, and 3909 mg/kg bw per day) starting from day 12 of gestation. The pups were weaned at age 4–5 weeks and were fed the same diet until week 130. Feed and drinking-water were available ad libitum. The initial numbers of mice assigned to the groups at 0, 2000, 8000, 16 000, and 32 000 ppm groups were 117, 103, 62, 64, and 83 for males and 102, 122, 73, 64, and 62 for females, respectively. Surviving animals (67 in total, equally distributed in groups and sex) were killed at age 130 weeks. The survival rate per group at study termination was not reported; however, the authors reported that there were no statistically significant differences in survival or in the breeding of the offspring for all groups of males and females compared with controls. No differences were observed in body weight in the treated groups compared with controls. Histopathology was performed on all organs and tissues (all were preserved in 70% ethanol, except for bone, which was preserved in 10% formalin). All slides were evaluated by a junior pathologist (with at least 4 years of experience), and all tumours and lesions of oncological interest were reviewed by two senior pathologists (with more than 20 years of experience) (Soffritti et al., 2010).

In male mice, there was a significant positive trend ($P < 0.01$, trend test [method not reported]) in the incidence of hepatocellular carcinoma – 6/117 (5.1%), 12/103 (11.7%), 9/62 (14.5%), 10/64 (15.6%), and 15/83 (18.1%) for the groups at 0 (control), 2000, 8000, 16 000 and 32 000 ppm, respectively – with the incidence being significantly increased at 16 000 and 32 000 ppm ($P < 0.05$ and $P < 0.01$, respectively, Cox proportional hazard model) and at 8000, 16 000, and 32 000 ppm [$P = 0.0331$, $P = 0.0196$, and $P = 0.0035$, respectively, Fisher exact test]. The incidence of hepatocellular carcinoma in all groups was within the upper bound of the range for historical controls (1047 male Swiss mice, overall incidence of 3.2%; range, 0–26.3%) from this laboratory. The incidence of hepatocellular adenoma or carcinoma (combined) – 15/117 (12.8%), 22/103 (21.4%), 13/62 (21.0%), 16/64 (25.0%), and 17/83 (20.5%) for the groups at 0 (control), 2000, 8000, 16 000 and 32 000 ppm, respectively – was significantly increased at 16 000 ppm ($P < 0.05$, logistic analysis; [$P = 0.0321$, Fisher exact test]). The incidence of hepatocellular adenoma, described as "typically loss of the normal lobular architecture, cells occurred in irregular plates, 1–3 cell layers thick, disposed perpendicular or obliquely on the surrounding parenchyma", did not show dose-related changes or a significant increase in all exposed groups. There was a significant positive trend ($P < 0.05$, trend test [method not reported]) in the incidence of bronchioloalveolar carcinoma – 7/117 (6.0%), 6/103 (5.8%), 7/62 (11.3%), 8/64 (12.5%), and 11/83 (13.3%) for the groups at 0 (control), 2000, 8000, 16 000 and 32 000 ppm, respectively – with the incidence being significantly increased at 32 000 ppm ($P < 0.01$, Cox proportional hazard model). The incidence of bronchioloalveolar carcinoma in all groups was within the upper bound of the range for historical controls (1047 male Swiss mice, overall incidence, 1.45%; range, 0–14.3%) from this laboratory. There was a significant positive trend ($P < 0.05$, logistic analysis) in the incidence

of bronchioloalveolar adenoma or carcinoma (combined) – 15/117 (12.9%), 15/103 (14.6%), 14/62 (22.6%), 15/64 (23.4%), and 17/83 (20.5%) for the groups at 0 (control), 2000, 8000, 16 000 and 32 000 ppm treated groups, respectively – with incidence being significantly increased at 32 000 ppm ($P < 0.05$, Cox proportional hazard model). The incidence in historical controls was not reported for adenoma or carcinoma (combined). The incidence of bronchioloalveolar adenoma did not show dose-related changes or a significant increase in any of the groups of males. In female mice, dietary exposure to aspartame did not cause a significant increase in the incidence of any tumours.

Regarding non-neoplastic lesions, no information was available to the Working Group.

[The Working Group noted that this long-term study used multiple dose groups, adequate numbers of animals of both sexes, and an adequate duration, and it provided purity and stability data for aspartame. However, the Working Group considered that there were uncertainties regarding litter effects (e.g. there was no information regarding the number of litters per treatment group, the number of pups per litter per treatment group, whether the treatments were loaded in a balanced fashion, and the method for animal selection), potentially resulting in false-positive results. The conditions of animal husbandry were unclear. In addition, the incidence of hepatocellular carcinoma and bronchioloalveolar carcinoma in all groups was within the upper bound of the range for historical controls for male Swiss mice from this institute. The incidence in historical controls was not reported for bronchioloalveolar adenoma or carcinoma (combined). There were concerns about the reliability of histological diagnoses of solid tumours in autolytic tissues. A minority of the Working Group considered, that although concerns were expressed about potential litter effects in prenatal studies, the large sample size per group ($n = 70–150$), the significant

dose–response trend, and pairwise testing of multiple tumour types suggested the absence of a potential bias caused by litter effects. Although the incidence of hepatocellular carcinoma and bronchioloalveolar carcinoma in all groups was within the upper bound of the range for historical controls for male Swiss mice from this institute, the Preamble to the *IARC Monographs* (see the front matter of the present volume; IARC, 2019) notes that concurrent controls are generally preferred over historical controls, except in the case of rare tumours. While concerns were expressed about the reliability of histological diagnoses in autolytic tissues, a minority of the Working Group considered that these bore no significance because the moribund animals were monitored two to three times daily to avoid such concerns.]

[To help with the interpretation of the study results, the Working Group deemed it important that the data be reanalysed and thus encouraged the Ramazzini Institute to provide a more detailed statistical analysis of haemolymphoreticular tumour diagnoses not previously reported in the Soffritti et al. (2010) study in mice treated with aspartame.] The statistical reanalysis of data for males by Gnudi et al. (2023) showed a significant positive trend for leukaemia (all types) ($P = 0.0492$, Cochran–Armitage trend test), with incidence being significantly increased) at all doses ($P = 0.018$, $P = 0.001$, $P = 0.001$, and $P = 0.007$, Fisher exact test; for the groups at 2000, 8000, 16 000 and 32 000 ppm treated groups, respectively). A significant increase in the incidence of lymphoblastic leukaemia ($P = 0.012$, $P = 0.001$, and $P = 0.021$, Fisher exact test; for the groups at 2000, 8000, and 32 000 ppm, respectively), of monocytic leukaemia ($P = 0.043$, Fisher exact test; for the group at 16 000 ppm), and for total myeloid tumours ($P = 0.024$, Fisher exact test; for the group at 16 000 ppm) was also reported. A significant increase in lymphoblastic lymphoma or lymphoblastic leukaemia (combined) was

observed at 8000 and 32 000 ppm [P = 0.0039 and 0.0330, respectively, Fisher exact test].

In females, Gnudi et al. (2023) showed a significant increase in the incidence of leukaemia (all types) (P = 0.007, Fisher exact test; for the group at 2000 ppm).

[The Working Group noted that the incidence of lymphoma or leukaemia (combined) in the treated groups did not exceed the incidence in historical controls, and no indication of the historical control incidence for individual haematolymphoid tumours was available, especially for rare tumours with low incidence. A minority of the Working Group noted that the Preamble to the *IARC Monographs* (see the front matter of the present volume) states that concurrent controls are generally preferred over historical controls, except in the case of rare tumours. This minority also emphasized that the diagnostic criteria were adequate for myeloid and lymphoid tumours and leukaemia, with the exception of immunoblastic lymphoma of the lung, for which the differentiation between reactive hyperplasia and neoplasia has been recognized as challenging by the NTP, Ramazzini Institute, and pathologists from other institutes (Malarkey and Bucher, 2011; Tibaldi et al., 2020).]

3.1.4 Intravesical pellet implantation

In a 26-week study, a total of 400 female Swiss albino mice (age, 60–90 days) were implanted intravesically (urinary bladder) with a pellet containing one part aspartame (purity not reported) at a dose of 0 or 4.0–4.4 mg per mouse and four parts cholesterol, for up to 26 weeks. The control group of 200 mice (group A, n = 100; group B, n = 100) was exposed only to pellets of purified cholesterol. Survival and body weight did not differ statistically between the control and treated groups (EFSA_E58, 2011; also reported by Bryan, 1984).

Urinary bladder neoplasia was observed at 175 days post-implantation. There were no statistical differences in tumour stage or incidence of neoplasia in the urinary bladder between the control and treated groups. The incidence of other tumours observed in each group was low, and histopathological data were not available. [The Working Group noted that this study was limited by the use of a single dose, the short duration (26 weeks), the lack of information on purity, and the use of an uncommon route of exposure in a single sex. Therefore, the Working Group judged this study to be inadequate for the evaluation of the carcinogenicity of aspartame in experimental animals.]

In a concurrent study, a total of 200 (group A, n = 100; group B, n = 100) female Swiss albino mice (age, 60–90 days) were implanted intravesically (urinary bladder) with a pellet containing one part aspartame (purity not reported) at a dose of 0 or 4.0–4.4 mg per mouse and four parts cholesterol, for up to 56 weeks. The control group of 200 mice (group A, n = 100; group B, n = 100) was exposed only to pellets of purified cholesterol. Survival and body weight did not differ statistically between the control and treated groups. All mice underwent complete necropsy with histopathological evaluation (EFSA_E72, 2011; also reported by Bryan, 1984).

There were no statistical differences in tumour stage or the incidence of neoplasia in the urinary bladder between the control and treated groups. There was no difference in the incidence of other tumours observed in each group (Prejean et al., 1973). No data on non-neoplastic lesions were provided. [The Working Group noted that this study was limited by the use of a single dose, the lack of information on purity, and the use of an uncommon route of exposure in a single sex. Therefore, the Working Group judged this study to be inadequate for the evaluation of the carcinogenicity of aspartame in experimental animals.]

3.2 Rat

See Table 3.2.

3.2.1 Oral administration (feed)

In studies published by Soffritti et al. (2005, 2006), also reported by Belpoggi et al. (2006), groups of 100 or 150 male and female Sprague-Dawley rats (age, 8 weeks) were treated with feed containing aspartame (purity, > 98%; impurities, concentrations of the decomposition products diketopiperazine and L-phenylalanine were 1.5% and < 0.5%, respectively) at a concentration of 0, 80, 400, 2000, 10 000, 50 000, or 100 000 ppm (approximately equivalent to daily intakes of 0, 4, 20, 100, 500, 2500, and 5000 mg/kg bw). The Sprague-Dawley rats were obtained from the breeding colony maintained at the Ramazzini Institute, Italy. At age 4–5 weeks, the rats were randomized to include no more than one male and one female from each litter in the same group, to control for litter effects. The exposure lasted until the natural death of the rats, and all rats found moribund or dead were promptly necropsied for gross and microscopic evaluation of the extensive list of tissues identified in the study protocol. The in-life phase of the study lasted 159 weeks (Soffritti et al., 2005, 2006; also reported by Belpoggi et al., 2006). There were no significant differences in water consumption, slight dose-dependent decreases in feed consumption, and no differences in body-weight gain or survival across groups. The rats at 100 000 ppm developed yellowing of the hair coat, which was reported to be also observed in a study from the Ramazzini Institute in rats treated with formaldehyde (Soffritti et al., 2002).

In male rats, an overall increase in the incidence of animals bearing malignant tumours was observed, with a significant positive trend ($P \leq 0.05$, Cochran–Armitage trend test). There was a significant positive trend ($P \leq 0.05$, Cochran–Armitage trend test; $P \leq 0.05$, poly-3 trend test)

in the incidence of lymphoma and leukaemia (combined) (multiple organs) – 31/150 (20.7%), 23/150 (15.3%), 25/150 (16.7%), 33/150 (22%), 15/100 (15%), 20/100 (20%), and 29/100 (29%), at 0 (control), 80, 400, 2000, 10 000, 50 000, and 100 000 ppm, respectively – with the incidence in all treated groups being within the upper bound of the range observed in historical controls (2265 males, 20.6%; range, 8.0–30.9%) from this laboratory (Soffritti et al., 2005, 2006). There was a significant positive trend ($P \leq 0.05$, Cochran–Armitage trend test; $P \leq 0.05$, poly-3 trend test) in the incidence of malignant schwannoma of the peripheral nerve, most frequently observed in cranial nerves and other sites (mainly spinal nerve roots) – 1/150 (0.7%), 1/150 (0.7%), 3/150 (2%), 2/150 (1.3%), 2/100 (2%), 3/100 (3%), and 4/100 (4%) at 0 (control), 80, 400, 2000, 10 000, 50 000, and 100 000 ppm, respectively – with the incidence at 50 000 and 100 000 ppm exceeding the upper bound of the range observed in historical controls (2265 males, 0.5%; range, 0–2.0%) from this laboratory (Soffritti et al., 2005, 2006). All the schwannoma diagnoses were supported by positive immunohistochemical staining for S100 protein. [The Working Group noted that malignant schwannoma is a rare tumour in this animal model.] Metastases of malignant schwannoma were observed in three rats at 100 000 ppm. The incidence of dysplastic papilloma and carcinoma (combined) of the renal pelvis and ureter exceeded that observed in historical controls (2265 males, 0%) from this laboratory (Soffritti et al., 2005, 2006). [The Working Group considered it satisfactory to combine the "dysplastic papillomas" with the carcinomas, while excluding "dysplastic hyperplasia".] In addition, two transitional cell carcinomas of the urinary bladder were reported in male rats at 10 000 ppm (Soffritti et al., 2006). [The Working Group noted that transitional cell carcinoma is a rare tumour among historical controls in this colony of Sprague-Dawley rats.]

Table 3.2 Studies of carcinogenicity with aspartame in rats

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (M) 8 wk Lifetime Soffritti et al. (2006)	Oral administration (feed) Purity, > 98% (impurities, diketopiperazine, < 1.5%, and L-phenylalanine, < 0.5%) Feed 0, 80, 400, 2000, 10 000, 50 000, 100 000 ppm, daily 150, 150, 150, 150, 100, 100, 100 150, 150, 150, 150, 100, 100, 100	*Systemic (multiple organs)* Lymphoma or leukaemia (combined) 31/150 (20.7%), 23/150 (15.3%), 25/150 (16.7%), 33/150 (22%), 15/100 (15%), 20/100 (20%), 29/100 (29%) *Peripheral nerve (cranial and others)* Malignant schwannoma 1/150 (0.7%), 1/150 (0.7%), 3/150 (2%), 2/150 (1.3%), 2/100 (2%), 3/100 (3%), 4/100 (4%) *Renal pelvis and ureter* Dysplastic papilloma 0/150, 0/149, 1/149 (0.7%), 0/150, 0/100, 0/100, 0/100 Carcinoma 0/150, 0/149, 0/149, 1/150 (0.7%), 1/100 (1%), 1/100 (1%), 1/100 (1%) Dysplastic papilloma and carcinoma (combined) 0/150, 0/149, 1/149 (0.7%), 1/150 (0.7%), 1/100 (1%), 1/100 (1%), 1/100 (1%)	$P \leq 0.05$, Cochran–Armitage trend test; $P \leq 0.05$ poly-3 trend test $P \leq 0.05$, Cochran–Armitage trend test; $P \leq 0.05$, poly-3 trend test NS NS NS	*Principal strengths*: multiple-dose study; males and females used; large group size (100–150/group); lifetime exposure (> 2 yr). *Principal limitations*: details of pathology descriptions not available. *Other comments*: combinations of some lesions in this study are not commonly used, e.g. (i) histiocytic sarcoma was combined with lymphoma/leukaemia; (ii) hyperplasia was combined with tumours (dysplastic hyperplasia lesions of renal pelvis and ureter were combined with dysplastic papilloma and carcinoma). See text for details on the histological reanalysis by Tibaldi et al. (2020), and the statistical reanalysis by Gnudi et al. (2023). Historical controls: lymphoma and leukaemia (combined), 2265 males, 20.6% (range, 8.0–30.9%); malignant schwannoma, 2265 males, 0.5% (range, 0–2.0%); olfactory neuroblastoma, 2265 males, 0.1% (range, 0–1.8%); renal pelvis and ureter transitional cell carcinomas (combined), 2265 males, 0%.

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (F) 8 wk Lifetime Soffritti et al. (2006)	Oral administration (feed) Purity, > 98% (impurities, diketopiperazine, < 1.5%, and L-phenylalanine, < 0.5%) Feed 0, 80, 400, 2000, 10 000, 50 000, 100 000 ppm, daily 150, 150, 150, 150, 100, 100, 100 150, 150, 150, 150, 100, 100, 100	*Systemic (multiple organs)* Lymphoblastic lymphoma 2/150 (1.3%), 3/150 (2%), 7/150 (4.7%), 5/150 (3.3%), 2/100 (2%), 2/100 (2%), 1/100 (1%) Lymphoblastic leukaemia 0/150, 0/150, 0/150, 1/150 (0.7%), 0/100, 0/100, 0/100 Lymphocytic lymphoma 2/150 (1.3%), 5/150 (3.3%), 2/150 (1.3%), 1/150 (0.7%), 2/100 (2%), 0/100, 2/100 (2%) Lymphoimmunoblastic lymphoma 5/150 (3.3%), 6/150 (4%), 8/150 (5.3%), 8/150 (5.3%), 3/100 (3%), 10/100 (10%), 11/100 (11%) Histiocytic sarcoma 4/150 (2.7%), 6/150 (4%), 9/150 (6%), 8/150 (5.3), 10/100 (10%), 8/100 (8%), 7/100 (7%)	NS [One animal at 400 ppm dose group had lymphoblastic lymphoma and histiocytic sarcoma] NS NS NS NS	*Principal strengths*: multiple-dose study; males and females used; large group size (100–150/group); lifetime exposure (> 2 yr). *Principal limitations*: details of pathology descriptions were not available. *Other comments*: combinations of some lesions in this study are not commonly used, e.g. (i) histiocytic sarcoma was combined with lymphoma/leukaemia; (ii) hyperplasia was combined with tumours (dysplastic hyperplasia lesions of renal pelvis and ureter were combined with dysplastic papilloma and carcinoma). See the text for details on the histological reanalysis by Tibaldi et al. (2020), and the statistical reanalysis by Gnudi et al. (2023). Historical controls: lymphoma and leukaemia (combined), 2274 females, 13.3% (range, 4.0–25.0%); malignant schwannoma, 2274 females, 0.1% (range, 0–2.0%); renal pelvis and ureter transitional cell carcinomas, 2274 females, 0.04% (range, 0–1.0%); olfactory neuroblastoma, 2274 females, 0.1% (range, 0–1.8%).

Table 3.2   (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (F) 8 wk Lifetime Soffritti et al. (2006) (cont.)		Monocytic leukaemia 0/150, 2/150 (1.3%), 5/150 (3.3%), 4/150 (2.7%), 2/100 (2%), 4/100 (4%), 2/100 (2%)	NS	
		Myeloid leukaemia 0/150, 0/150, 0/150, 1/150 (0.7%), 0/100, 1/100 (1%), 2/100 (2%)	NS	
		Lymphoma or leukaemia (combined) 13/150 (8.7%), 22/150 (14.7%), 30/150 (20%)**, 28/150 (18.7%)*, 19/100 (19%)*, 25/100 (25%)**, 25/100 (25%)**	$P \leq 0.01$, Cochran–Armitage trend test; $P \leq 0.05$, poly-3 trend test *$P \leq 0.05$, poly-3 test; [$P \leq 0.01$, Fisher exact test] **$P \leq 0.01$, poly-3 test; [$P \leq 0.01$, Fisher exact test]	
		Peripheral nerve (cranial and others) Malignant schwannoma 0/150, 2/150 (1.3%), 0/150, 3/150 (2%), 1/100 (1%), 1/100 (1%), 2/100 (2%)	NS	

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (F) 8 wk Lifetime Soffritti et al. (2006) (cont.)		*Renal pelvis and ureter* Dysplastic papilloma 0/150, 1/150 (0.7%), 1/150 (0.7%), 1/150 (0.7%), 1/100 (1%), 1/100 (1%), 3/100 (3%)	$P \leq 0.05$, Cochran–Armitage trend test	
		Carcinoma 0/150, 1/150 (0.7%), 3/150 (2%), 3/150 (2%), 3/100 (3%), 3/100 (3%), 4/100 (4%)*	*$P \leq 0.05$, poly-3 test; [$P = 0.0247$, Fisher exact test] [One rat at 2000 and at 10 000 ppm had tumours in both kidneys]	
		Dysplastic papilloma or carcinoma (combined) 0/150 (0%), 2/150 (1.3%), 4/150 (2.7%), 4/150 (2.7%), 4/100 (4%), 4/100 (4%), 7/100 (7%)*	[$P = 0.041$, Cochran–Armitage trend test] [*$P = 0.0014$, Fisher exact test] [One rat at 2000 and 10 000 ppm had tumours in both kidneys]	
		Mammary glands Carcinoma 8/150 (5.3%), 15/150 (10%), 16/150 (10.7%), 12/150 (8%), 7/100 (7%), 18/100 (18%)*, 7/100 (7%)	[*$P = 0.0015$, Fisher exact test]	

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, SLC Wistar (M) 6 wk 104 wk Ishii (1981)	Oral administration (feed) Purity, NR Feed 0, 1, 2, 4 g/kg bw per day 60, 60, 60, 60 26, 16, 28, 25	*Brain* Glioma (astrocytoma, atypical astrocytoma, oligodendroglioma, combined) 0/60, 1/60 (1.7%), 0/60, 1/60 (1.7%)	NS	*Principal strengths*: males and females used; multiple-dose study; adequate number of animals used; adequate duration of exposure and observation. *Principal limitations*: purity, NR; lack of detailed histopathology. Other comments: histological reanalysis by Shibui et al. (2019) and EFSA_UA04 (2011)
Full carcinogenicity Rat, SLC Wistar (F) 6 wk 104 wk Ishii (1981)	Oral administration (feed) Purity, NR Feed 0, 1, 2, 4 g/kg bw per day 60, 60, 60, 60 49, 40, 51, 43	*Brain* Glioma (astrocytoma, atypical astrocytoma, oligodendroglioma, combined) 1/60 (1.7%), 0/60, 1/60 (1.7%), 0/60	NS	*Principal strengths*: males and females used; multiple-dose study; adequate number of animals used; adequate duration of exposure and observation. *Principal limitations*: purity, NR; lack of detailed histopathology. Other comments: histological reanalysis by Shibui et al. (2019) and EFSA_UA04 (2011).
Full carcinogenicity Rat, SLC Wistar (M) 6 wk 52 wk Ishii (1981)	Oral administration (feed) Purity, NR Feed 0, 1, 2, 4 g/kg bw per day 16, 16, 16, 16 16, 16, 16, 16	No significant increase in tumour incidence in treated animals		*Principal strengths*: males and females used; multiple-dose study. *Principal limitations*: purity, NR.
Full carcinogenicity Rat, SLC Wistar (F) 6 wk 52 wk Ishii (1981)	Oral administration (feed) Purity, NR Feed 0, 1, 2, 4 g/kg bw per day 16, 16, 16, 16 16, 16, 16, 16	No significant increase in tumour incidence in treated animals		*Principal strengths*: males and females used; multiple-dose study. *Principal limitations*: purity, NR.

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, SLC Wistar (M) 6 wk 26 wk Ishii (1981)	Oral administration (feed) Purity, NR Feed 0, 1, 2, 4 g/kg bw per day 16, 16, 16, 16 16, 16, 16, 16	No significant increase in tumour incidence in treated animals		*Principal strengths*: males and females used; multiple-dose study. *Principal limitations*: purity, NR
Full carcinogenicity Rat, SLC Wistar (F) 6 wk 26 wk Ishii (1981)	Oral administration (feed) Purity, NR Feed 0, 1, 2, 4 g/kg bw per day 16, 16, 16, 16 16, 16, 16, 16	No significant increase in tumour incidence in treated animals		*Principal strengths*: males and females used; multiple-dose study. *Principal limitations*: purity, NR.
Full carcinogenicity Rat, SLC Wistar (M) 6 wk 26 wk Shibui et al. (2019)	Oral administration (feed) Purity, NR Feed 0, 1, 2, 4 g/kg bw per day 60, 60, 60, 60 60, 60, 60, 60	*Lung* Bronchioloalveolar adenoma 1/60 (1.7%), 3/60 (5%), 0/60, 2/60 (3.3%) Bronchioloalveolar carcinoma 0/60, 0/60, 2/60 (3.3%), 0/60 Bronchioloalveolar adenoma or carcinoma (combined) 1/60 (1.7%), 3/60 (5%), 2/60 (3.3%), 2/60 (3.3%)	NS NS NS	*Principal strengths*: males and females used; multiple-dose study. *Principal limitations*: purity, NR. *Other comments*: histological reanalysis of Ishii et al. (1981) by Shibui et al. (2019) also reported by EFSA_UA04 (2011).

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, SLC Wistar (M) 6 wk 26 wk Shibui et al. (2019) (cont.)		*Pituitary gland (pars distalis)* Adenoma 5/48 (10.4%), 8/48 (16.7%), 12/53 (22.6%), 5/53 (9.4%) Carcinoma 0/48, 1/48 (2%), 0/53, 0/53 *Adrenal gland* Benign pheochromocytoma 4/59 (6.8%), 9/59 (15.3%), 4/60 (6.7%), 10/60 (16.7%)	NS NS NS	
Full carcinogenicity Rat, SLC Wistar (F) 6 wk 26 wk Shibui et al. (2019)	Oral administration (feed) Purity, NR Feed 0, 1, 2, 4 g/kg bw per day 60, 60, 60, 60 60, 60, 60, 60	*Lung* Bronchioloalveolar adenoma 0/60 (1.7%), 1/60 (1.7%), 3/60 (5%), 3/60 (5%) *Adrenal gland* Benign pheochromocytoma 1/60 (1.7%), 1/60 (1.7%), 6/59 (10.2%), 2/60 (3.3%) Malignant pheochromocytoma 0/60, 0/60, 1/59 (1.7%), 1/60 (1.7%)	NS NS NS	*Principal strengths*: males and females used; multiple-dose study. *Principal limitations*: purity, NR. *Other comments*: histological reanalysis of Ishii et al. (1981) by Shibui et al. (2019), also reported by EFSA_UA04 (2011).

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Charles River albino (M) Weanling 104 wk EFSA_E34 (2011)	Oral administration (feed) Purity, NR (SC-18862; impurities, ≤ 1.5% of conversion product diketopiperazine, SC-191912) Feed 0, 1, 2, 4, 8 g/kg bw per day 60, 40, 40, 40, 40 23, 18, 21, 23, 21	*Brain* Glioma (astrocytoma, oligodendroglioma, combined) 0/60, 2/40 (5%), 1/40 (2.5%), 2/40 (5%), 0/40	NS	*Principal strengths*: 104-wk study; males and females used; multiple-dose study; adequate number of animals per group. *Principal limitations*: 35% and 25% survival in females at 4 g/kg bw and 8 g/kg bw; survival was about 50% in most groups; chronic murine pneumonia complex in control and treated animals; only 25% of animals examined microscopically in groups at 1 g/kg bw and 2 g/kg bw; study from pre-GLP era.
Full carcinogenicity Rat, Charles River albino (F) Weanling 104 wk EFSA_E34 (2011)	Oral administration (feed) Purity, NR (SC-18862; impurities, up to 1.5% of conversion product diketopiperazine, SC-191912) Feed 0, 1, 2, 4, 8 g/kg bw per day 60, 40, 40, 40, 40 26, 23, 20, 14, 10	*Brain* Glioma (astrocytoma, oligodendroglioma, glioma unclassified, combined) 0/60, 2/40 (5%), 0/40, 0/40, 1/40 (2.5%)	NS	*Principal strengths*: 104-wk study; males and females used; multiple-dose study; adequate number of animals per group. *Principal limitations*: 35% and 25% survival in females at 4 g/kg bw and 8 g/kg bw; survival was about 50% in most groups, chronic murine pneumonia complex in control and treated animals, only 25% of animals examined microscopically in 1 g/kg bw and 2 g/kg bw groups, study from the pre-GLP era.

Table 3.2   (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (M) Day 12 of gestation Lifetime Soffritti et al. (2007)	Oral administration (feed) Purity, > 98.7% (impurities, diketopiperazine, < 0.3%, and L-phenylalanine, < 0.5%) Feed 0, 400, 2000 ppm, daily 95, 70, 70 95, 70, 70	*Systemic (multiple organs)* Lymphoma or leukaemia (combined) 9/95 (9.5%), 11/70 (15.7%), 12/70 (17.1%)* *Renal pelvis* Papilloma 0, 2/70 (2.9%), 1/70 (1.4%) *Mammary gland* Carcinoma 0, 0, 2/70 (2.9%)	*P ≤ 0.05, Cox regression model [P = 0.0030, Fisher exact test] NS NS	*Principal strengths*: males and females used; prenatal exposure; large group size (70–95); lifetime exposure (> 2 yr); two doses, with the lower dose being relevant to human exposure. *Principal limitations*: details of pathology descriptions were not available. *Other comments*: no information on the incidence, distribution, and histotype of the lymphomas and leukaemias presented. See the text for details on the histological reanalysis by Tibaldi et al. (2020) and the statistical reanalysis by Gnudi et al. (2023). Historical controls: lymphoma and leukaemia (combined), 2265 males, 20.6% (range, 8.0–30.9%).

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (F) Day 12 of gestation Lifetime Soffritti et al. (2007)	Oral administration (feed) Purity, > 98.7% (impurities, diketopiperazine, < 0.3%, and L-phenylalanine, < 0.5%) Feed 0, 400, 2000 ppm, daily 95, 70, 70 95, 70, 70	*Systemic (multiple organs)* Lymphoma or leukaemia 12/95 (12.6%), 12/70 (17.6%), 22/70 (31.4%)* *Renal pelvis* Papilloma 0/95, 6/70 (8.6%)*, 0/70 *Mammary gland* Carcinoma 5/95 (5.3%), 5/70 (7.1%), 11/70 (15.7%)*	$P \leq 0.01$, Cox regression model (trend) *$P \leq 0.01$, Cox regression model; [$P = 0.0030$, Fisher exact test] [*$P = 0.0051$, Fisher exact test] $P \leq 0.05$, Cox regression model (trend) *$P \leq 0.05$, Cox regression model; [$P = 0.0245$, Fisher exact test]	*Principal strengths*: males and females used; prenatal exposure; large group size (70–95); lifetime exposure (> 2 yr); two doses, with the lower dose being relevant to human exposure. *Principal limitations*: details of pathology descriptions were not available. *Other comments*: no information on the incidence, distribution, and histotype of the lymphomas and leukaemias was presented. Historical controls: lymphoma and leukaemia (combined), 2274 females, 13.3% (range, 4.0–25.0%); mammary gland carcinoma, 2274 females, 9.2% (range, 4.0–14.2%); a statistical reanalysis by Gnudi et al. (2023) showed positive significant trends in the incidence of total lymphoid tumours, myeloid leukaemia, and total myeloid tumours, furthermore, significant increases were shown in the incidence of total lymphoid tumours, lymphoma (all types), and leukaemia (all types).

Table 3.2   (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Charles River albino (M) F₁ rats were obtained from parents exposed 60 days before mating 104 wk EFSA_E70 (2011)	Oral administration (feed) Purity, > 98.5% (SC-18862; impurities, conversion product diketopiperazine, SC-191912, 0.4–1.5%) Feed 0, 2, 4 g/kg bw per day 60, 40, 40 25, 20, 23	*Brain* Astrocytoma 3/60 (5%), 1/40 (2.5%), 1/40 (2.5%)	NS	*Principal strengths*: 104-wk study; males and females used; multiple-dose study; adequate duration of exposure and observation. *Principal limitations*: survival was about 50% in most groups; chronic murine pneumonia complex in control and treated animals; study did not adjust for litter effects.
Full carcinogenicity Rat, Charles River albino (F) F₁ rats were obtained from parents exposed 60 days before mating 104 wk EFSA_E70 (2011)	Oral administration (feed) Purity, > 98.5% (SC-18862; impurities, conversion product diketopiperazine, SC-191912, 0.4–1.5%) Feed 0, 2, 4 g/kg bw per day 60, 40, 40 28, 18, 21	*Brain* Astrocytoma 1/60 (1.7%), 1/40 (2.5%), 0/40	NS	*Principal strengths*: 104-wk study; males and females used; multiple-dose study; adequate duration of exposure and observation. *Principal limitations*: survival was about 50% in most groups; chronic murine pneumonia complex in control and treated animals; study did not adjust for litter effects.

approx., approximately; bw, body weight; F, female; GLP, Good Laboratory Practice; M, male; NR, not reported; NS, not significant; ppm, parts per million; wk, week(s); yr, year(s).

In female rats, an overall increase in the incidence of animals bearing malignant tumours was observed, with a significant positive trend ($P \leq 0.01$, Cochran–Armitage trend test); the incidence in rats at 50 000 ppm was significantly higher than that in the control group ($P \leq 0.01$, poly-3 test). There was a significant positive trend ($P \leq 0.01$, Cochran–Armitage trend test; $P \leq 0.05$, poly-3 trend test) in the incidence of lymphoma and leukaemia (combined) (multiple organs) – 13/150 (9%), 22/150 (15%), 30/150 (20%), 28/150 (19%), 19/100 (19%), 25/100 (25%), and 25/100 (25%) at 0 (control), 80, 400, 2000, 10 000, 50 000, and 100 000 ppm, respectively – with the incidence being statistically significant at 2000 and 10 000 ppm ($P \leq 0.05$, poly-3 test; [$P \leq 0.01$, Fisher exact test]) and at 50 000 and 100 000 ppm ($P \leq 0.01$, poly-3 test; [$P \leq 0.01$, Fisher exact test]), and within the upper bound of the range observed in historical controls (2274 males, 13.3%; range, 4.0–25.0%) from this laboratory (Soffritti et al., 2005, 2006). [The Working Group noted that the lymphoid and myeloid tumour classification was originally performed according to the IARC Classification of Rodent Tumours (IARC, 1993) and subsequently according to the International Harmonization of Nomenclature and Diagnostic Criteria for Lesions Project (INHAND), both on the basis of haematoxylin-and-eosin–stained sections only, defining lymphoid proliferations (lymphoblastic leukaemia, lymphocytic lymphoma, lymphoimmunoblastic lymphoma) and myeloid proliferations (histiocytic sarcoma, monocytic leukaemia, myeloid leukaemia). The main types reported were lymphocytic lymphomas and lymphoimmunoblastic lymphomas that involved the thymus, lung, spleen, and peripheral lymph nodes. Although details on the incidence of the different morphologies or localization were not reported in Soffritti et al. (2006), they were subsequently provided in Gnudi et al. (2023), at the request of the Working Group. The Working Group members were in agreement on the combinations of lymphoid tumours and lymphoid leukaemias, and combinations of myeloid leukaemias, but not on the combinations of lymphoid and myeloid tumours, and combinations of lymphoid leukaemias and myeloid leukaemias.] The incidence of peripheral nerve malignant schwannoma (cranial and other) – 0/150, 2/150 (1.3%), 0/150, 3/150 (2%), 1/100 (1%), 1/100 (1%), 2/100 (2%), at 0 (control), 80, 400, 2000, 10 000, 50 000, and 100 000 ppm, respectively – was within the upper bound of the range observed in historical controls (2274 females, 0.1%; range, 0–2.0%) from this laboratory (Soffritti et al., 2005, 2006). [The Working Group noted that all the schwannoma diagnoses were supported by positive immunohistochemical staining for S100 protein.] There were significant exposure-specific increases in the incidence of proliferative urothelial lesions within the renal pelvis and ureter in female rats. There was a significant positive trend ($P \leq 0.05$, Cochran–Armitage trend test) in the incidence of dysplastic papilloma – 0/150, 1/150 (0.7%), 1/150 (0.7%), 1/150 (0.7%), 1/100 (1%), 1/100 (1%), 3/100 (3%), at 0 (control), 80, 400, 2000, 10 000, 50 000, and 100 000 ppm, respectively. The incidence of carcinoma of the renal pelvis and ureter – 0/150, 1/150 (0.7%), 3/150 (2%), 3/150 (2%), 3/100 (3%), 3/100 (3%), 4/100 (4%), at 0 (control), 80, 400, 2000, 10 000, 50 000, and 100 000 ppm, respectively – was significantly increased at 100 000 ppm ($P \leq 0.05$, poly-3 test; [$P = 0.0247$, Fisher exact test]), and exceeded the upper bound of the range observed in historical controls (2274 females, 0.04%; range, 0–1.0%) from this laboratory at all doses except for 80 ppm. There was a significant positive trend in the incidence of papilloma or carcinoma (combined) of the renal pelvis and ureter [$P = 0.041$, Cochran–Armitage trend test], with the incidence – 0/150, 2/150 (1.3%), 4/150 (2.7%), 4/150 (2.7%), 4/100 (4%), 4/100 (4%), 7/100 (7%), at 0 (control), 80, 400, 2000, 10 000, 50 000, and 100 000 ppm, respectively – being significantly increased at 10 000 and 50 000 ppm [$P = 0.0247$, Fisher exact test] and 100 000 ppm [$P = 0.0014$,

Fisher exact test]. There was a significant increase [$P = 0.0015$, Fisher exact test] in the incidence of mammary gland carcinoma in the group at 50 000 ppm – 18/100 (18%) compared with 8/150 (5%) in controls. In addition, one transitional cell carcinoma of the urinary bladder was reported in female rats at 2000 ppm. There was a single incidence each of adenoma and of olfactory neuroblastoma in females at 100 000 ppm.

Exposure to aspartame increased the incidence of malignant brain tumours (10 gliomas, 1 medulloblastoma, and 1 malignant meningioma) in male and female rats, with none being reported in concurrent controls (Soffritti et al., 2006). [The Working Group noted that gliomas are rare tumours (incidence, approximately 0.5–1%) among historical controls in this colony of Sprague-Dawley rats.] The incidence of glioma in males and females in the study by Belpoggi et al. (2006) was reported as follows: males, 0/150, 2/150, 0/150, 1/150, 0/100, 2/100, 1/100; females, 0/150, 1/150, 0/150, 1/150, 0/100, 1/100, 1/100 at 0 (control), 80, 400, 2000, 10 000, 50 000, and 100 000 ppm, respectively. The incidence of brain tumours exhibited no dose–response trends. The data on brain neoplasms were based on the examination of three sagittal sections rather than the seven or eight coronal sections used in other studies. [The Working Group noted that males and females were combined when counting malignant brain tumours. The Working Group also noted that incidence was reported separately for each sex in the study by Belpoggi et al. (2006) reported above.]

Regarding non-neoplastic lesions, there was also an increase in the incidence of dysplastic hyperplasia of the transitional cell epithelium of the renal pelvis and ureter in males (no significant trend) and in females (significant positive trend). There was an increase in the incidence of calcification (mineralization) within the renal pelvis and ureter of female rats, especially at the higher doses (10 000 ppm, 19%; 50 000 ppm, 25%; and 100 000 ppm, 39%) compared with the concurrent controls (8%). A similar effect was not reported in the male rat kidney. Information on the severity and distribution of this mineralization was not available, and its contribution to the proliferative lesions remains to be determined. The incidence of hyperplasia of the olfactory epithelium exhibited a significant positive trend in males, and was significantly increased at 10 000, 50 000, and 100 000 ppm in males and females, and at 400 ppm in males. [The Working Group noted that this was a well-conducted long-term study that used multiple dose groups, both sexes, a large group size, and with a duration that covered most of the rat lifespan. However, it was limited by the lack of detailed pathology descriptions for some haematolymphoid lesions, the combination of non-neoplastic and neoplastic proliferative lesions of the renal pelvis and ureter for statistical analyses, and the use of combinations of lymphoid and myeloid neoplastic lesions that are not common. The Working Group noted that the practice of combining lymphoid and myeloid proliferations was unwarranted since these lesions derive from different cell lineages. It was noted that lung lymphomas (primary pulmonary lymphomas, with isolated or dominant lung involvement) are rare in all species, including rats, and are difficult to distinguish from non-neoplastic proliferative lesions. However, in the present study, this presentation and diagnosis was highly prevalent. The Working Group also noted that the arguments used for some lymphoma diagnoses reported in Soffritti et al. (2006) and the histological reanalysis by Tibaldi et al. (2020) were not sufficient to allow differentiation between inflammatory or neoplastic proliferations, especially those in the lung (see Section 3.2.2). The diagnostic criteria were adequate for myeloid and lymphoid tumours and leukaemia, with the exception of immunoblastic lymphoma of the lung, for which the differentiation between reactive hyperplasia and neoplasia has been recognized as challenging by the NTP, Ramazzini Institute, and pathologists

from other laboratories (Malarkey and Bucher, 2011; Tibaldi et al., 2020).]

Gnudi et al. (2023) reported on the details of diagnoses of haemolymphoreticular tumours and related statistics for the study by Soffritti et al. (2006) in rats treated with aspartame. In males, this reanalysis showed a significant positive trend in the incidence of immunoblastic lymphoma (P = 0.0006, Cochran–Armitage trend test) and total lymphoid tumours (P = 0.0022, Cochran–Armitage trend test). There was a significant increase in the incidence of lymphoma (all types) (P = 0.016, Fisher exact test) at 100 000 ppm, immunoblastic lymphoma (P = 0.016, Fisher exact test) at 100 000 ppm, and total lymphoid tumours (P = 0.023, Fisher exact test) at 100 000 ppm.

In females, there was a significant positive trend in the incidence of immunoblastic lymphoma (P = 0.0015, Cochran–Armitage trend test), myeloid leukaemia (P = 0.0060, Cochran–Armitage trend test), and total myeloid tumours (P = 0.0473, Cochran–Armitage trend test). There was a significant increase in the incidence of lymphoma (all types) at 100 000 ppm (P = 0.028, Fisher exact test), of immunoblastic lymphoma at 50 000 and 100 000 ppm (P = 0.030, P = 0.016, respectively, Fisher exact test), of total lymphoid tumours at 100 000 ppm (P = 0.028, Fisher exact test), of monocytic leukaemia at 50 000 ppm and 400 ppm (P = 0.025, P = 0.030, respectively, Fisher exact test), of histiocytic sarcoma at 10 000 and 50 000 ppm (P = 0.015, P = 0.053, respectively, Fisher exact test), and of total myeloid tumours at 400, 2000, 10 000, 50 000, and 100 000 ppm (P = 0.013, P = 0.021, P = 0.004, P = 0.002, P = 0.008, respectively, Fisher exact test). [The Working Group acknowledged that histological diagnoses of the original study were retained for this analysis. The diagnostic criteria were adequate for myeloid and lymphoid tumours and leukaemia, with the exception of immunoblastic lymphoma of the lung, for which the differentiation between reactive hyperplasia and neoplasia

has been recognized as challenging by the NTP, the Ramazzini Institute, and pathologists from other institutes (Malarkey and Bucher, 2011; Tibaldi et al., 2020). The Working Group noted that the histological diagnoses of immunoblastic lymphoma of the lung remained inconclusive. Although the majority of the Working Group considered that the histological diagnoses in autolytic tissues were not reliable, a minority of the Working Group considered that these bore no significance, because the moribund animals were monitored two to three times daily to avoid such concerns.]

In a study by Ishii (1981), also reported by Ishii et al. (1981), groups of 60 male and 60 female SLC Wistar rats (age, 6 weeks) were treated with feed containing aspartame (purity not reported) at a concentration of 0 (control), 1, 2, or 4 g/kg bw per day for 104 weeks. All surviving rats in these groups were killed after 104 weeks. Two additional groups of 16 males and 16 females or 10 males and 10 females were scheduled for interim evaluation at experimental weeks 52 and 26, respectively. Survival at 104 weeks was lower in males (26/60, 16/60, 28/60, 25/60) than in females (49/60, 40/60, 51/60, 43/60) at 0 (control), 1, 2, and 4 g/kg bw per day, respectively. Mean feed consumption was lower in all dosed rats than in controls. A dose-dependent decrease in body-weight gain was observed in groups of males at 2 and 4 g/kg bw per day, and in all dosed groups of females. At 52 weeks, an increase in relative spleen weight was reported only in males at 2 and 4 g/kg bw per day, but no histological correlates were described (Ishii, 1981; also reported by Ishii et al., 1981). Dietary administration of aspartame did not cause a significant increase in the incidence of any type of neoplasm in male or female rats at either interim time of 26 or 52 weeks Ishii (1981).

At 104 weeks, dietary administration of aspartame did not cause a significant increase in the incidence of any type of neoplasm in male or female rats Ishii et al. (1981). There were no

differences in the incidence of glioma across all groups in males – 0/60, 1/60 (1.7%), 0/60, 1/60 (1.7%) or females, 1/60 (1.7%), 0/60, 1/60 (1.7%), 0/60, at 0 (control), 1, 2, and 4 g/kg bw, respectively (Ishii, 1981).

Shibui et al. (2019), also reported by EFSA_UA04 (2011), subsequently re-evaluated the rat tissues from groups at 0 (control), 1, 2, and 4 g/kg bw per day from the study by Ishii et al. (1981). [The Working Group noted that the study by Shibui et al. (2019) was a comprehensive re-evaluation of the study by Ishii et al. (1981), carried out by cutting new slides.] In males, there was a non-significant increase in the incidence of bronchioloalveolar adenoma – 1/60 (1.7%), 3/60 (5%), 0%, 2/60 (3.3%) at 0 (control), 1, 2, and 4 g/kg bw per day, respectively. In addition, there were two bronchioloalveolar carcinomas reported in the group at 2 g/kg bw per day. When the bronchioloalveolar adenomas and carcinomas were combined, their incidence was 1/60 (1.7%), 3/60 (5%), 2/60 (3.3%), 2/60 (3.3%) at 0 (control), 1, 2, and 4 g/kg bw per day, respectively. There were non-significant exposure-related increases in the incidence of adenoma in the pars distalis of the pituitary gland – 5/48 (10.4%), 8/48 (16.7%), 12/53 (22.6%), 5/53 (9.4%) at 0 (control), 1, 2, and 4 g/kg bw per day, respectively. In addition, there was one carcinoma in the pars distalis of the pituitary gland in the group at 1 g/kg bw per day. There were non-significant increases in the incidence of benign pheochromocytoma – 4/59 (6.8%), 9/59 (15.3%), 4/60 (6.7%), 10/60 (16.7%) at 0 (control), 1, 2, and 4 g/kg bw per day, respectively.

In females, there was a non-significant increase in the incidence of bronchioloalveolar adenoma – 0%, 1/60 (1.7%), 3/60 (5%), 3/60 (5%) – that approached statistical significance. There were no differences in the incidence of pituitary tumours in female rats across all groups. There were non-significant increases in the incidence of benign pheochromocytoma in females – 1/60 (1.7%), 1/60 (1.7%), 6/59 (10.2%), 2/60 (3.3%) at 0 (control), 1, 2, and 4 g/kg bw per day, respectively.

There were single instances of malignant pheochromocytoma in the groups at 2 and 4 g/kg bw per day.

Regarding the non-neoplastic lesions, renal pelvis mineralization was observed in males and females. Mineralization in the renal pelvis did not correlate with transitional cell hyperplasia in the kidney in either male or female rats (Shibui et al., 2019). [The Working Group noted that the study by Ishii (1981), also reported by Ishii et al. (1981), used both sexes, multiple doses, an adequate number of animals per group, and an adequate duration of exposure and observation. However, it was a pre-GLP study, although aligned to OECD TG451 (OECD, 2018). It was limited by the lack of information on purity and the sparse details on histopathology in the original study. These data showed increases in the incidence of bronchioloalveolar tumours that approached statistical significance in female rats and non-significant increases in the incidence of benign pheochromocytoma in male and female rats, suggesting a potential effect of chronic exposure to aspartame.]

The first studies on aspartame (SC-18862 compound) were completed in 1973 (EFSA_E33, 2011; EFSA_E34, 2011; EFSA_E87, 2011) and 1974 (EFSA_E70, 2011).

In the 1973 study (EFSA_E34, 2011; also reported by EFSA_E33, 2011; and EFSA_E87, 2011), groups of 40 male and 40 female Charles River Albino rats (60 males and 60 females for the control group) were treated with feed containing aspartame [SC-18862 compound, purity not reported; impurities, 0–1.5% of conversion product SC-191912] at a dose of 0 (control), 1, 2, 4, or 8 g/kg bw per day for 104 weeks. The overall survival was around 50% in both male and female rats but significantly lower (25%) in females at 8 g/kg bw per day. Body-weight gains were significantly lower in the groups of males and females at 8 g/kg bw per day than in the controls, owing to lower feed consumption. Relative kidney weights were increased in male

rats at 2, 4, and 8 mg/kg bw per day, and relative uterus weights were increased in female rats at 2 and 4 mg/kg bw per day, but there were no histological correlates.

There were no significant differences in the incidence of grossly detected tumours in controls and all the exposed dose groups in males and females. Histopathology examinations were performed on all gross lesions identified at necropsy, as well as on 20–25 tissues in the control group and groups at 4 and 8 g/kg bw per day and about 25% of the animals in the groups at 1 and 2 mg/kg bw per day. There was a non-significant increase in the incidence of glioma (astrocytoma, oligodendroglioma, glioma not otherwise specified) in the treated groups of males and females (EFSA_E87, 2011). There were two ependymomas in male rats at 4 g/kg bw per day and one ependymoma and one meningeal sarcoma in female rats at 4 g/kg and 8 g/kg bw per day, respectively. There were no other major differences in tumour incidence between controls and all the exposed groups.

Regarding non-neoplastic lesions, renal pelvis epithelial hyperplasia was observed in males. Nodular hyperplasia in the pancreas, a non-neoplastic proliferative lesion, was observed in female rats. [The Working Group noted that this study used both sexes, multiple doses, and an adequate duration of exposure and observation. However, this was a pre-GLP study, and was limited by the lack of information on purity, and an infection (murine pneumonia) observed in controls and treated animals.]

3.2.2 Transplacental and perinatal administration followed by oral administration (feed)

In a study published by Soffritti et al. (2007), also reported by Chiozzotto et al. (2011), groups of 70–95 male and 70–95 female Sprague-Dawley rats were treated with feed containing aspartame (purity, > 98.7%; impurities, diketopiperazine

< 0.3%, and L-phenylalanine < 0.5%) for pregnant dams starting at day 12 of gestation and continuing in the pups until their natural death. This study focused on human-relevant aspartame dosage levels at 0, 400, and 2000 ppm that simulated a daily intake of 0, 20, and 100 mg/kg bw. At age 4–5 weeks, the pups were assigned to dose groups that reflected the exposure of their respective dams. The Sprague-Dawley rats were obtained from the breeding colony maintained at the Ramazzini Institute. The exposure lasted until the natural death of the animals, and all moribund or found dead animals were promptly necropsied for gross and microscopic evaluation of an extensive list of tissues identified in the study protocol. The in-life phase of the study lasted until 147 weeks. There appeared to a slight reduction (significance not reported) in survival in dosed groups compared with controls. There were no significant differences in water consumption, in feed consumption, and in body-weight gains (Soffritti et al., 2007; also reported by Chiozzotto et al., 2011).

In male rats, there was a significant positive trend ($P \leq 0.01$, Cox regression model) in the incidence of animals bearing malignant tumours, with the incidence – 23/95 (24.2%), 18/70 (25.7%), 28/70 (40%), for 0 (control), 400 and 2000 ppm, respectively – being significantly increased ($P \leq 0.01$, Cox regression model; [$P = 0.0231$, Fisher exact test]) at 2000 ppm. There was an increase in the incidence of lymphoma/leukaemia (combined) – 9/95 (9.5%), 11/70 (15.7%), 12/70 (17.1%), at 0 (control), 400, and 2000 ppm, respectively – with the incidence being significant ($P \leq 0.05$, Cox regression model, [$P = 0.003$, Fisher exact test]) at 2000 ppm, and these increases were within the upper bound of the range observed in historical controls (2265 males, 20.6%; range, 8–30.9%) from this laboratory (Soffritti et al., 2005, 2006). [The Working Group noted that lung lymphomas (primary lymphomas presenting in the lung, with isolated or dominant lung involvement) are rare in all species, including rats,

and notoriously difficult to differentiate from inflammatory conditions. In the present studies, however, this presentation and diagnosis was a predominant finding. The results of the subsequent study (Tibaldi et al., 2020) were insufficient to differentiate between inflammatory or neoplastic proliferations, since the immunohistochemical markers used were not appropriate for this purpose and the staining was of insufficient technical quality, on the basis of the figures provided. Additional supportive evidence for neoplasia using flow cytometry or molecular techniques such as polymerase chain reaction or Southern blot were not provided. Therefore, these diagnoses remain largely unconfirmed. The Working Group noted that the review of slides in this study confirmed the diagnoses in 72 out of 78 cases and revised the diagnoses from malignant to benign in 8% of evaluable cases.] No significant difference was observed in the incidence of renal pelvis papilloma and mammary gland carcinoma compared with concurrent controls.

In female rats, there was a significant positive trend ($P \leq 0.01$, Cox regression model) in the incidence of lymphoma/leukaemia (combined), with the incidence – 12/95 (12.6%), 12/70 (17.1%), 22/70 (31.4%), at 0 (control), 400, and 2000 ppm, respectively – being significantly increased at the highest dose ($P \leq 0.01$, Cox regression model; [$P = 0.0030$, Fisher exact test]), and exceeding the upper bound of the range in historical controls at the highest dose (2274 females, 13.3%; range, 4.0–25%) from this laboratory (Soffritti et al., 2005, 2006). There was a significant positive trend ($P \leq 0.05$, Cox regression model) in the incidence of carcinoma of the mammary gland, with the incidence – 5/95 (5.3%), 5/70 (7.1%), 11/70 (15.7%), at 0 (control), 400, and 2000 ppm, respectively – being significantly increased at the highest dose ($P \leq 0.05$, Cox regression model; [$P = 0.0245$, Fisher exact test]), and exceeding the upper bound of the range in historical controls at this dose (2274 females, 9.2%; range, 4.0–14.2%) from this laboratory (Soffritti et al., 2005, 2006).

The incidence of renal pelvis papilloma was significantly increased [$P = 0.0051$, Fisher exact test] at the lowest dose – 0/95, 6/70 (8.6%), 0/70, at 0 (control), 400, and 2000 ppm, respectively (Soffritti et al., 2007; also reported by Chiozzotto et al., 2011). There was a significant increase in the incidence of uterus polyps and of head osteoma in the group at 400 ppm (Soffritti et al., 2007; also reported by Chiozzotto et al., 2011).

Regarding non-neoplastic lesions, no data were available to the Working Group.

[The Working Group noted that this study used both sexes, a low dose relevant to human exposure, a high number of animals per group, and an adequate duration of exposure and observation (prenatal and lifetime exposure). The study was not conducted under GLP. The Working Group expressed concerns regarding the lack of adjustment for potential litter effects (e.g. there was no information regarding the number of litters per treatment group, the number of pups per litter per treatment group, or whether the treatments were loaded in a balance fashion), which could lead to false-positive results. A minority of the Working Group considered that these concerns might have been overcome because the large sample size per group ($n = 70$–150), significant dose–response trend, and pairwise testing of multiple tumour types suggested the absence of an potential bias due to litter effects. The diagnostic criteria were adequate for myeloid and lymphoid tumours and leukaemia, with the exception of immunoblastic lymphoma of the lung, for which the differentiation between reactive hyperplasia and neoplasia has been recognized as challenging by the NTP, the Ramazzini Institute, and other pathologists (Malarkey and Bucher, 2011; Tibaldi et al., 2020).]

Subsequently, all the haematopoietic and lymphoid tissue tumours from the prenatal exposure study (Soffritti et al., 2007) were re-evaluated according to updated pathological criteria and with the use of immunohistochemistry (Tibaldi et al., 2020). The analysis confirmed the

tumour diagnoses in 72 cases out of 78, identified 3 cases of lymphoid hyperplasia and categorized 3 cases as inflammatory lesions. A statistically significant increase in the incidence of total haematopoietic and lymphoid tissue tumours ($P = 0.006$, Fisher exact test), total lymphomas ($P = 0.032$, Fisher exact test), and total leukaemia ($P = 0.031$, Fisher exact test) in treated female rats was confirmed (high dose versus controls), and a statistically significant linear trend for each haematolymphoid tumour type was also observed. [The Working Group noted that the immunohistochemistry study by Tibaldi et al. (2020) provided immunophenotypical characterization of the constituent cell populations in the proliferative haematolymphoid lesions, but this information did not provide definitive data to support or refute whether a haematolymphoid proliferative lesion in the lung was neoplastic or reactive. The Working Group agreed that determining clonality, for establishing the neoplastic nature of haematolymphoid proliferative lesions in the lung, was beyond the scope of routine rodent carcinogenicity assays.]

The statistical reanalysis performed by Gnudi et al. (2023) reported positive significant trends in females: total lymphoid tumours ($P = 0.0368$, Cochran–Armitage trend test), myeloid leukaemia ($P = 0.0324$, Cochran–Armitage trend test), total myeloid tumours ($P = 0.0485$, Cochran–Armitage trend test), and lymphoma all types ($P = 0.0368$, Cochran–Armitage trend test). Furthermore, statistically significant increases in the incidence of total lymphoid tumours ($P = 0.027$, Fisher exact test), and leukaemia all types ($P = 0.007$, Fisher exact test) were observed in females at 2000 ppm females compared with their respective controls. The constituent lesions comprising the lymphoma or leukaemia (combined) diagnosis included various lymphoid tumours (lymphoblastic lymphoma, lymphocytic lymphoma, lymphoimmunoblastic lymphoma) and myeloid tumours (histiocytic sarcoma, monocytic leukaemia, myeloid leukae-

mia). [The Working Group acknowledged that revised histological diagnoses of the Tibaldi et al. (2020) study were retained for this analysis. The diagnostic criteria were adequate for myeloid and lymphoid tumours and leukaemia, with the exception of immunoblastic lymphoma of the lung, for which the differentiation between reactive hyperplasia and neoplasia has been recognized as challenging by the NTP, the Ramazzini Institute, and pathologists from other institutes (Malarkey and Bucher, 2011; Tibaldi et al., 2020).]

The 1974 study (EFSA_E70, 2011; also reported by EFSA_E87, 2011) included prenatal exposure to aspartame (SC-18862 compound; impurities, conversion product SC-191912, 0.4–1.5%) of at a dose of 0 (control), 2, or 4 g/kg bw per day (60 days before mating) and subsequent exposure for 104 weeks through maternal milk and through feed after weaning in male and female Charles River Albino rats per day. The controls included 60 males and 60 females, and the treatment groups included 40 males and 40 females. Compared with controls, body-weight gains were significantly lower in males at 4 g/kg bw and were related to lower feed consumption in this group. Survival was around 50% in both the control and exposed groups. Histopathology was performed on all gross lesions, as well as on 20–25 tissues in the control and treated groups.

The incidence of astrocytoma was 3/60 (5%), 1/40 (2.5%), 1/40 (2.5%) in male rats and 1/60 (1.7%), 1/40 (2.5%) and 0/40 in female rats at 0 (control), 2, and 4 g/kg bw, respectively (EFSA_E87, 2011). There was one ependymoma in male rats at 2 g/kg bw and one meningioma in female rats at 4 g/kg bw. No significant differences in tumour incidence were observed between control and treated groups.

Regarding non-neoplastic lesions, adrenal nodular hyperplasia was observed in males, and liver hyperplastic nodules were observed in females. [The Working Group noted that this study used both sexes, multiple doses, and an adequate duration of exposure and observation.

However, there was no adjustment for litter effects and the study was limited by low survival, and an infection (murine pneumonia) observed in controls and treated animals. In addition, this study was performed in a pre-GLP era, and the pathology diagnoses and criteria may not satisfy current practices.]

3.2.3 Urinary bladder tumour initiation with N-*butyl*-N-*(4-hydroxybutyl) nitrosamine and promotion by aspartame*

In the study by Hagiwara et al. (1984), also reported by Ito et al. (1984), groups of 30 male F344/DuCrj rats (age, 6 weeks) were treated with drinking-water containing 0.01% *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN) for 4 weeks followed by feed containing 5% aspartame for 32 weeks, to determine the tumour promotion effect of aspartame. [No information on the purity of BBN or aspartame was provided.] Controls received BBN alone for 4 weeks or aspartame alone for 32 weeks. At necropsy, urinary bladders were examined for evidence of proliferative urothelial lesions such as papillary or nodular hyperplasia, papilloma, or carcinoma.

There was no evidence of increased proliferative urothelial lesions that could be attributed to aspartame exposure. [The Working Group noted that the study by Hagiwara et al. (1984) used an adequate number of animals per group. It was limited by the use of a single sex and a single dose, pathology examinations on one tissue only, the lack of information on purity, and the inadequate duration of exposure and observation for this initiation–promotion model. This study was judged inadequate for the evaluation of the carcinogenicity of aspartame in experimental animals.]

3.3 Hamster

See Table 3.3.

Oral administration (diet)

Groups of 35 male and 35 female Syrian Golden hamsters (weanling; age, 21 days), housed individually, were treated with feed containing aspartame (purity not reported; diketopiperazine, < 1%) at a dose of 1, 2, 4, or 12 g/kg bw per day for 46 weeks (EFSA_E27, 2011; EFSA_E35, 2011; EFSA_E36, 2011). The control group (aspartame, 0 g/kg bw per day) contained 70 males and 70 females. Although the bioassay was scheduled to last 104 weeks, it was terminated after 46 weeks because of extensive mortality (males, 57–77%; females, 69–80%). The deaths occurred across all treatment groups, were not related to aspartame exposure, and were attributed to an unidentified infection, possibly "wet tail" syndrome, a condition to which hamsters are quite susceptible. Body weights were typically within 10% of that of the control group for both males and females. Food consumption did not appear to be affected by treatment with aspartame. The measured consumption of aspartame was 1.1, 2.4, 4.9, and 8.4 g/kg bw per day in males and 1.2, 2.5, 4.4, and 8.1 g/kg bw per day in females. The dietary intake of aspartame in the highest dose group was less than the target of 12 g/kg bw per day in both males and females because the hamsters were fed aspartame at 8 g/kg bw per day during weeks 1–14, 8 g/kg bw per day during weeks 15–18, 10 g/kg bw per day during weeks 19–22, and 12 g/kg bw per day from week 23 onward. Gross and microscopic examinations were conducted on all dead and surviving hamsters.

Dietary administration of aspartame did not cause a significant increase in the incidence of any type of malignant neoplasm in hamsters removed before 46 weeks. A low incidence of adrenal cortex adenoma, which was not related to

Table 3.3 Studies of carcinogenicity with aspartame in hamsters and dogs

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Hamster, Syrian golden (M) Weanling (age, 21 days) 46 wk EFSA_E27 (2011)	Oral administration (feed) Purity, NR (< 1% diketopiperazine) Feed 0, 1.1, 2.4, 4.9, 8.4 g/kg bw per day 70, 35, 35, 35, 35 24, 15, 13, 14, 8	*Adrenal cortex* Adenoma 1/22, 0/8, 0/13, 1/13, 1/15	NS	*Principal strengths*: appropriate experimental design, including pathological analysis; sufficient number of animals per group, randomly allocated. *Principal limitations*: extensive mortality due to infection; insufficient duration of exposure; stability and homogeneity of aspartame in the diet not measured; uncertainty regarding purity. *Other comments*: incidence of adrenal cortex adenoma was based upon surviving animals.
Full carcinogenicity Hamster, Syrian golden (F) Weanling (age, 21 days) 46 wk EFSA_E27 (2011)	Oral administration (feed) Purity, NR (< 1% diketopiperazine) Feed 0, 1.2, 2.5, 4.4, 8.1 g/kg bw per day 70, 35, 35, 35, 35 14, 7, 6, 11, 11	No significant increase in tumour incidence in treated animals		*Principal strengths*: appropriate experimental design, including pathological analysis; sufficient number of animals per group, randomly allocated. *Principal limitations*: extensive mortality due to infection; insufficient duration of exposure; stability and homogeneity of aspartame in the diet not measured; uncertainty regarding purity.
Full carcinogenicity Dog, Beagle (M) 150–160 days 106 wk EFSA_E28 (2011)	Oral administration (feed) Purity, NR (0.1–1% diketopiperazine) from week 20 onwards Feed 0, 1, 2, 4 mg/kg bw per day 5, 5, 5, 5 5, 5, 5, 5	No significant increase in tumour incidence in treated animals		*Principal strengths*: appropriate pathological analyses. *Principal limitations*: limited number of animals per dose group; insufficient duration of exposure; uncertainty regarding purity, from week 20 until the end of week 106 of the feeding period, the test material contained 0.1–1% diketopiperazine (by weight).
Full carcinogenicity Dog, Beagle (F) 150–160 days 106 wk EFSA_E28 (2011)	Oral administration (feed) Purity, NR (0.1–1% diketopiperazine) from week 20 onwards Feed 0, 1, 2, 4 mg/kg bw per day 5, 5, 5, 5 5, 5, 5, 5	No significant increase in tumour incidence in treated animals		*Principal strengths*: appropriate pathological analyses. *Principal limitations*: limited number of animals per dose group; insufficient duration of exposure; uncertainty regarding purity, from week 20 until the end of week 106 of the feeding period, the test material contained 0.1–1% diketopiperazine (by weight).

approx., approximately; bw, body weight; F, female; M, male; NR, not reported; NS, not significant; ppm, parts per million.

treatment, was found in surviving male hamsters (EFSA_E27, 2011; EFSA_E35, 2011; EFSA_E36, 2011).

[The Working Group noted that this study used an adequate number of animals per group (randomly allocated) and an appropriate experimental design, including pathological analysis. However, the study was limited by the lack of information on purity, the lack of measurement of the stability and homogeneity of aspartame in the diet, and the extensive mortality due to infection. The Working Group also noted that the 46-week study period was insufficient for tumour induction on the basis of the lifespan of a hamster, which is 2–3 years.]

3.4 Dog

Oral administration (diet)

Groups of five male and five female Beagle dogs (age, 150–160 days), randomized and housed individually, were treated with feed containing aspartame at a dose of 0, 1, 2, or 4 g/kg bw per day for 106 weeks. Although the purity of the aspartame was not indicated, the test material contained 0.1–1.0% diketopiperazine (by weight) from week 20 until the end of the 106-week feeding period (EFSA_E28, 2011; EFSA_E86, 2011). In males, the geometric mean body weight in the group at 2 g/kg bw per day from approximately week 14 was ≤ 90% that of the control group; by week 106, it was 79% that of the control group. The body weight of males in the group at 4 g/kg bw per day was 90% (geometric mean) that of males in the control group from week 88 onward. The geometric mean body weight of treated females was ≥ 90% that of the control group. At the end of the 106-week feeding period, the dogs were killed and a complete macroscopic and microscopic (histological) evaluation was conducted. Subsequently, selected sections from the brain were subjected to an additional histopathological evaluation with an emphasis on

ependymal neoplasms because brain ependymal glial proliferation was observed in two animals at 2 g/kg bw per day.

Dietary administration of aspartame did not cause a significant increase in the incidence of any type of neoplasm in this study (EFSA_E28, 2011; EFSA_E86, 2011). [The Working Group noted that this study was limited by the number of dogs used, the lack of information on purity, and the lack of measurement of the stability and homogeneity of aspartame in the diet. The Working Group also noted that the 106-week study period represents < 20% of the lifespan of a Beagle dog and that the induction of tumours in bioassays in Beagle dogs typically takes more than 3 years. Based on the limitations noted, the Working Group considered that the absence of tumour induction was insufficient to assess the carcinogenicity of aspartame.]

3.5 Evidence synthesis for cancer in experimental animals

There were numerous studies available to the Working Group regarding cancer in multiple species (mouse, rat, dog, and hamster). The carcinogenicity of aspartame was assessed in well-conducted GLP studies on oral administration (feed) in male and female transgenic mice in three models: a FVB/N-TgN(v-Ha-*ras*) Led (Tg.AC) hemizygous mouse model (NTP, 2005); a B6.129-*Trp53*^tm1Brd (N5) haploinsufficient mouse model (NTP, 2005); and a B6.129-*Cdkn2a*^tm1Rdp (N2) deficient mouse model (NTP, 2005). No significant increase in the incidence of tumours was observed. The NTP concluded and the Working Group noted that these were new transgenic mouse models, and the duration of exposure may not have been sufficiently sensitive to detect a carcinogenic effect after chronic exposure to aspartame.

Additional studies included oral administration (feed) in male and female HAM-ICR Swiss mice (EFSA_E75, 2011), in male and female SLC Wistar rats (Ishii, 1981, also reported by Ishii et al., 1981, and reanalysed histologically by Shibui et al., 2019), in male and female Charles River Albino rats (EFSA_E34, 2011; also reported by EFSA_E33, 2011; and EFSA_E87, 2011), and in male and female Charles River Albino rats (EFSA_E70, 2011; also reported by EFSA_E87, 2011); in utero exposure followed by oral administration (drinking-water) in C57BL/6 Ela1-Tag mice (Dooley et al., 2017); and intravesical pellet implantation in female Swiss albino mice (EFSA_E58, 2011; EFSA_E72, 2011 both reported by Bryan, 1984). In addition, initiation–promotion studies were conducted in male Fischer 344 rats (Hagiwara et al., 1984; also reported by Ito et al., 1984).

The Working Group noted that the studies with negative results in mice (EFSA_E75, 2011), rats (EFSA_E34, 2011, and EFSA_E70, 2011); hamsters (EFSA_E27, 2011), and dogs (EFSA_E28, 2011) and by Ishii et al. (1981) were conducted before the advent of GLP guidelines and had some limitations, e.g. lack of information on test substance purity and selective histopathology. Studies on in utero exposure followed by oral administration (drinking-water) in C57BL/6 Ela1-Tag mice (Dooley et al., 2017), by intravesical pellet implantation in female Swiss albino mice (EFSA_E58, 2011; EFSA_E72, 2011; both reported by Bryan, 1984), and the one initiation–promotion study in male Fischer 344 rats (Hagiwara et al., 1984; also reported by Ito et al., 1984) were judged to be inadequate for the evaluation of the carcinogenicity of aspartame in experimental animals.

The carcinogenicity of aspartame has been also assessed by other routes of exposure in studies that did not comply with GLP. Specifically, there were studies on oral administration (feed) in male and female Sprague-Dawley rats (Soffritti et al., 2005, 2006; also reported by Belpoggi et al., 2006; statistical reanalysis by Gnudi et al., 2023) and on transplacental and perinatal exposure followed by oral administration (feed) in male and female Swiss mice (Soffritti et al., 2010; statistical reanalysis by Gnudi et al., 2023) and in male and female Sprague-Dawley rats (Soffritti et al., 2007; reanalysis by Tibaldi et al., 2020, and Gnudi et al., 2023; also reported by Chiozzotto et al., 2011).

During the review of the set of studies conducted by the Ramazzini Institute, Italy (Soffritti et al., 2005, 2006, 2007, 2010, and subsequent reanalyses), the Working Group raised concerns regarding diagnoses of lymphomas located predominantly, but not exclusively, in the lung. Therefore, the Working Group focused its evaluation of the potential carcinogenicity of aspartame in experimental animals on all neoplastic lesions (all solid tumours, myeloid tumours, and leukaemia) except lymphoid tumours and related combinations.

In a study on transplacental and perinatal followed by oral administration (feed) in male and female Swiss mice (Soffritti et al., 2010), there was a significant positive trend in the incidence of hepatocellular carcinoma in males, with the incidence being significantly increased at all doses except the lowest (2000 ppm). There was a significant increase in the incidence of hepatocellular adenoma or carcinoma (combined) at the higher intermediate dose (16 000 ppm) in males. There was a significant positive trend in the incidence of bronchioloalveolar carcinoma in males, with the incidence being significantly increased at the highest dose. There was a significant positive trend in the incidence of bronchioloalveolar adenoma or carcinoma (combined) in males, with incidence being significantly increased at the highest dose. In female mice, dietary administration of aspartame did not cause a significant increase in the incidence of any type of neoplasm (Soffritti et al., 2010). The reanalysis by Gnudi et al. (2023) reported a significant positive trend for leukaemia (all types), with incidence being

significantly increased at all doses in males. Also in males, there was a significant increase in the incidence of lymphoblastic leukaemia at the highest three doses, and a significant increase in the incidence of monocytic leukaemia and total myeloid tumours at the higher intermediate dose (16 000 ppm). In females, there was a significant increase in the incidence of lymphoblastic leukaemia and leukaemia (all types) at the lowest dose.

In the study on oral administration (feed) in male and female Sprague-Dawley rats (Soffritti et al., 2005, 2006; also reported by Belpoggi et al., 2006), there was an overall increase in the incidence of rats bearing malignant tumours, with a significant positive trend for malignant schwannoma of the peripheral nerve, a rare type of tumour, in males. In females, there was an overall increase in the incidence of rats bearing malignant tumours, with a significant positive trend. There was a significant positive trend in the incidence of dysplastic papilloma of the renal pelvis and ureter, a rare type of tumour. The incidence of carcinoma of the renal pelvis and ureter was significantly increased at the highest dose. There was a significant positive trend in the incidence of papilloma and carcinoma (combined) of the renal pelvis and ureter, with incidence being significantly increased at the highest dose. There was a significant increase in the incidence of mammary gland carcinoma in the highest intermediate dose group (50 000 ppm). In addition, one transitional cell carcinoma of the urinary bladder, a rare type of tumour, was reported in female rats at 2000 ppm. The reanalysis by Gnudi et al. (2023) reported a significant positive trend for myeloid leukaemia and total myeloid tumours in females. There was a significant increase in the incidence of monocytic leukaemia at 400 and 50 000 ppm, histiocytic sarcoma at 10 000 and 50 000 ppm, and total myeloid tumours at all doses except the lowest (80 ppm).

In the study on transplacental and perinatal exposure followed by oral administration (feed)

in male and female Sprague-Dawley rats (Soffritti et al., 2007; also reported by Chiozzotto et al., 2011), there was a significant positive trend in the incidence of carcinoma of the mammary gland in females, with the incidence being significantly increased at the highest dose (2000 ppm). The incidence of renal pelvis papilloma was significantly increased at the lowest dose (400 ppm). A reanalysis of these data by Gnudi et al. (2023) reported positive significant trends in incidence for leukaemia (all types), myeloid leukaemia, and total myeloid tumours in females. There was a significant increase in the incidence of leukaemia (all types) at 2000 ppm.

Overall, the Working Group considered that there were unresolved questions about the adequacy of the design, conduct, interpretation, and/or reporting of the available studies (Soffritti et al., 2006, 2007, 2010, and subsequent reanalyses). For example, no adjustments were made for litter effects, which can lead to false-positive results for incidence and trend. A minority of the Working Group considered that these limitations were minor and would not affect the overall interpretation of these studies.

4. Mechanistic Evidence

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

The absorption, distribution, metabolism, and excretion of aspartame (Chemical Abstracts Service, CAS No. 22839-47-0; α-aspartame; L-α-aspartyl-L-phenylalanine methyl ester) have been intensively investigated in humans after dietary exposure.

Aspartame is normally hydrolysed in the gastrointestinal tract to its major constituents: aspartate, phenylalanine, and methanol (EFSA E15, 2011a) (Fig. 1.1). It has also been reported that

esterases and peptidases (e.g. aminopeptidase A) in the gastrointestinal tract yield equimolar amounts of the three constituents, as shown in microvillar membranes prepared from human duodenum, jejunum, and ileum in vitro. The jejunal section of the human intestinal microvillar membranes has been reported to be more than twice as active as the duodenal sections at hydrolysing aspartame (Hooper et al., 1994).

The three hydrolytes undergo absorption from the intestinal lumen and reach the systemic circulation, similarly to amino acids and methanol obtained from dietary sources (SteginK, 1987; Butchko et al., 2002; Magnuson et al., 2007). Alternatively, ester hydrolysis in the lumen may yield methanol and the dipeptide, aspartyl-phenylalanine, which is also absorbed into intestinal mucosal cells via a peptide transport mechanism and subsequently undergoes hydrolysis to aspartic acid and phenylalanine in the enterocyte (SteginK, 1984, 1987; Tobey and Heizer, 1986). The hydrolysis of aspartame has been considered efficient, with the three metabolites being absorbed, entering the bloodstream, distributed, and finally excreted through expired air, faeces, or urine, or entering the amino acid pool.

Several studies have failed to detect free aspartame or aspartyl-phenylalanine in the plasma (Ranney et al., 1976; Stegink et al., 1981a; Zhang et al., 2016). Ranney et al. reported that between 10–24% of ^{14}C from radiolabelled aspartame was expired in 24 hours, with 0.7–11% being excreted in the faeces or urine and 10% incorporated into the plasma amino acid pool (Ranney et al., 1976). Only one study, in rodents (Creppy et al., 1998), has measured absorption of aspartame as the intact compound (by high-performance liquid chromatography, HPLC) (see Section 4.1.2).

More recently, in humans, Zhang et al. (2016) used highly sensitive HPLC coupled with isotope dilution tandem mass spectrometry to quantify aspartame and some other artificial sweeteners (acesulfame-K, saccharin, cyclamate) in paired samples of blood and urine from healthy adults and in liver samples from liver cancer patients. Aspartame was not found in any analysed sample, although acesulfame-K, saccharin, and cyclamate were reported to be within the limits of quantification (LOQs) at 0.001–0.01 ng/mL in urine, 0.01–0.1 ng/mL in blood, and 0.01–0.1 ng/g in liver tissue. [The Working Group noted that the specific LOQs for each analyte were not reported.] In contrast, Liu et al. (2022a) recently reported the detection of aspartame in serum samples from 218 pregnant women with and without gestational diabetes. Using ultra-performance liquid chromatography coupled with tandem mass spectrometry, they found serum aspartame levels in the range of 0.0459 to 0.0692 nmol/L (median, 0.0563 nmol/L [16.57 ng/L]), with no significant differences between the two groups. [The Working Group noted that the aspartame levels reported by Liu et al. (2022a) were several orders of magnitude lower than the reported increases in serum levels of aspartame-derived phenylalanine after aspartame intake; however, the Working Group expressed concerns about the quality of this study (see Section 1.4.3).]

Aspartame has not been detected in breast milk from lactating women (Stegink et al., 1979a; Sylvetsky et al., 2015). [The Working Group noted that in the studies from Stegink et al., aspartame was mainly provided in orange juice.]

After dosing with aspartame (34 mg/kg body weight (bw), in orange juice), absorbed phenylalanine was shown to enter the plasma pool of free amino acids from the portal blood, after partial conversion to tyrosine in the liver mediated by phenylalanine hydroxylase. Aspartic acid underwent transamination in the enterocyte, producing oxaloacetate, an intermediate in the citric acid cycle, thus decreasing the amount of aspartate entering the portal circulation (Filer and Stegink, 1989). Methanol does not undergo metabolism in the enterocyte but enters the portal circulation and is oxidized to formaldehyde by hepatic alcohol dehydrogenase.

Formaldehyde quickly undergoes formaldehyde dehydrogenase-mediated oxidation to formic acid ($t_{1/2}$ = 1–2 minutes) in the portal circulation (reviewed by Magnuson et al., 2007). Ultimately, formic acid has been reported to convert to carbon dioxide and water via formation of 10-formyl tetrahydrofolate (Barceloux et al., 2002). [The Working Group noted that methanol released from aspartame hydrolysis corresponds to 10% of aspartame by weight. It has been estimated that the overall exposure to methanol from aspartame at 40 mg/kg bw per day (the established acceptable daily intake, ADI) could range from 1% to 10% in the general population, with exposure from other food sources accounting for < 10% and exposure to endogenous pathways accounting for > 80% (EFSA, 2013). Although the Working Group also noted that formaldehyde has been classified by IARC as *carcinogenic to humans* (Group 1), no evidence was available as to whether exposure to formaldehyde at the levels arising from aspartame consumption would significantly alter normal endogenous formaldehyde concentrations.]

The metabolism of aspartame and of each of its components has been studied in healthy adults and infants, lactating women, adults who are heterozygous and homozygous for phenylketonuria, and in people with diabetes, high body mass index (BMI), or non-fatty liver disease. These studies have included a broad range of doses, single, repeated, or long-term dosing, and co-administration with meals (Stegink et al., 1982, 1983a, 1987b, 1991; Romano et al., 1989; Burns et al., 1991). There were no significant effects of aspartame on the plasma concentrations of any amino acids. The following paragraphs summarize the relevant information.

(a) Metabolism in healthy volunteers

In an early study from the 1970s by the manufacturer, for example, the absorption, distribution, and metabolism of aspartame were determined in three men given a single oral dose of 500 mg of [^{14}C-Phe]aspartame (18.7 μCi) in water (EFSA_E15, 2011a). As a result of rapid hydrolysis of aspartame in the gut, plasma radiolabel levels rose quickly; half-maximal levels were reached after about 15 minutes and peak levels after 4–8 hours after dosing. The elimination half-life from the plasma was relatively slow ($t_{1/2}$ = 47.5 hours), consistent with incorporation into body constituents; plasma radiolabel was detected in naturally occurring polar compounds that included proteins, peptides, and free amino acids (phenylalanine and tyrosine). Total urinary excretion of the radiolabel represented a very small fraction of the administered dose (0.46 ± 0.04%, measured 0–48 hours after dosing) and occurred primarily in the first 4 hours (EFSA_E15, 2011a).

Plasma or erythrocyte aspartate concentrations were not significantly affected by single doses of aspartame, except for slight increases in mean peak plasma concentrations at higher doses (Stegink et al., 1977, 1979a, b, 1987a, 1980, 1981a). In lactating women, a small, but statistically significant, difference in overall milk aspartate levels was noted 4 hours after dosing but did not persist over 24 hours (Stegink et al., 1979a). [The Working Group noted that aspartame was provided in orange juice, and a clear negative control was not used in the study.]

Plasma phenylalanine concentrations were not significantly increased after a single dose of aspartame (about 4 mg/kg bw) (Wolf-Novak et al., 1990), although there were brief significant increases at higher doses (10 mg/kg bw) (Stegink et al., 1979b, 1987a). Erythrocyte phenylalanine levels exhibited similar, but smaller changes (Stegink et al., 1979b). After very high doses of aspartame (100, 150, and 200 mg/kg bw), mean peak plasma phenylalanine concentrations were approximately proportional to the administered dose, and considerably higher than the normal postprandial range (Stegink et al., 1980, 1981a). Erythrocyte phenylalanine concentrations showed similar changes (Stegink et al., 1981a).

[The Working Group noted that the aspartame was provided in orange juice, and a clear negative control was not used in several of the above studies.]

Like aspartate in the same study, a small, but statistically significant, difference in overall milk phenylalanine levels was noted 4 hours after dosing but did not persist over 24 hours (Stegink et al., 1979a).

Regarding methanol metabolism, in men and women given a single dose of aspartame (34, 100, 150, or 200 mg/kg bw), blood methanol concentrations were below the limit of detection (0.4 mg/dL) at the lowest dose [the Working Group noted that currently available methods would have better sensitivity] but significantly increased after each higher dose, and mean peak blood methanol concentrations and blood methanol AUCs increased proportionally to dose (Stegink et al., 1981b). Concentrations returned to baseline by 24 hours after aspartame administration for all dosing levels (Stegink et al., 1981b). Formate analyses for participants at the highest dose showed no significant changes in blood formate concentration, but urinary formate excretion was significantly increased in urine samples taken 0–4 hours and 4–8 hours after aspartame ingestion, returning to baseline values by 8–24 hours after aspartame loading. [The Working Group noted that the urinary excretion data were consistent with the conversion of methanol to formate, with the rate of formate production not exceeding the rate of formate excretion. The Working Group further noted that the data suggest that the metabolism of methanol to formate and the renal excretion of formate are efficient.]

The effects of long-term dosing have been assessed in a 24-week randomized, double-blind, placebo-controlled, parallel-group study (Leon et al., 1989). After a large daily dose of aspartame (75 mg/kg bw), no persistent changes were noted in clinical and standard laboratory tests throughout the study. Over time, mean fasting plasma concentrations of aspartate and phenylalanine did not show any statistically significant differences from baseline. Likewise, plasma levels of all other amino acids did not indicate any consistent pattern of change in either group, except for tyrosine, for which plasma levels were slightly higher (although within the normal range) at weeks 3 and 24 in the aspartame group compared with controls ($P < 0.05$). Likewise, the ratio of plasma levels of phenylalanine to other large neutral amino acids (Phe/LNAA) did not change in either group over the course of the study. Most blood methanol concentrations were below the limit of detection (0.31 mmol/L) and there was no indication of aspartame-related accumulation of methanol. There were no significant differences in blood formate levels, in mean 24-hour concentrations of urinary formate, or in urinary formate to creatinine ratios, consistent with no significant increase in formate production after a long-term, high-dose aspartate regimen.

Aspartame metabolism in infants was reported to be generally similar to that in adults receiving aspartame at the same dose levels (Filer et al., 1983; Stegink et al., 1983b). In older people, the volume of distribution and clearance of phenylalanine were significantly decreased, but the elimination half-life ($t_{1/2}$) was not significantly different from that in younger adults (Puthrasingam et al., 1996).

(b) Metabolism in people with certain health conditions

(i) Phenylketonuria

Phenylketonuria (PKU) is an autosomal recessive disorder of phenylalanine metabolism characterized by mutations in the gene for hepatic phenylalanine hydroxylase that decrease or inactivate its function. Since this enzyme is necessary for the metabolism of phenylalanine to tyrosine, homozygous PKU subjects are at risk for build-up of dietary phenylalanine to potentially

toxic levels, ultimately leading to brain dysfunction (van Spronsen et al., 2021).

The ability to metabolize aspartame-derived phenylalanine has been studied in individuals who are PKU homozygous (e.g. EFSA_E26, 2011; Koch et al., 1976; Caballero et al., 1986) (on low phenylalanine diets or otherwise, although these individuals are not expected to ingest aspartame-sweetened products, which carry a warning label) and particularly in PKU heterozygous (e.g. single doses: Stegink et al., 1979b, 1980, 1981c, 1987a; Caballero et al., 1986; Filer and Stegink, 1989; Curtius et al., 1994; da Silva et al., 2000; EFSA_E108, 2011; EFSA_E109, 2011; repeated doses: Stegink et al., 1989, 1990; and with meals: EFSA_E25, 2011; EFSA_E67, 2011). In general, plasma phenylalanine levels and AUCs were significantly higher in the PKU heterozygotes than in the normal participants, and one study suggested that PKU heterozygotes metabolized aspartame-derived phenylalanine approximately twice as slowly as do normal individuals (Stegink et al., 1980; EFSA_E109, 2011). Plasma levels of aspartate remained within the normal range and no other significant differences were observed.

(ii) Diabetes, high BMI, and alcoholic liver disease

Aspartame metabolism has also been investigated in diabetic patients (Stern et al., 1976; EFSA_E65, 2011; Gupta et al., 1989), adolescents (Knopp et al., 1976) and adults (EFSA_E65, 2011) with a high BMI, and patients with alcoholic liver disease (Hertelendy et al., 1993; EFSA_UN09, 2011). Despite the different aspartame dosages and regimens used in the various settings, no significant effects were observed in the disposition of the three aspartame metabolites or in plasma phenylalanine and tyrosine levels.

(c) Absorption, distribution, metabolism, and excretion of impurities of aspartame

(i) Diketopiperazine

5-Benzyl-3,6-dioxo-2-piperazine acetic acid (or diketopiperazine, DKP; CAS No. 55102-13-1) is an impurity that may be found in commercial preparations of aspartame for use as a sweetener. DKP is formed via the intramolecular reaction of the primary amine with the methyl ester group of aspartame, which can be formed during the manufacturing process, resulting in the liberation of methanol (Lin and Cheng, 2000; EFSA, 2013) (Fig. 1.1). DKP content may increase substantially in aspartame-containing food and drinks during processing and storage, depending on the temperature and duration of storage, and is typically present at concentrations ranging from 0.1% to 4% (EFSA, 2013).

DKP is poorly absorbed from the gut and is found in the faeces, primarily as unchanged DKP. In vitro, DKP is not hydrolysed by human intestinal microvillar membrane preparations or by purified preparations of aminopeptidases A and W (Hooper et al., 1994). After oral administration, DKP is metabolized in the gastrointestinal tract to phenylacetic acid, which undergoes absorption and is then rapidly excreted in the urine, both as intact phenylacetic acid and as phenylacetylglutamine, a naturally occurring urinary metabolite, after conjugation with glutamate. In humans, the combined urinary excretion of phenylacetic acid and phenylacetylglutamine accounts for about 50% of orally administered DKP (EFSA, 2013).

Cho et al. (1987) measured plasma and urinary concentrations of DKP (by HPLC) in six adults who ingested a dose of 200 mg/kg bw of aspartame containing 1.1% DKP. Over 24 hours, DKP was detected in all collected samples of urine but was below the limit of detection (< 1 µg/mL) in all plasma samples. Mean total urinary DKP excreted over the 24 hours after dosing was 6.68 ± 1.30 mg (corresponding to 4.83 ± 0.23% of

the ingested DKP), and about 44% of total urinary excretion of DKP occurred in the first 4 hours after dosing (EFSA_E15, 2011b), consistent with the data reported above.

(ii)   β-Aspartame

β-Aspartame (β-ʟ-aspartyl-ʟ-phenylalanine methyl ester) (Fig. 1.1) is another aspartame impurity that is formed in small quantities during the manufacture and storage of aspartame-sweetened beverages. The pharmacokinetics and metabolism of β-aspartame (administered as [^{14}C-Phe]β-aspartame) in humans were studied in the late 1980s (EFSA, 2013). It was estimated that more than 90% of the administered radiolabel was absorbed: over 7 days, 9.6% of the administered dose was excreted in the faeces, parent radiolabelled β-aspartame was not detected in the plasma, and less than 0.15% was excreted unchanged in the urine. Hydrolysis of the methyl ester yielded the major metabolite [^{14}C]β-ʟ-aspartyl-ʟ-phenylalanine) (a normal constituent of human plasma and urine; Burton et al., 1989), which was detected in the plasma (t_{max} = 1.5 hours; $t_{1/2}$ = 1.1 hours) and in the urine, where it accounted for 7% of the administered radiolabel. In plasma, phenylacetylglutamine was another major metabolite, and free ^{14}C-labelled phenylalanine was a minor one. Urine was the major route of excretion of the radiolabel (42% of the administered dose), and phenylacetylglutamine was the major urinary metabolite (30.8% of the administered dose). The only metabolite recovered in the faeces was ^{14}C-labelled phenylalanine. No clinical effects associated with the administration of [^{14}C-Phe]β-aspartame were observed in the study participants.

4.1.2 Absorption, distribution, metabolism, and excretion in experimental systems

This section describes the available evidence on the absorption, distribution, metabolism, and excretion of aspartame in experimental systems. The disposition of aspartame and its metabolites in experimental systems was originally studied in rats, mice, rabbits, dogs, and monkeys in the early 1970s (Oppermann et al., 1973a, b; Ranney et al., 1976; EFSA_E15, 2011a; EFSA_E17, 2011; EFSA_E18, 2011; EFSA_E80, 2011). The available studies used various radio-labelled versions of aspartame (Fig. 4.1) and of its metabolites (i.e. methanol, phenylalanine methyl ester, aspartyl-phenylalanine, phenylalanine, and aspartic acid). As briefly mentioned in Section 4.1.1, evidence for the absorption of aspartame as intact compound was reported in one study from the late 1990s in rodents (Creppy et al., 1998). The authors reported that 10–12% of the administered dose of aspartame (25 mg/kg), as measured by HPLC, was absorbed as free aspartame and distributed to various tissues (kidney, 73 ± 6 μg/g; liver, 1.8 ± 0.1 μg/g; brain, 156 ± 9 μg/g; testis, 34 ± 2 μg/g; urine, 66 ± 5 μg/mL; and serum, 19.2 ± 2 μg/mL).

Many of the studies were conducted before the establishment of Good Laboratory Practice (GLP) and internationally recognized guidelines for testing. [The Working Group noted that although relatively minor deficiencies in reporting have been highlighted compared with standards established for modern GLP-compliant studies, independent reviews of the toxicokinetic studies on aspartame concluded that there were no significant issues concerning how the results were reported (EFSA_E91, 2011; Stanley, 2013).]

(a)   Non-human mammals in vivo

(i)   Kinetics of methanol

The metabolism of methanol derived from aspartame has been studied in rats, pigs, and monkeys. Based on the available evidence, methanol is completely cleaved from aspartame in the small intestine (Burgert et al., 1991) and rapidly excreted in expired air (EFSA_E15, 2011a). Low levels of methanol from aspartame are retained (EFSA_E15, 2011a). Davoli et al. (1986) showed

Fig. 4.1 Chemical structure of aspartame and diagram of the various radiolabelled compounds used in the toxicokinetic studies
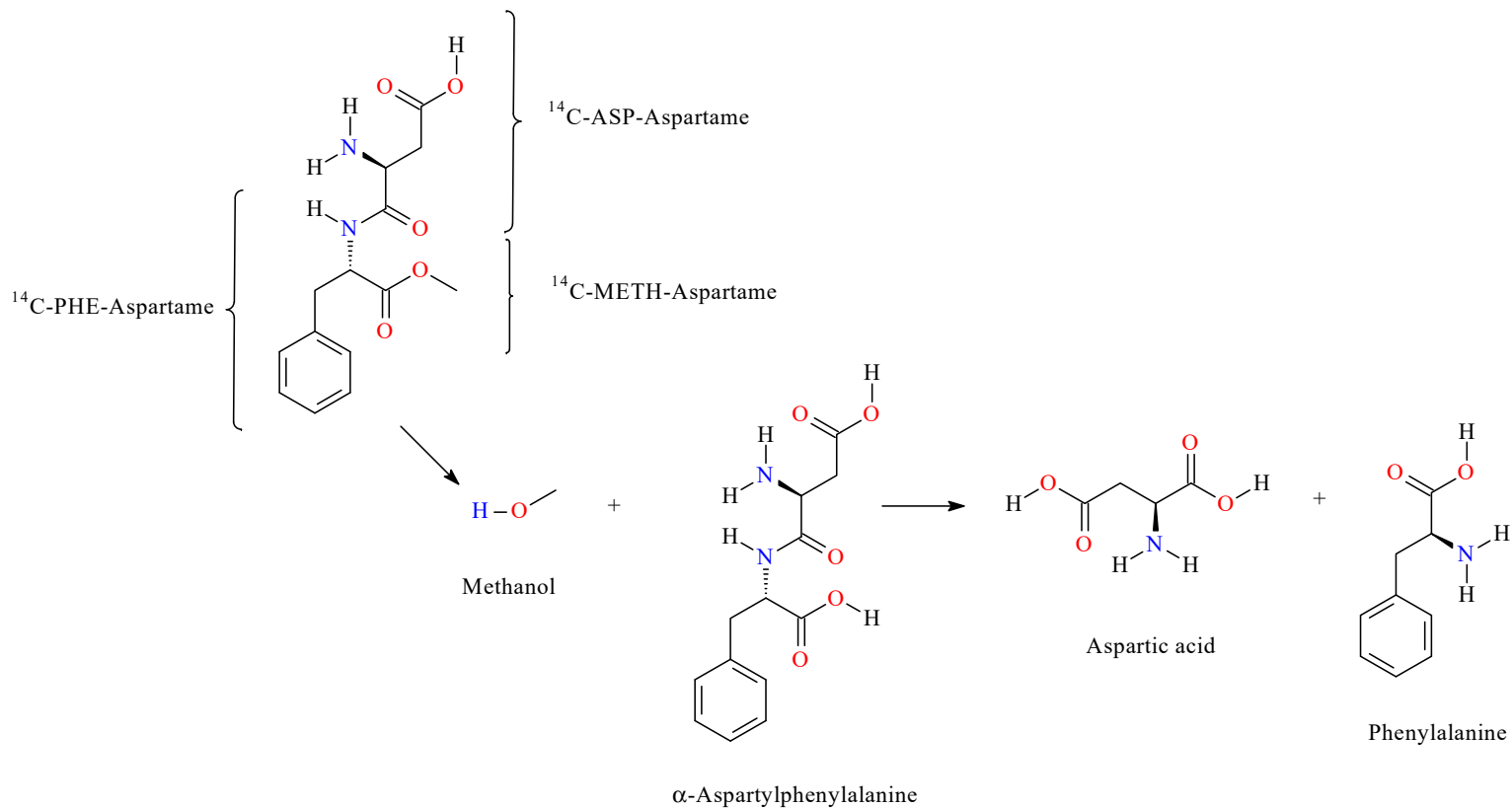
Structure of aspartame and its metabolites: radiolabelled methyl component of aspartame (^{14}C-METH-aspartame); radiolabelled aspartic acid component of aspartame (^{14}C-ASP-aspartame); radiolabelled phenylalanine component of aspartame (^{14}C-PHE-aspartame).
Created by the Working Group.

that a single dose of aspartame (34 mg/kg bw) causes a briefly increase in endogenous serum concentrations of methanol in rats, and Trocho et al. (1998) has suggested that methanol from aspartame is capable of binding with proteins and nucleic acids in the liver and blood, or of being incorporated into these molecules via the one-carbon metabolic pool.

When comparing the disposition of ^{14}C-METH-aspartame (aspartame labelled with ^{14}C in the methyl component) versus that of ^{14}C-methanol (oral exposure, 0.068 mmol/kg bw) in young female Rhesus monkeys (weight, 2–3 kg), the proportion of radiolabel in the cumulative expired air of monkeys exposed to ^{14}C-METH-aspartame (67.12% after 8 hours) was very similar to that in monkeys exposed to ^{14}C-methanol (73.02% after 8 hours; see Fig. 4.1 and Fig. 4.2). Plasma radioactivity in monkeys exposed to ^{14}C-METH-aspartame or ^{14}C-methanol was low, and the clearance of radioactivity was slow. The amount of radiolabel recovered from the urine 8 hours after dosing was low in both groups (^{14}C-METH-aspartame, 1.57%; ^{14}C-methanol, 3.17%). The amounts of radiolabel estimated to remain in the carcass of monkeys exposed to ^{14}C-METH-aspartame or ^{14}C-methanol were not significantly different ($P > 0.05$). No faeces samples were produced within the 8-hour observation interval (Oppermann et al., 1973a; EFSA_E15, 2011a).

(ii) Kinetics of aspartic acid
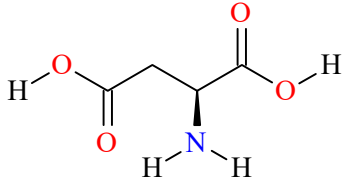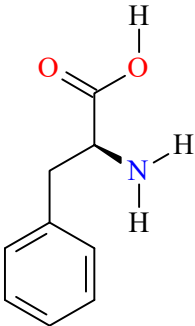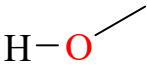
The metabolism of aspartic acid derived from aspartame has been studied in rats (Matsuzawa and O'Hara, 1984; EFSA_E15, 2011a), pigs (Burgert et al., 1991), and monkeys (Oppermann et al., 1973a; EFSA_E17, 2011). Based on the available evidence, the aspartame peptide bond is completely cleaved in the small intestine (Burgert et al., 1991). Some of the resulting aspartic acid enters cellular metabolism, but most is rapidly excreted in the expired air (EFSA_E15, 2011a; EFSA_E17, 2011).

Female Rhesus monkeys (*Macaca mulatta*) (four per group; weight, 5–7 kg) were pre-exposed to aspartame or aspartic acid (approximately 0.068 mmol/kg bw) mixed with peanut butter and spread on a quarter slice of bread for 5 days. On day 6, the monkeys were exposed to aspartame labelled on the aspartyl component, ^{14}C-ASP-aspartame, or ^{14}C-aspartic acid at approximately 0.068 mmol/kg bw by gavage, and expired air, plasma, urine, and faeces were collected and analysed for radioactivity. Most of the radiolabel was rapidly (peak, 1 hour) detected in expired air as CO_2 after exposure to either compound (after 12 hours: ^{14}C-ASP-aspartame, 77%; and ^{14}C-aspartic acid, 67%). The plasma radioactivity time curves appeared biphasic in animals exposed to ^{14}C-ASP-aspartame or ^{14}C-aspartic acid. Urinary and faecal analysis showed low radioactivity for both ^{14}C-ASP-aspartame and ^{14}C-aspartic acid (e.g. < 4 and < 2%, respectively) (Fig. 4.2). The authors noted that there appeared to be considerable individual variation in levels of radioactivity in the plasma and CO_2 after exposure to ^{14}C-ASP-aspartame and ^{14}C-aspartic acid (Oppermann et al., 1973a; EFSA_E17, 2011).

(iii) Kinetics of phenylalanine

The metabolism of phenylalanine derived from aspartame has been studied in mice (EFSA_E18, 2011; Hjelle et al., 1992), rats (Oppermann, 1984; EFSA_E15, 2011a; EFSA_E17, 2011; EFSA_E18, 2011; Hjelle et al., 1992; Romano et al., 1989, 1990; Reilly and Lajtha, 1995; Matsuzawa and O'Hara, 1984), rabbits (EFSA_E18, 2011; EFSA_E80, 2011), dogs (Oppermann, 1984; EFSA_E17, 2011), pigs (Burgert et al., 1991), and monkeys (Reynolds et al., 1980; Oppermann, 1984; Oppermann et al., 1973a, b; EFSA_E15, 2011a; EFSA_E17, 2011). On the basis of the available evidence, phenylalanine from aspartame is completely cleaved in the small intestine (Burgert et al., 1991) and primarily excreted in expired air (EFSA_E15, 2011a; EFSA_E17, 2011; EFSA_E18,

Fig. 4.2 Distribution of metabolites of aspartame

Metabolites	Expired air/breath (as CO_2)	Carcass	Excreta (urine)
Aspartate	77% (65%)	19% (30%)	4% (5%)
Phenylalanine	18% (18%)	78% (74%)	4% (8%)
Methanol	67% (73%)	31% (24%)	2% (3%)

Distribution of metabolites of aspartame (measured as a percentage of ^{14}C) in various body compartments in monkeys exposed to radiolabelled aspartame. Up to 78% of phenylalanine is retained in the body (carcass). Aspartate and methanol are mainly excreted as CO_2 in expired air or breath. The percentages in parentheses refer to the distribution of these metabolites when they are administered directly. The highest percentages of distribution are in red.
Data were extracted from Oppermann et al. (1973a) and EFSA_E15 (2011a).
Adapted from EFSA_E15 (2011a).

2011). However, a portion of the absorbed phenyl-alanine is incorporated into plasma proteins (e.g. Oppermann, 1984; EFSA_E17, 2011; EFSA_E80, 2011) and exhibits a half-life of about 25 hours in rats (EFSA_E15, 2011a).

The disposition of aspartame labelled on the phenyl component ^{14}C-PHE-aspartame or ^{14}C-phenylalanine in young female Rhesus monkeys (weight, 3–7 kg) was examined after exposure to an oral dose of approximately 0.068 mmol/kg bw (equivalent to ~20 mg/kg bw for ^{14}C-PHE-aspartame or ~11 mg/kg bw

for ^{14}C-phenylalanine). Animals exposed to ^{14}C-PHE-aspartame showed similar amounts of radiolabel total recovery in the expired air, urine, and faeces (i.e. 21.89%) compared with ^{14}C-phenylalanine exposed animals (26.56%), with most of the recovered radiolabel being detected as CO_2 in the expired air (e.g. 17.52% versus 18.00%). Detection of radioactivity in the plasma after exposure to ^{14}C-PHE-aspartame or ^{14}C-phenylalanine peaked at 5 hours, showed an apparent biphasic decay, and a levelling off 48 hours after exposure. On thin-layer chromatography, the majority (~95%) of radioactivity in the plasma of monkeys exposed orally to ^{14}C-PHE-aspartame was present at the origin (i.e. there was very little migration to the mobile phase) (Oppermann et al., 1973a; EFSA_E15, 2011a).

To determine whether repeated doses of aspartame had any effect on the normal metabolism of phenylalanine, a study was conducted in monkeys exposed to unlabelled aspartame or ^{14}C-phenylalanine (Oppermann et al., 1973b; EFSA_E17, 2011). Female Rhesus monkeys (*Macaca mulatta*) (three per dose; weight, 4.2–6.4 kg) were pre-exposed to unlabelled aspartame at 0, 15, or 60 mg/kg bw, with a mixture of peanut butter on a quarter slice of bread, for 10 days. On day 11, ^{14}C-phenylalanine at a dose of approximately 11 mg/kg bw (0.068 mmol/kg bw) was administered via intravenous injection. The disposition of the radiolabelled portion of ^{14}C-phenylalanine was determined in expired air and in the plasma. Additionally, the concentrations of phenylalanine and tyrosine in the plasma and the level of radiolabel incorporated into protein precipitate were determined. No significant differences in any of the parameters measured were noted between aspartame-exposed and non-exposed monkeys; however, a slightly lower amount of total radioactivity was observed in the plasma of control animals compared with aspartame-exposed animals. Also, small amounts of aspartame were detected in the faeces of Rhesus monkeys exposed to aspartame at 20 mg/kg (Oppermann et al., 1973a).

Reynolds et al. (1980) exposed groups of neonatal Rhesus monkeys (three species: *Macaca mulatta*, *M. fascicularis*, and *M. arctoides*) (age 1–22 days; weight, 280–820 g) to aspartame as a single dose at 2000 mg/kg bw by gavage (2 males; 6 females) or a combination of aspartame at 2000 mg/kg bw and monosodium glutamate at 1000 mg/kg bw (3 males; 3 females). A vehicle control group (water; 2 males; 3 females) and non-exposed control group (5 males; 1 female) were also investigated. Blood samples were taken at various time points between 4 and 5 hours after dosing, and concentrations of aspartic acid, phenylalanine, and tyrosine were determined using an automated amino acid analyser. After dosing with aspartame, the plasma concentrations of aspartic acid, phenylalanine, and tyrosine increased significantly. Maximum mean plasma concentrations of aspartic acid and phenylalanine in the neonatal monkeys were observed 60 and 90 minutes, respectively, after exposure to aspartame. Plasma concentrations of tyrosine in the neonatal monkeys steadily increased over the observation period, and the maximum mean plasma tyrosine concentration was observed at the last observation interval of 240 minutes. [The Working Group noted that there was considerable individual variation in the levels of each amino acid, which may have been due to the dose formulation (e.g. administration as a slurry not a solution). For example, Stegink et al. (1979c) showed that dosing as a slurry in humans produced more variable results than did dosing in solution.]

(iv) Effects on enzymes

Studies in rats suggest that equimolar doses of aspartame and phenylalanine show similar changes in plasma phenylalanine and phenylalanine hydroxylase activity (EFSA_E17, 2011; EFSA_E80, 2011). Aspartame exposure does not appear to significantly alter gastric juice

secretion, concentration of gastric acid, acid output, or proteolytic activity in rats (Bianchi et al., 1980).

Although no significant increase in cytochrome P450 (CYP) content or the activity of any of the enzymes tested was observed in rats exposed to aspartame at 0, 40 or 4000 mg/kg bw per day for 90 days, (Tutelyan et al., 1990), Vences-Mejía et al. (2006) showed increased levels of CYPs proteins (1A1, 1A2, 2B, 3A2) and enzymatic activities (ethoxyresorufin O-deethylase, methoxyresorufin O-demethylase, pentoxyresorufin O-dealkylase, benzyloxyresorufin O-debenzylase, 4-nitrophenol hydroxylase, and erythromycin-N-demethylase) in the cerebrum and cerebellum after aspartame exposure (75 or 125 mg/kg bw per day by gavage) for 30 days.

(b)   Non-human mammalian in vitro studies

Consistent with the in vivo data, in vitro incubation of aspartame with pepsin or dog gastric juice showed that pepsin and gastric juices had no effect on aspartame concentrations in the medium (EFSA_E15, 2011a). Bianchi et al. (1980) reported that aspartame did not affect the proteolytic activity of pepsin or the lipolytic activity of pancreatic lipase at concentrations of 143 μg/mL and 1.25 mg/mL, respectively, in vitro.

Hooper et al. (1994) reported that microvillar membranes from pig small intestines (duodenum) and kidney were able to rapidly hydrolyse aspartame in vitro.

Incubation of ^{14}C-METH-aspartame with rat plasma caused removal of the radiolabelled methyl group (EFSA_E15, 2011a). Incubation of ^{14}C-PHE-aspartame with dog plasma showed that less than 2.5% of the added radiolabel was incorporated into the high-molecular-weight fraction (EFSA_E17, 2011). This was in direct contrast to the results observed after in vivo exposure; for example, in dogs exposed orally to ^{14}C-PHE-aspartame at 20 mg/kg bw per day (0.068 mmol/kg), up to 70% of the radioactivity

was incorporated into the high-molecular-weight fraction of dog plasma within 5 hours (EFSA_E17, 2011).

The potential for nitrosamide formation after incubation of aspartame with nitrite was investigated. No nitrosamide formation occurred under simulated conditions of use or simulated physiological conditions (EFSA_E71, 2011).

(c)   In silico

Fatoki et al. (2020) used computer modelling to determine the binding affinity of aspartame to CYPs (1A2, 3A4, 2C9, 2C19, 2D6, 2E1) and P-glycoprotein; the results suggested that aspartame would not be substantially metabolized by the specified CYPs or transported by P-glycoprotein.

(d)   Absorption, distribution, metabolism, and excretion of impurities

The kinetics of DKP have been studied in rats, rabbits, and monkeys (Lipton et al., 1991; EFSA_E15, 2011b; EFSA_E18, 2011; EFSA_E80, 2011). Some DKP is metabolized by gut bacteria to phenylacetic acid, which is then excreted in the urine as phenylacetylglutamine. Unchanged DKP is also recovered in the urine and faeces. [The Working Group noted that no toxicity (including neoplasms) was reported when DKP was administered in combination with aspartame in rats (Ishii, 1981).]

The kinetics of β-aspartame have been studied in rabbits, dogs, and monkeys (using ^{14}C-β-aspartame). β-Aspartame is readily metabolized in the gut before absorption and no intact β-aspartame has been detected in the plasma. Once cleaved, the metabolites of β-aspartame are absorbed and primarily excreted in the urine as phenylacetylglutamine and β-L-aspartyl-L-phenylalanine (EFSA, 2013). [The Working Group noted that the primary studies (i.e. E170, E169, and E171) that formed the basis for the EFSA (2013) opinion regarding the

toxicokinetics of β-aspartame were not available to the Working Group for review.]

4.2 Evidence relevant to key characteristics of carcinogens

This section reviews the mechanistic data for the key characteristics of carcinogens (Smith et al., 2016) encompassed by aspartame. Evidence was available on whether aspartame exhibits the key characteristics "is electrophilic or can be metabolically activated to an electrophile", "is genotoxic", "induces oxidative stress", "induces chronic inflammation", "is immunosuppressive", and "modulates receptor-mediated effects", "causes immortalization", and "alters cell proliferation, cell death, or nutrient supply". Insufficient data were available for the evaluation of the other key characteristics of carcinogens. In addition, other relevant evidence on the potential effects of aspartame on insulin and glucose metabolism are reported in Section 4.3. Within each section, the most informative studies are described first. The exposure assessments for mechanistic studies in humans are reported in Table S1.3 (see Annex 1, Supplementary material for Section 1, Exposure Characterization, online only, available from: https://publications.iarc. who.int/627).

4.2.1 Is electrophilic or can be metabolically activated to an electrophile

(a) Humans

No data on DNA adducts or protein adducts were available to the Working Group.

(b) Experimental systems

(i) Non-human mammals in vivo

The only available study assessing the formation of adducts in vivo investigated the binding of formaldehyde derived from dietary aspartame to tissue components (Trocho et al., 1998). Adult male rats were exposed to aspartame radiolabelled with ^{14}C on the methanol carbon at an oral dose of 10 mg/kg. Most of the radioactivity detected (> 98% in the plasma, > 75% in the liver) was bound to protein. DNA radioactivity was essentially located on a single adduct base, not the usual bases present in DNA: after acid hydrolysis, the radiolabel incorporated into DNA migrated with a single unidentified spot near the origin in a two-dimensional thin-layer chromatogram system and not near the positions of the standards, adenine, guanosine, or thymine. [The Working Group noted that the authors described the spot as an unidentified DNA base adduct.] The accumulation of radioactivity is considered to be a direct consequence of the binding of formaldehyde to tissue structures. The chronic exposure of rats to non-labelled aspartame at 200 mg/kg for 10 days before administration of the radiolabelled dose resulted in increased accumulation of radiolabel, suggesting that there may be a cumulative increase in the number of formaldehyde adducts derived from aspartame in tissue proteins and nucleic acids (Trocho et al., 1998). [The Working Group noted that the methods used could not differentiate between formaldehyde binding and incorporation versus metabolism of methanol and metabolic incorporation of the methyl group; therefore, it was not possible to derive a clear picture of the potential to form DNA adducts.]

(ii) Non-human mammalian cells in vitro

No data were available to the Working Group.

(iii) Acellular systems in vitro

In an acellular system in vitro, the binding of aspartame to human serum albumin was studied at physiological pH using spectrophotometric spectrofluorometric competition experiments and circular dichroism techniques. The results indicated that the binding of aspartame to human serum albumin caused fluorescence quenching of the protein through a static

quenching mechanism, and the number of binding sites was approximately one. The study of molecular docking at the albumin binding site also indicated that aspartame can strongly bind to Sudlow site I (subdomain IIA) of human serum albumin, mainly by hydrophobic interaction, and that hydrogen bond interactions exist between aspartame and human serum albumin (Kheirdoosh et al., 2021).

Relative non-covalent binding affinities between calf thymus DNA (0.05 mg/mL) and aspartame (0.12–0.50 mg/mL), L-aspartic acid (0.25 mg/mL), L-phenylalanine (0.25 mg/mL), L-alanine (0.25 mg/mL), or doxorubicin (as positive control, 0.12–0.50 mg/mL) were investigated using HPLC (Karikas et al., 1998). A moderate DNA-binding affinity (39.8% DNA peak exclusion) was observed when aspartame at 0.25 mg/mL was tested with DNA. Analogous effects were exhibited by L-aspartic acid (39.3%) and L-phenylalanine (31.6%), and, to a lesser extent, by L-alanine (12.3%), whereas a 65.5% DNA-binding affinity was observed with aspartame at 0.50 mg/mL. Doxorubicin exhibited complete binding affinity (100% DNA peak exclusion) at 0.25 and 0.5 mg/mL. [The Working Group noted that several studies were available, but all described non-covalent binding to proteins, which is not relevant to electrophilicity.]

The nitrosation and alkylation reactions were investigated for three natural amino acids (alanine, glycine, aspartic acid), aspartame, and glycine ethyl ester in an acellular system (Meier et al., 1990). Aspartame showed a relatively high nitrosation rate constant ($K_2 = 1.4$, at pH 3.2) compared with aspartic acid ($K_2 = 0.08$, at pH 3.7) or glycine ethyl ester ($K_2 = 0.2$, at pH 2.5). Alkylation of 4-(p-nitrobenzyl)pyridine (used as a surrogate for pyridine base DNA and binding to DNA) by nitrosated aspartame showed rapid formation of a relatively stable alkylating product.

4.2.2 Is genotoxic

(a) Humans

(i) Exposed humans

No data in exposed humans were available to the Working Group.

(ii) Human primary cells in vitro

See Table 4.1.

Two studies were conducted using cultured human peripheral blood lymphocytes from healthy donors. Aspartame, at a single concentration of 287.342 μg/mL, equivalent to the half-maximal inhibitory concentration (IC$_{50}$), calculated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) metabolic activity assay, induced a significant increase in the frequency of chromosomal aberrations after 72 hours of treatment (Çadirci et al., 2020). [The Working Group noted that the limitations of the study included the use of a single concentration level, the lack of information on aberrant types and mitotic index, and the evaluation of cytotoxicity (by MTT assay), rather than cell replication.] However, in another study, a statistically significant increase in the frequency of chromosomal aberrations was also reported at all three tested concentrations (500, 1000, and 2000 μg/mL) in human lymphocytes treated with aspartame for 24 or 48 hours (Rencüzoğullari et al., 2004). A small but statistically significant increase in the frequency of micronuclei was also observed at the highest concentration (2000 μg/mL) after treatment for both 24 and 48 hours. In the same study, aspartame at concentrations of up to 2000 μg/mL did not induce sister-chromatid exchange (SCE) at 24 or 48 hours. No changes in the pH or osmolality of the treatment medium were observed (Rencüzoğullari et al., 2004). [The Working Group noted that the micronucleus study was of limited informativeness since the t-test employed was not corrected for multiple comparisons relative to the small increase; also, the genotoxicity

Table 4.1 End-points relevant to genotoxicity in human cells in vitro exposed to aspartame

End-point (assay)	Tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Primary cells						
Chromosomal aberrations	Human primary lymphocytes	(+)	NT	287.34 µg/mL	Single concentration (at IC_{50} by MTT assay); no information on aberrant types and mitotic index.	Çadirci et al. (2020)
Chromosomal aberrations	Human primary lymphocytes	+	NT	500 µg/mL		Rencüzoğullari et al. (2004)
Micronucleus formation	Human primary lymphocytes	(+)	NT	2000 µg/mL	Not corrected for multiple comparisons.	
Sister-chromatid exchange	Human primary lymphocytes	–	NT	2000 µg/mL		
Cell lines						
DNA strand breaks (alkaline comet assay)	Caco-2, HT-29, HEK-293 cells	–	NT	2943 µg/mL [10 mM]	No short (2–6 h) treatment.	van Eyk (2015)
DNA strand breaks (alkaline comet assay)	HL-60 cells	+	NT	15 µg/mL [0.05 mM]		Mateo-Fernández et al. (2022)
DNA strand breaks (alkaline comet assay)	HeLa cells	(+)	NT	2943 µg/mL [10 mM]	No quantitative/semiquantitative evaluation.	Pandurangan et al. (2016a)
DNA strand breaks (γH2AX)	HepG2 cells	(+)	NT	320 µg/mL	DNA fragmentation was observed at concentrations that also caused significant cytotoxicity and/or apoptosis.	Qu et al. (2019)

Caco-2, human colorectal adenocarcinoma cell line; h, hour(s); γH2AX, phosphorylated histone H2AX; HEK-293, human embryonic kidney cell line; HeLa, human cervical adenocarcinoma cell line; HepG2, human hepatoblastoma cell line; HIC, highest ineffective concentration; HL-60, human promyelocytic leukaemia cell line; HT-29, human colorectal adenocarcinoma cell line; IC_{50}, half-maximal inhibitory concentration; LEC, lowest effective concentration, MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide; NT, not tested.

[a] +, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study); (+) or (–), positive or negative results in a study of limited quality.

community considers results from the SCE assay to be of less relevance than other currently available genotoxicity tests.]

(iii)   Human cell lines in vitro

Aspartame treatment of Caco-2 (human colorectal adenocarcinoma), HT-29 (human colorectal adenocarcinoma), and HEK-293 (human embryonic kidney) cells to aspartame at 29.4, 294.3, or 2943 µg/mL (10 mM) for 24, 48, or 72 hours did not induce DNA strand breaks according to the alkaline comet assay (van Eyk, 2015). The appearance of comets was evaluated by a score of no damage to severe damage (levels 0–4). Aspartame-treated cells yielded no or little DNA fragmentation after any incubation period in any of the cell lines tested (score, level 0 or 1) (van Eyk, 2015).

In contrast, DNA strand breaks were observed in HL-60 (human promyelocytic leukaemia) cells treated with aspartame at 15 µg/mL (0.5 mM) for 5 hours (Mateo-Fernández et al., 2022) and in HeLa (human cervical adenocarcinoma) cells treated with aspartame at 2943 µg/mL (10 mM) or more (Pandurangan et al., 2016a). [The Working Group noted that the latter study was limited by the lack of quantitative or semiquantitative evaluation.] After a 24-hour at 320 µg/mL (IC_{25}) or 480 µg/mL (IC_{50}), aspartame induced phosphorylation of histone H2AX (γH2AX), a biomarker of DNA double-strand breaks, in HepG2 (human hepatoblastoma) cells (Qu et al., 2019). [The Working Group considered that the results of this study were of limited value because of evidence of cytotoxicity and apoptosis at the concentrations at which results were positive, thus preventing the clear distinction between necrosis, apoptosis, and genotoxicity.]

(b)   Experimental systems

(i)   Non-human mammals in vivo

See Table 4.2.

Aspartame induced a small but statistically significant increase in DNA strand breaks according to the alkaline comet assay in bone marrow of mice exposed to a single oral dose at 35 mg/kg bw by gavage (Bandyopadhyay et al., 2008). Positive findings were also obtained in the heart of Wistar albino rats exposed at 75 and 150 mg/kg bw per day by oral gavage for 4 weeks (Al-Eisa et al., 2018) and in the liver of rats exposed at 75 and 150 mg/kg bw per day by oral gavage for 30 days (Hamza et al., 2019). Histopathological observation of the liver or the heart in the groups exposed to aspartame revealed degenerative modifications (Hamza et al., 2019) or alterations in nuclear size and shape of the cardiomyocytes (Al-Eisa et al., 2018), respectively. [The Working Group noted that each study had limitations because of the poor quality of the microphotographs, which hindered the interpretation of results. In the study from Hamza et al., the rat strain was not reported.] Aspartame caused DNA damage in the sperm of male NMRI mice exposed at doses of 40, 80 or 160 mg/kg bw per day by oral gavage for 90 days (Anbara et al., 2020, 2021). On the other hand, negative findings in the comet assay were obtained in eight organs (glandular stomach, colon, liver, kidney, urinary bladder, lung, brain, and bone marrow) of male ddY mice after exposure to aspartame as a single oral dose at 2000 mg/kg bw by gavage for 3 and 24 hours (Sasaki et al., 2002). [The Working Group noted that this study did not score an adequate number of cells.]

DNA fragmentation was observed in the liver of rat dams and their offspring, at birth or at 3 or 9 weeks after birth, after oral administration of aspartame to the dams at a dose of 50.4 mg/animal per day [about 265 mg/kg bw per day] by gavage during gestation or during gestation plus 3 or 9 weeks after birth (Abd Elfatah et al., 2012). [The Working Group noted that the day of gestation on which treatment began was not clearly mentioned.] DNA fragmentation was not observed in the spleen, thymus, or lymph nodes of rats exposed to aspartame at 40 mg/kg

Table 4.2 End-points relevant to genotoxicity in non-human mammals in vivo exposed to aspartame or its impurity, diketopiperazine

End-point (assay)	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
DNA strand breaks (alkaline comet assay)	Mouse, Swiss albino (M)	Bone marrow	+	35 mg/kg bw	Single oral gavage (7, 14, 28, 35 mg/kg bw), sampling at 18 h after treatment		Bandyopadhyay et al. (2008)
DNA strand breaks (alkaline comet assay)	Rat, Wistar albino (M)	Heart (cardiac myocytes)	(+)	75 mg/kg bw per day	Oral gavage for 4 wk (75, 150 mg/kg bw per day)	Difficult to interpret the results based on the pictures provided.	Al-Eisa et al. (2018)
DNA strand breaks (alkaline comet assay)	Rat (M)	Liver	(+)	75 mg/kg bw per day	Oral gavage for 30 days (75 and 150 mg/kg bw per day)	Difficult to interpret the results based on the pictures provided; rat strain was not reported.	Hamza et al. (2019)
DNA strand breaks (alkaline comet assay)	Mouse, ddY (M)	Glandular stomach, colon, liver, kidney, urinary bladder, lung, brain, bone marrow	–	2000 mg/kg bw	Single oral gavage, sampling at 3 h and 24 h after the treatment	Too few cells scored.	Sasaki et al. (2002)
DNA damage (alkaline comet assay)	Mouse, NMRI (M)	Sperm	+	80 mg/kg bw per day	Oral gavage for 90 days (40, 80, 160 mg/kg bw per day)	DNA damage was due to apoptosis.	Anbara et al. (2020, 2021)
DNA fragmentation	Rat, Wistar albino (M)	Spleen, thymus, lymph node	–	40 mg/kg bw per day	Oral gavage for 90 days	The study was looking for evidence of apoptosis. Single dose tested only.	Choudhary and Sheela Devi (2016)
DNA fragmentation	Rat, albino (F; pregnant and after delivery)	Liver	+	50.4 mg/animal per day [about 265 mg/kg bw per day]	Gavage for GP, or for GP + 3 wk or 9 wk after delivery	Use of a commercial tablet.	Abd Elfatah et al. (2012)
DNA fragmentation	Rat, albino, offspring at birth or at 3 wk or 9 wk after birth	Liver	+	50.4 mg/animal per day to mother [about 265 mg/kg bw per day]	Gavage to mother for GP + 3 wk or 9 wk after delivery	Use of a commercial tablet.	Abd Elfatah et al. (2012)
Dominant lethal mutation	Rat, CD, albino (M)	Offspring	–	2000 mg/kg bw	Oral gavage (1000 mg/kg bw × 2, 2 h interval)	Purity, 99.8% (contained 0.2% DKP).	EFSA_E40 (2011)

Table 4.2   (continued)

End-point (assay)	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Dominant lethal mutation	Rat, CD, albino (M)	Offspring	–	2000 mg/kg bw	Oral gavage (1000 mg/kg bw × 2, 2 h interval)	Purity, 99.25% (contained 0.75% DKP).	EFSA_E41 (2011)
Reverse mutation (host-mediated)	Rat, Purina Caesarean-derived, albino (M)	i.p. injection of *Salmonella typhimurium* G46	–	4000 mg/kg bw per day	Oral gavage for 5 days, three equally divided doses every 3 h (500, 1000, 2000, 4000 mg/kg bw per day)	Purity, 99.3% (contained 0.7% DKP).	EFSA_E44 (2011)
Reverse mutation (host-mediated)	Mouse, Ha/ICR random-bred Swiss (M)	i.p. injection of *Salmonella typhimurium* G46	–	8000 mg/kg bw per day	Oral gavage for 5 days, three equally divided doses every 3 h (1000, 3000, 4000, 8000 mg/kg bw per day)	Purity unknown.	EFSA_E81 (2011)
Chromosomal aberrations	Rat, albino (F, pregnant and after delivery)	Bone marrow	(+)	50.4 mg/animal per day [about 265 mg/kg bw per day]	Gavage for GP, or for GP + 3 wk or 9 wk after delivery	Used a commercial tablet. Included gaps and scored total aberrations.	Abd Elfatah et al. (2012)
Chromosomal aberrations	Rat, albino, offspring at birth or at 3 wk or 9 wk after birth	Liver	(+)	50.4 mg/animal per day to mother [about 265 mg/kg bw per day]	Gavage for gestation period to mother + 3 wk or 9 wk after delivery to mother	Used a commercial tablet. Included gaps and scored total aberrations.	Abd Elfatah et al. (2012)
Chromosomal aberrations	Mouse, Swiss (M)	Bone marrow	(+)	35 mg/kg bw	Single oral gavage (3.5, 35, 350 mg/kg bw), sampling at 24 h after treatment	Questions regarding the validity of the results.	Alsuhaibani (2010)
Chromosomal aberrations	Mouse, C57BL/6	Bone marrow	–	400 mg/kg bw per day	Oral gavage for 5 days (40, 400 mg/kg bw per day), sampling at 6 h after the final treatment		Durnev et al. (1995)

Table 4.2 (continued)

End-point (assay)	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Chromosomal aberrations	Rat, Holtzman, albino (M)	Bone marrow, spermatogonia	(−)	8000 mg/kg bw per day	Oral gavage for 5 days (2000, 4000, 6000, 8000 mg/kg bw per day), sampling at 29 h after the final treatment	Delayed sampling time; only 50 cells/animal were analysed.	EFSA_E12 (2011)
Chromosomal aberrations	Rat, Purina Caesarean-derived, albino (M)	Bone marrow	(−)	4000 mg/kg bw per day	Oral gavage for 5 days, three equally divided doses every 3 h (500, 1000, 2000, 4000 mg/kg bw per day), sampling at 29 h after the final treatment	Purity, 99.3% (contained 0.7% DKP); only 50 cells/animal were analysed.	EFSA_E43 (2011)
Chromosomal aberrations	Mouse, Swiss albino (M, F)	Bone marrow	(+)	250 mg/kg bw at 72 h sampling	Single oral gavage (250, 455, 500, 1000 mg/kg bw), sampling at 24, 48, and 72 h after treatment	Difficult to interpret the results based on the pictures provided; no criteria on "aberrations" were provided; inappropriately included gaps.	Kamath et al. (2010)
Chromosomal aberrations	Rat, albino (M)	Bone marrow	(+)	250 mg/kg bw per day	Oral gavage for 90 days	Used a commercial tablet; difficult to interpret the results based on the pictures provided. Single dose tested only.	Elballat and Abas (2020)
Micronucleus formation	Mouse, Swiss albino (M, F)	Bone marrow	(+)	455 mg/kg bw at 24 h sampling, 455 mg/kg bw at 48 h sampling, 250 mg/kg bw at 72 h sampling	Single oral gavage (250, 455, 500, 1000 mg/kg bw), sampling at 24, 48, and 72 h after treatment	Difficult to interpret the results based on the pictures provided; signs of toxicity; altered NCE/PCE ratio.	Kamath et al. (2010)
		Peripheral blood	(+)	500 mg/kg bw at 24 h sampling, 455 mg/kg bw at 48 h sampling, 250 mg/kg bw at 72 h sampling			

Table 4.2   (continued)

End-point (assay)	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Micronucleus formation	Rat, F344/N (M)	Bone marrow	–	2000 mg/kg bw per day	Oral gavage, 3 × at 24 h intervals (500, 1000, 2000 mg/kg bw per day), sampling at 24 h after the final treatment		NTP (2005)
Micronucleus formation	Mouse, *Tg-AC* hemizygous (M, F), *Cdkn2a*-deficient (M, F), *p53*-haploinsufficient (M)	Peripheral blood	–	50 000 ppm [7280–9560 mg/kg bw per day, depending on the strain or sex]	Feeding for 9 mo (3125, 6250, 12 500, 25 000, 50 000 ppm)		NTP (2005)
Micronucleus formation	Mouse, *p53*-haploinsufficient (F)	Peripheral blood	(+)	50 000 ppm, [9620 mg/kg bw per day]	Oral administration (feed) for 9 mo (3125, 6250, 12 500, 25 000, 50 000 ppm)	A single animal at the highest dose was responsible for the increase.	NTP (2005)
Micronucleus formation	Mouse, CD-1 (M)	Bone marrow	–	2000 mg/kg bw	Single oral gavage (500, 1000, 2000 mg/kg bw), sampling at 24 h and 48 h after treatment		Otabe et al. (2019)
Sister-chromatid exchange	Mouse, Swiss albino (M)	Bone marrow	–	350 mg/kg bw	Single oral gavage (3.5, 35, 350 mg/kg bw), sampling at 24 h after treatment		Alsuhaibani (2010)
Results for the impurity, diketopiperazine							
Dominant lethal mutation	Rat, CD, albino (M)	Offspring	–	1000 mg/kg bw	Oral gavage (500 mg/kg bw × 2, 2 h interval)		EFSA_E42 (2011)
Reverse mutation (host-mediated)	Rat, Purina Caesarean-derived, albino (M)	i.p injection of *Salmonella typhimurium* G46	–	2000 mg/kg bw per day	Oral gavage for 5 days, three equally divided doses every 3 h (250, 500, 1000, 2000 mg/kg bw per day)		EFSA_E31 (2011)

Table 4.2 (continued)

End-point (assay)	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Reverse mutation (host-mediated)	Mouse, Ha/ICR random-bred Swiss (M)	i.p. injection of *Salmonella typhimurium* G46	–	8000 mg/kg bw per day	Oral gavage for 5 days, three equally divided doses every 3 h (1000, 3000, 4000, 8000 mg/kg bw per day)		EFSA_E82 (2011)
Chromosomal aberrations	Rat, Purina Caesarean-derived, albino (M)	Bone marrow	–	2000 mg/kg bw per day	Oral gavage for 5 days (250, 500, 1000, 2000 mg/kg bw per day), sampling at 29 h after the final treatment		EFSA_E30 (2011)

bw, body weight; F, female; GLP, Good Laboratory Practice; GP, gestation period; i.p., intraperitoneal; h, hour(s); HID, highest ineffective dose; LED, lowest effective dose; M, male; mo, month(s); NCE/PCE, normochromatic erythrocytes/polychromatic erythrocytes; NT, not tested; ppm, parts per million; wk, week(s).

[a] +, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study); (+) or (–), positive or negative result in a study of limited quality.

bw per day by oral gavage for 90 days (Choudhary and Sheela Devi, 2016).

Aspartame did not induce dominant lethal mutation in male CD albino rats exposed, in two separate studies, to aspartame of two different purities (purity, 99.8% or 99.25%) at 2000 mg/kg bw by oral gavage, given twice at 1000 mg/kg bw with a 2-hour interval. Each male was mated with two females weekly for 8 weeks. On day 14 of gestation, the mated females were killed in order to analyse fetal deaths. Paternal body-weight changes and maternal pregnancy rate were assessed, and the uterus and ovaries were examined. No effects were observed (EFSA_E40, 2011; EFSA_E41, 2011).

Aspartame gave negative results in the host-mediated assay in male Purina Caesarean-derived albino rats, Ha/ICR random-bred Swiss mice, and *Salmonella typhimurium* strain G46 (EFSA_E44, 2011; EFSA_E81, 2011). Aspartame was administered by oral gavage for 5 days at a dose of 1000, 2000, and 4000 mg/kg bw per day (rats) or 1000, 3000, 4000, and 8000 mg/kg bw per day (mice) in three equally divided doses every 3 hours. After the final dose, the rodents were inoculated with bacteria by intraperitoneal injection. No increase in mutants was observed among bacteria recovered after 3 hours.

Many cytogenetics studies (chromosomal aberrations and micronucleus formation) have been conducted with aspartame administered orally. Induction of chromosomal aberrations was observed in the bone marrow of albino rat dams and the liver of their offspring at birth or after 3 or 9 weeks. Aspartame was administered to the mother by oral gavage at a dose of 50.4 mg/animal per day [about 265 mg/kg bw per day] during gestation or during gestation plus 3 or 9 weeks after birth (Abd Elfatah et al., 2012). [The Working Group noted that the day of gestation on which exposure began was not clearly mentioned and that the study was of limited quality because of inappropriately included gaps

and the scoring of total aberrations, not cells with aberrations.]

Aspartame was administered to male and female Swiss albino mice as a single oral dose at 250–1000 mg/kg bw by gavage, and bone marrow samples were obtained at 24, 48, or 72 hours after dosing. Chromosomal aberrations were observed in mice at 250 mg/kg bw at 72 hours (Kamath et al., 2010). [The Working Group noted that the study limitations included difficulties in the interpretation of the results on the basis of the pictures provided, lack of criteria on "aberration", or inappropriately included gaps.]

Chromosomal aberrations were induced in the bone marrow of male albino rats exposed to aspartame at 250 mg/kg bw per day by oral gavage for 90 days (Elballat and Abas, 2020). [The Working Group noted that the interpretation of results was limited by the quality of the pictures provided; however, data were provided with statistical analysis. An unusual background of 12% of cells with numerical aberrations was reported; normally, numerical aberrations are few or absent. The background of 3.4% of bone marrow cells with structural aberrations was not consistent with the usual value of 0.2–2.0% (Tice et al., 1994).]

In the bone marrow of male Swiss albino mice exposed to aspartame as a single oral dose at 3.5, 35 or 350 mg/kg bw by oral gavage, a small but statistically significant increase in the frequency of chromosomal aberrations was observed at 35 mg/kg bw or more at 24 hours after dosing (Alsuhaibani, 2010). [The Working Group considered the study to be of low informativeness because of questions regarding the validity of the results.] In contrast, negative results were obtained in the chromosomal aberrations assay in bone marrow cells or spermatogonia of male Holtzman albino rats. Aspartame was administered at a dose of 2000, 4000, 6000, or 8000 mg/kg bw per day by oral gavage for 5 days. No chromosomal aberrations were observed

in the bone marrow cells or spermatogonia at 29 hours after the final dose (EFSA_E12, 2011).

In a similar study, no chromosomal aberrations were observed in the bone marrow cells of male Purina Caesarean-derived albino rats exposed to aspartame at 500, 1000, 2000, or 4000 mg/kg bw per day by oral gavage for 5 days (EFSA_E43, 2011). Aspartame did not induce chromosomal aberrations in the bone marrow cells of C57BL/6 mice exposed at a dose of 40 or 400 mg/kg bw per day by oral gavage for 5 days (Durnev et al., 1995).

In a micronucleus assay in mice, aspartame was administered as a single oral dose at 250–1000 mg/kg bw by gavage, and bone marrow and peripheral blood samples were obtained at 24, 48, or 72 hours after dosing. The most effective induction of micronucleus formation was observed at 72 hours in both bone marrow cells and peripheral blood (Kamath et al., 2010). [The Working Group noted that the study was of limited informativeness because of difficulties in the interpretation of the microphotographs; however, data results were provided with statistical analysis, and there were signs of toxicity. In addition, the increase in micronucleus frequency occurred earlier (at 24 hours) in the peripheral blood than in the bone marrow, whereas, physiologically, changes in peripheral blood micronuclei ratios would be expected to be detected 12–24 hours later than changes in the bone marrow.]

Several micronucleus assays, performed in accordance with GLP, gave negative results. Aspartame was administered at a dose of 500, 1000, or 2000 mg/kg bw per day by oral gavage for 3 days to male F344/N rats. No induction of micronuclei was observed in the bone marrow at 24 hours after the final dose (NTP, 2005). Three strains of transgenic mouse were fed diets containing aspartame at a concentration of 0, 3125, 6250, 12 500, 25 000, or 50 000 ppm (equivalent to average daily doses of approximately 500–8000 or 9000 mg/kg bw per day, depending

on the strain and sex) for 9 months. There was no micronucleus formation in the peripheral blood (normochromatic erythrocytes) in Tg-AC hemizygous (male and female), *Cdkn2a*-deficient (male and female), or *p53*-haploinsufficient (male) mice exposed to aspartame at concentrations up to 50 000 ppm (about 7280–9560 mg/kg bw per day). However, a small but statistically significant increase in micronucleus induction was observed in female *p53*-haploinsufficient mice at 50 000 ppm (about 9620 mg/kg bw per day) (NTP, 2005). [The Working Group noted that the increase in frequency of micronucleated normochromatic erythrocytes in female *p53*-haploinsufficient mice at 50 000 ppm was small (1.80‰ versus concurrent female control 0.79‰, male control 1.18‰), and the biological relevance of this extremely high dose was questionable. The Working Group also noted that the study did not provide historical control data.]

In another micronucleus assay in mice, aspartame was administered as a single oral dose of 500, 1000, or 2000 mg/kg bw by gavage to male CD-1 mice. No micronucleus induction was observed in the bone marrow at 24 and 48 hours after dosing (Otabe et al., 2019).

Negative findings were also reported for SCE investigated in the bone marrow of Swiss albino mice exposed to aspartame as a single oral dose of up to 350 mg/kg bw by gavage (at 24 hours after dosing) (Alsuhaibani, 2010). [The Working Group noted that the SCE assay is now considered of less relevance than other currently available genotoxicity tests.]

The genotoxicity of the aspartame impurity DKP was also investigated for different genotoxicity end-points in vivo. Overall, the findings of these studies were negative. In a test for dominant lethal mutations, DKP was administered at a dose of 2000 mg/kg bw per day (two doses of 1000 mg/kg bw with a 2-hour interval) by oral gavage to male CD albino rats. After mating male and female rats, mated females were killed for the analysis of dominant lethal mutations on day

14 of gestation. No dominant lethal effect was observed (EFSA_E42, 2011). In a host-mediated assay, similar strains of male Purina Caesarean-derived albino rats or Ha/ICR random-bred Swiss mice were exposed to DKP at a dose of 1000, 3000, 4000, and 8000 mg/kg bw per day, or 250, 500, 1000, and 2000 mg/kg bw per day, respectively, in three equally divided doses, every 3 hours, by oral gavage, for 5 days. After the final dose, the rodents were inoculated with *S. typhimurium* G46 by intraperitoneal injection. The bacteria were recovered 3 hours later, and the presence of mutants was investigated. No statistically significant increases in mutants were observed (EFSA_E31, 2011; EFSA_E82, 2011). In a test for chromosomal aberrations, DKP was administered at a dose of 250, 500, 1000, or 2000 mg/kg bw per day by oral gavage for 5 days to male Purina Caesarean-derived albino rats. No chromosomal aberrations were observed in the bone marrow at 29 hours after the final dose (EFSA_E30, 2011).

(ii)   Non-human mammalian cells in vitro

See Table 4.3.

An alkaline comet assay in MDCK (Madin–Darby canine kidney) cells gave a positive result for DNA damage with aspartame at 2943 µg/mL (10 mM) (Pandurangan et al., 2017). [The Working Group noted that the study was limited by the lack of quantitative or semiquantitative evaluation and difficulties in the interpretation of the results based on the pictures provided.]

Aspartame did not induce unscheduled DNA synthesis (UDS) in rat primary hepatocytes (Jeffrey and Williams, 2000). Cells prepared from the liver of male Sprague-Dawley rats were exposed to aspartame at a concentration of 1472 or 2943 µg/mL (5 or 10 mM) for 20 hours. Unscheduled DNA synthesis was measured by an autoradiographic method and 150 cells were scored per concentration. [The Working Group noted that the study was limited by the lack of short time-point sampling.]

(iii)   Non-mammalian experimental systems

See Table 4.4.

Aspartame induced a concentration-related increase in DNA fragmentation in *Danio rerio* (zebra fish) exposed to aspartame at 62.5–500 mg/mL for 5 days (Reshman et al., 2015). [The Working Group noted that the study reported only a qualitative evaluation of the data.] Aspartame induced concentration-related increases in the percentage of DNA fragmentation in *Drosophila melanogaster* exposed to medium containing aspartame at concentrations of 62.5–500 mg/mL for 72 hours (Reshman et al., 2015). [The Working Group noted that the informativeness of the study was low because of the poor quantitative evaluation and because increases were observed only in one duplicate.]

A study was carried out using SMART (somatic mutation and recombination by wing spot test) in two *D. melanogaster* strains (*flr3* females and *mwh* males) with genetic markers that affect the wing-hair phenotype (Mateo-Fernández et al., 2022). After crossing females with males, trans-heterozygous larvae were obtained. After synchronization, the larvae were fed instant medium containing aspartame at 7.4 or 241.3 µg/mL until pupae hatching (10–12 days), when the emerged flies were collected and mutant wing spots (single, large, or twin) were assessed. At concentrations of up to 241.3 µg/mL, aspartame did not induce mutations in SMART.

Aspartame at concentrations up to 5000 µg/plate did not induce reverse mutation with or without metabolic activation in *S. typhimurium* strains TA1535, TA100, TA1538, TA97, and TA1537 (EFSA_E101, 2011; EFSA_E97, 2011). In a study that complied with GLP, negative results were obtained with aspartame at concentrations up to 10 000 µg/plate in *S. typhimurium* strains TA1535, TA100, TA97, TA98, and TA1537, with or without metabolic activation (NTP, 2005). The most recent study conducted under GLP and compliant with OECD test guideline 471

Table 4.3 End-points relevant to genotoxicity in non-human mammalian cells in vitro exposed to aspartame

End-point (assay)	Species, tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	**With metabolic activation**			
DNA strand breaks (alkaline comet assay)	MDCK cells	(+)	NT	2943 µg/mL [10 mM]	No quantitative/ semiquantitative evaluation; difficult to interpret the results based on the pictures provided.	Pandurangan et al. (2017)
Unscheduled DNA synthesis	Primary hepatocyte (male Sprague-Dawley rat, liver)	(−)	NT	2943 µg/mL [10 mM]	No short time sampling.	Jeffrey and Williams (2000)

HIC, highest ineffective concentration; LEC, lowest effective concentration; MDCK, Madin–Darby canine kidney; NT, not tested.

[a] +, positive; −, negative; +/−, equivocal (variable response in several experiments within an adequate study); (+) or (−), positive or negative result in a study of limited quality.

Table 4.4 End-points relevant to genotoxicity in non-mammalian experimental systems exposed to aspartame or its impurity, diketopiperazine

Test system (species, strain)	End-point (assay)	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Danio rerio (zebra fish)	DNA fragmentation	(+)	NA	250 mg/mL	Only qualitative evaluation.	Reshman et al. (2015)
Drosophila melanogaster	DNA fragmentation	(+)	NA	500 mg/mL	Quantitative evaluation; increase seen in only one duplicate.	Reshman et al. (2015)
Drosophila melanogaster (*flr3* female, *mwh* male)	SMART (somatic mutation and recombination by wing spot test)	–	NA	0.82 mM, [241.3 µg/mL]		Mateo-Fernández et al. (2022)
Salmonella typhimurium TA1535, TA1537, TA1538, TA98, TA100	Reverse mutation	–	–	5000 µg/plate	Purity unknown.	EFSA_E97 (2011); EFSA_E101 (2011)
Salmonella typhimurium TA98, TA100	Reverse mutation	(–)	(–)	2000 µg/plate	Top concentration[b] tested was not suitable.	Rencüzoğullari et al. (2004)
Salmonella typhimurium TA1535, TA97, TA98, TA100, TA 1537	Reverse mutation	–	(+)	10 000 µg/plate	Equivocal result in TA97 with 30% rat S9.	NTP (2005)
Salmonella typhimurium TA1535, TA1537, TA98, TA100	Reverse mutation	–	–	5000 µg/plate		Otabe et al. (2019)
Escherichia coli WP2 *uvr*A	Reverse mutation	–	–	5000 µg/plate		Otabe et al. (2019)
Salmonella typhimurium TA97a, TA100	Reverse mutation	(–)	(–)	1000 µg/plate	Top concentration[b] tested was not suitable.	Bandyopadhyay et al. (2008)
Salmonella typhimurium TA98	Reverse mutation	(–)	(–)	2400 µg/plate	Purity unknown; top concentration[b] tested was not suitable.	Najam et al. (2017)
Salmonella typhimurium TA100	Reverse mutation	(–)	(+)	150 µg/plate	Unknown reproducibility at low concentrations; top concentration[b] tested was not suitable.	

Table 4.4   (continued)

Test system (species, strain)	End-point (assay)	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Salmonella typhimurium TA100	Reverse mutation	+	+	8 mM [2355 μg/mL]	A nitrosated form of aspartame was tested.	Shephard et al. (1993)
Salmonella typhimurium TA98	Reverse mutation	+	–	20 mM [5886 μg/mL]	A nitrosated form of aspartame was tested.	
Salmonella typhimurium TA102	Reverse mutation	–	NT	20 mM [5886 μg/mL]	A nitrosated form of aspartame was tested.	
Results for the impurity diketopiperazine						
Salmonella typhimurium TA1535, TA1537, TA1538, TA98, TA100	Reverse mutation	–	–	5000 μg/plate		EFSA_E98 (2011)
Salmonella typhimurium TA1535, TA1537, TA1538, TA98, TA100	Reverse mutation	–	–	10 000 μg/plate	Precipitate was observed at 5000 and 10 000 μg/plate.	EFSA_E106 (2011)

h, hour(s); HIC, highest ineffective concentration; LEC, lowest effective concentration; NA, not available; NT, not tested; S9, 9000 × *g* supernatant.

[a] +, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study); (+) or (–), positive or negative result in a study of limited quality.

[b] "Top" concentration indicates the highest recommended concentration when toxicity is not limiting.

gave negative results with aspartame at concentrations up to 5000 µg/plate in *S. typhimurium* strains TA1535, TA100, TA97, and TA1537, and *Escherichia coli* strain WP2 *uvrA*, with or without metabolic activation (Otabe et al., 2019). Negative results were also obtained at concentrations up to 1000–2400 µg/plate in *S. typhimurium* strains TA97a, TA98, and TA100, with or without metabolic activation (Rencüzoğullari et al., 2004; Bandyopadhyay et al., 2008; Najam et al., 2017). [The Working Group noted that the studies with aspartame at concentrations up to 2400 µg/plate had limitations because a non-suitable top concentration (the highest recommended concentration when toxicity is not limiting) was tested.] One study, however, reported positive responses at 150 and 300 µg/plate, but not at 600, 1200, or 2400 µg/plate, in *S. typhimurium* TA100 with metabolic activation (Najam et al., 2017). [The Working Group noted that the study had limitations because of unknown reproducibility at low concentrations and testing of a non-suitable top concentration.]

The mutagenicity of nitrosated aspartame was investigated in a bacterial reverse mutation assay in *S. typhimurium* strains TA100, TA98, TA102, and TA104 (Shephard et al., 1993). Aspartame (420 mM in reaction mixtures) was nitrosated with sodium nitrite (40 mM) at pH 3.0 or pH 3.5 for 30 minutes at 37 °C. Concentration-related increases in the number of revertant colonies were obtained in TA100 without metabolic activation. Nitrosated aspartame (20 mM in reaction mixtures) showed mutagenic activity in strain TA100, TA104, and TA98, but not in TA102, without metabolic activation. The effects were decreased with metabolic activation in TA100 or TA98. The most sensitive strain was TA100.

The aspartame impurity DKP did not induce reverse mutations, with or without metabolic activation, in *S. typhimurium* strains TA1535, TA100, TA1538, TA97, or TA1537, at concentrations up to 5000 or 10 000 µg/plate (EFSA_E98, 2011; EFSA_E106, 2011).

4.2.3 Induces oxidative stress

(a) Humans

(i) Exposed humans

No data on induction of oxidative stress in humans were available to the Working Group.

(ii) Human cells in vitro

See Table 4.5.

Several studies evaluated cells for markers of oxidative stress at cytotoxic concentrations of aspartame. There were no signs of increased cellular reactive oxygen species (ROS) in primary human peripheral blood mononuclear cells using colourimetric analysis of a proprietary reagent based on oxidation of a ferrous compound at aspartame concentrations of up to 100 mg/L (339.8 µM). The higher concentrations reduced metabolic activity – oxidative phosphorylation, measured using (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide, MTT – and caused significant leakage of lactate dehydrogenase (LDH) enzymatic activity into the tissue culture medium. Total antioxidant capacity (TAC) and total antioxidant status (TOS) assays were also performed to determine the antioxidative/oxidative effects of aspartame; no significant changes were reported (Çadirci et al., 2020) [The Working Group noted that this study was limited by the failure to report the numbers of replicates used in the experiment and the lack of statistical analyses of the data, particularly of what appeared to be a concentration-related increase in ROS.]

In contrast, whereas exposure of a human neuroblastoma cell line to either aspartame at 271.7 µM or a mixture containing phenylalanine, aspartic acid, and methanol each at 271.7 µM, caused no cytotoxicity as measured by LDH activity leakage, both exposures led to mitochondrial damage and increased intracellular concentrations of ROS (measured by aminophenyl fluorescein, APF). Transmission electron microscopy was used to demonstrate

Table 4.5 End-points relevant to oxidative stress in human cells in vitro exposed to aspartame

End-points	Assay	Tissue, cell line	Results[a]	Concentration (LEC or HIC) or range	Comments	Reference
Primary cells						
ROS Antioxidant activity Cytotoxicity	TOS TAC MTT, LDH leakage	Human primary peripheral blood mononuclear cells	No change No change ↑: MTT, LDH	10.6–339.8 µM[b]	Number of replicates was not stated; no statistical analysis of the data; a trend towards an increase in "TOS" was observed.	Çadirci et al. (2020)
Cell lines						
ROS SOD1 SOD2 Cytotoxicity Mito-toxicity: cardiolipin FIS1 PINK1	APF qRT-PCR qRT-PCR LDH leakage qRT-PCR qRT-PCR qRT-PCR	Human neuroblastoma cells	↑ ↑ (↑) No changes ↓ ↑ ↑	271.7 µM (aspartame or an isomolar mix of phenylalanine, aspartic acid, and methanol)	Aspartame and the metabolite mix had similar effects for each end-point measured; while there was no cytotoxicity as measured by LDH leakage into the medium, photomicrographs showed significant mitochondrial toxicity and accumulation of cytoplasmic lipid particles. Only a single concentration was used.	Griebsch et al. (2023)
ROS	DCFDA	Caco-2 cells	↑	100 µM	A single non-cytotoxic concentration was used; 1.5 h treatment; the magnitude of the increase was similar to that in the LPS positive control (ROS was restored to vehicle control levels by co-incubation with NAC, T1R3 knockdown, or CLDN3 overexpression).	Shil et al. (2020)
ROS	DCFDA	HUVEC cells IMR-90 cells	↑ No changes	20–100 µM	The biological significance of the HUVEC cell response was questionable due to the flat response over five concentrations.	Alleva et al. (2011)

Table 4.5 (continued)

End-points	Assay	Tissue, cell line	Results[a]	Concentration (LEC or HIC) or range	Comments	Reference
Lipid peroxidation	TBARS	HTR-8/SV neo cells	No changes	0.5 mM	Single concentration used only; 24 h treatment; no cytotoxicity (MTT or LDH leakage assays).	Rodrigues et al. (2022)
Aspartame: ROS SOD2 Cytotoxicity *Phenylalanine:* ROS Cytotoxicity	DCFDA Western blot MTT DCDFDA MTT	3A-subE trophoblast cells	↑ ↓ ↑ ↑ ↑	34 mM[b] 0.34 μM[b] 8.5–34 mM[b] 34 mM[b] 8.5–34 mM[b]	Pre-treatment with NAC provided only slight protection against cytotoxicity, suggesting that ROS was a minor or secondary factor in causing cytotoxicity. SOD2 expression was not examined with either lactisole or gynemic acid alone, so the impact of co-exposure with aspartame is uninterpretable.	Huang et al. (2023a)
ROS Cytotoxicity	CellROX MTT	HepG2 cells	(↑) ↑	LEC, 713.1 μM	Significant cytotoxicity (the three concentrations tested were the IC_{10}, IC_{25}, and IC_{50}) and evidence of apoptosis made it impossible to tell whether the ROS increase was caused by cytotoxicity or vice versa.	Qu et al. (2019)
ROS Cytotoxicity Necrosis	DCFDA SRB PI	HeLa cells	(↑) ↑ ↑	LEC, 10 mM LEC, 100 μM LEC, 10 mM	The ROS increases occurred at concentrations that were cytotoxic both by SRB–protein-binding and PI entry into necrotic cells. Thus, the ROS liberated during cell death may be the cause of or secondary to cytotoxicity.	Pandurangan et al. (2016a)

Table 4.5   (continued)

End-points	Assay	Tissue, cell line	Results[a]	Concentration (LEC or HIC) or range	Comments	Reference
Cytotoxicity	CCK-8	GMVEC	No changes	0.1–100 μM		Enuwosa et al. (2021)
Membrane permeability	Fluorescence, EVOM2		No changes	100 μM		
			↓	50 ng/mL VEGF + 10 μM aspartame		
ROS	DCFDA		↑	10 μM		
Oxidative stress	GSH reduced/GSH oxidized		↓	10 μM		

ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid; APF, aminophenyl fluorescein; bw, body weight; Caco-2, epithelial cell line from a human colon carcinoma; CAT, catalase; CCK, cholecystokinin; CLDN3, claudin-3; DMSO, dimethyl sulfoxide; CellRox, a proprietary intracellular dye that fluoresces when oxidized; DCFDA, 2′7′-dichlorofluorescein diacetate; EVOM2, Epithelial Volt Ohm Meter 2; γ-GC, γ-glutamylcysteine; GMVEC, glomerular microvascular endothelial cells; GPx, glutathione peroxidase; GSH, glutathione; GSH RI, glutathione redox index; GSR, glutathione reductase; GSSG, glutathione disulfide; GST, glutathione *S*-transferase; HepG2, human hepatocellular carcinoma cell line; HeLa, human cervical cancer cell line; 3A-subE human trophoblasts; human placental cells transformed by SV40; HUVEC, human umbilical vein endothelial cells; h, hour(s); HTR-8/SV neo, SV40-transformed extravillous trophoblasts from human placenta explants; HCS, high content screening; HIC, highest ineffective concentration; IC, inhibitory concentration; IMR-90, human fetal lung fibroblasts; LDH, lactate dehydrogenase; LEC, lowest effective concentration; LPO, lipid peroxidation; MDA, malondialdehyde; MPO, myeloperoxidase; MTT, dimethylthiazolyl)-diphenyltetrazolium bromide; NAC, *N*-acetyl-ʟ-cysteine; NO, nitric oxide; NPSH, non-protein thiol; NR, not reported; PI, propidium iodide; qRT-PCR, quantitative gene transcription based on reverse transcriptase polymerase chain reaction; ROS, reactive oxygen species; SOD, superoxide dismutase; SOD1, type 1 SOD, found in cytoplasm in human cells; SOD2, type 2 (or Mn) SOD, associated with mitochondria in humans; SRB, sulforhodamine B protein-binding dye; TBARS, thiobarbituric acid reactive substances; T1R3, taste receptor type 1 member 3; TOS, total oxidant status; VEGF, vascular endothelial growth factor.

[a] ↓, decrease; ↑, increase.

[b] Concentration was converted to micromolar or millimolar (μM or mM) for consistency.

morphological changes in mitochondria. These changes were accompanied by increased transcription of superoxide dismutase isoenzyme 1 (cytoplasmic) and isoenzyme 2 (mitochondrial). Evidence of mitochondrial toxicity also included downregulation of cardiolipin and increased expression of genes associated with mitochondrial fission (*FIS1*) and mitophagy (*PINK1*). There were also signs of disruption of lipid metabolism (see also Section 4.2.8) (Griebsch et al., 2023). [The Working Group noted that the limitations of this study included the lack of concentration–response data, the lack of positive controls or antioxidants to determine whether mitochondrial toxicity led to the increase in ROS or vice versa, and the lack of experiments clarifying the relationships between these end-points and the observed changes in cellular lipids.]

Aspartame exposure was reported to disrupt intestinal cell barrier function, an effect that was dependent on oxidative damage and expression of the T1R3 sweet taste receptor (Shil et al., 2020). Exposure to aspartame at high concentrations (up to 10 mM) reduced metabolic activity (measured by the MTT assay) in Caco-2 cells, a human epithelial cell line derived from a colon carcinoma. Intracellular ROS, measured as fluorescence by 2′,7′-dichlorodifluorescein diacetate (DCFDA), was associated with exposure to aspartame at 0.1 mM (100 μM), a non-cytotoxic concentration. A series of well-controlled experiments linked the increase in ROS to reduced permeability in Caco-2 cell monolayers. The permeability reduction was reversed by pre-treatment of the cells with the antioxidant *N*-acetyl cysteine (NAC), by knocking down expression of claudin-3 (a component of the tight junction in the cell membrane) or the T1R3 taste receptor. [The Working Group noted that the study findings, examined in more detail in Section 4.2.8, presented evidence that generation of ROS is associated with and possibly necessary for disruption of membrane permeability in vitro.]

In contrast, Alleva et al. (2011) reported no increase in intracellular ROS, measured as fluorescence by DCFDA, in human fetal lung fibroblasts (IMR-90) exposed to aspartame at non-cytotoxic concentrations of up to 100 μM, although there was a small increase in fluorescence in primary human umbilical vein endothelial cells (HUVECs). [The Working Group noted that the increase observed in HUVEC cells might not be biologically significant because it was small in magnitude and did not increase with increasing concentration.]

No increase in thiobarbituric acid reactive substances (TBARS), a measurement of lipid peroxidation (LPO), was detected by Rodrigues et al. (2022) when HTR-8/SV neo cells (an SV40-transformed cell line derived from extravillous trophoblasts from human placenta explants) were exposed to aspartame at a non-cytotoxic concentration of 0.5 mM for 24 hours.

Increases in markers associated with ROS at cytotoxic aspartame exposures were featured in several reports. In the 3A-subE human trophoblast cell line, aspartame (10 mg/mL) was cytotoxic (measured by the MTT assay), increased intracellular ROS and decreased superoxide dismutase 2 (SOD2) expression (measured by western blot), increased phosphorylation of AKT serine/threonine kinase (AKT), and also inhibited the ability of cells to migrate into a "wound" scratched into the monolayer on the tissue culture plate. The cytotoxicity of aspartame was partially inhibited by exposure of the cells to NAC, and co-incubation of aspartame and either lactisole or gynemic acid increased SOD2 expression to levels higher than those seen in non-exposed cells. The results for lactisole and gynemic acid were attributed to their ability to inhibit binding of aspartame to the sweet taste receptor (Huang et al., 2023a). [The Working Group noted that the antioxidant NAC reversed only a small fraction of the cytotoxicity, thus a secondary effect of ROS could not be excluded. Also, there was no control experiment in which cells were exposed

to lactisol or gynemic acid without aspartame, nor was any evidence presented that these cells expressed a functional sweet taste receptor, which would support a role for the receptor.]

Qu et al. (2019) found a concentration-related increase in ROS using the proprietary fluorescence dye in the CellRox system in HepG2 cells (a human hepatoblastoma cell line) exposed to aspartame at 0.21, 0.32, or 0.48 g/L (713.1, 1088, and 1630 µM), concentrations that also induced signs of apoptosis and corresponded respectively to the IC_{10}, IC_{25}, and IC_{50} for cytotoxicity, measured as MTT reduction. Similarly, exposing a human cervical carcinoma cell line to aspartame at 10 mM resulted in significant cytotoxicity, measured by binding of sulforhodamine B to cellular proteins, binding of propidium iodide to DNA in necrotic cells, and measurement of intracellular ROS (DCFDA fluorescence) (Pandurangan et al., 2016a). [The Working Group noted that, in the absence of additional experimental results, it was impossible to determine whether ROS directly resulting from aspartame exposure led to cytotoxicity or whether toxicity caused by aspartame led to ROS generation secondary to necrotic and/or apoptotic cell death.]

The impact of aspartame on membrane permeability was studied in primary glomerular microvascular endothelial cells (Enuwosa et al., 2021). Exposure of these cells to aspartame at concentrations of up to 100 µM for 24 hours did not reduce metabolic activity as measured by colourimetric evaluation of WST-8 reagent (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, monosodium salt). In these experiments, the cells were exposed to vascular endothelial growth factor (VEGF) at 50 ng/mL to model renal damage and to assess the potential protective effects of pre- or co-exposure to aspartame. Each experiment consisted of a single trial with six replicates. Membrane permeability was assessed by migration of fluorescently labelled

dextran across the monolayer in a double-chambered cell. Exposure to VEGF significantly increased membrane permeability. Exposure of the cells to aspartame at concentrations as high as 100 µM had no impact on membrane permeability. Co-incubation of VEGF with aspartame at 10 or 100 µM blocked the permeability increase observed with VEGF alone. The protective effect of aspartame on VEGF-induced membrane leakage (decreased permeability) was reversed by blocking the T1R3 sweet taste receptor, either by addition of lactisole to inhibit receptor binding or by small interfering RNA (siRNA) knockdown of receptor expression. VEGF stimulation of intracellular cyclic adenosine monophosphate (cAMP) was not altered by co-incubation with aspartame at 10 µM. Exposure to aspartame at 10 µM increased intracellular ROS (measured as DCDFA fluorescence) and also depleted intracellular glutathione (GSH). Exposure to VEGF also increased intracellular ROS and depleted intracellular GSH, but co-incubation of VEGF (50 ng/mL) and aspartame (10 µM) resulted in ROS and GSH levels comparable to those in the non-exposed controls. Surface expression of VE-cadherin (detected by enzyme-linked immunosorbent assay, ELISA, of cells fixed to the plates) decreased after exposure to VEGF. Co-incubation with aspartame blocked the reduction in VE-cadherin surface expression. Enuwosa et al. (2021) concluded that, on the basis of the cAMP and VE-cadherin results, aspartame does not act through the signalling pathways associated with T1R3 signalling or VEGF injury.

(b)   *Experimental systems*

(i)   *Non-human mammals in vivo*

See Table 4.6.

In rats, administration of aspartame at 40 mg/kg bw per day by gavage for 6 weeks resulted in increased concentrations of GSH, catalase (CAT), or lipid peroxidation by thiobarbituric acid reactive substances (LPO/TBARS)

Table 4.6 End-points relevant to oxidative stress in non-human mammals in vivo exposed to aspartame

End-point	Species, strain (sex)/cell line	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
LPO/TBARS, SOD, CAT, GSH, O_2^-, H_2O_2, NO_2^-, $ONOO^-$	Rat, Wistar albino (M)	Erythrocytes	↑: O_2^-, H_2O_2, $ONOO^-$, LPO/TBARS, GSH, CAT No changes: NO_2^-, SOD	40 mg/kg bw per day	Oral gavage for 6 wk	Single dose.	Prokić et al. (2014)
LPO/MDA/TBARS, NO, GSH, GSSG, GSH RI	Rat, Wistar albino (M)	Blood	↓: GSH, GSH RI ↑: NO, LPO, GSSG	40 mg/kg bw per day	Drinking-water, 40 mg/kg bw per day for 6 wk	Single dose.	Prokić et al. (2015)
CAT, TAC	Rat, Wistar albino (M)	Blood	↓: CAT, TAC	250 mg/kg bw per day	Oral gavage for 8 wk (250, 1000 mg/kg bw per day)		Alkafafy et al. (2015)
LPO/MDA, NO, SOD, GPx, GSH, CAT, vit-C	Rat, Wistar albino (M)	Blood (serum)	↓: GSH, SOD, CAT, GPx, vit-C ↑: LPO/MDA, NO	40 mg/kg bw per day	Oral gavage for 90 days	Single dose.	Choudhary and Sheela Devi (2015)
Hsp70	Rat, Wistar albino (M)	Spleen, thymus, lymph node	↑	40 mg/kg bw per day	Oral gavage for 90 days	Indirect measurement of oxidative stress.	Choudhary and Sheela Devi (2016)
TAC, CAT, SOD, GPx, LPO/MDA, NO	Mouse, NMRI (M)	Blood (serum)	↓: TAC, GPx, SOD, CAT ↑: MDA, NO	80 mg/kg bw per day	Oral gavage for 90 days (40, 80 and 160 mg/kg bw per day)	Age 8–10 wk.	Anbara et al. (2020)
MDA, NO, SOD, TAC	Mouse, NMRI (M)	Blood (serum)	↓: TAC, SOD ↑: NO, MDA	80 mg/kg bw per day (SOD, NO, MDA), 160 mg/kg bw per day (TAC)	Oral gavage for 90 days (40, 80, 160 mg/kg bw per day)	Age 10–12 wk.	Anbara et al. (2022)
GSH, GSSG, γ-GC	Mouse, Swiss (M)	Liver	↓: GSH, GSSG, γ-GC	80 mg/kg bw per day	Oral gavage for 90 days	Single dose.	Finamor et al. (2017)
MDA, SOD, CAT, GPx	Mouse, Swiss (M)	Liver	↓: SOD, CAT, GPx ↑: MDA	80 mg/kg bw per day	Oral gavage for 12 wk	Single dose.	Finamor et al. (2021)
LPO/MDA, SOD, CAT, GPx, GSH	Rat (M)	Liver	↓: SOD, CAT, GPx, GSH ↑: LPO/MDA	75 mg/kg bw per day	Oral gavage for 30 days (75, 150 mg/kg bw per day)		Hamza et al. (2019)

Table 4.6   (continued)

End-point	Species, strain (sex)/cell line	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
LPO/TBARS, SOD, CAT, GPx, GSR, GSH	Rat, Wistar (M)	Liver	↓: GPx, GSR, GSH No changes: LPO/TBARS, SOD, CAT	500 mg/kg bw per day (GSH), 1000 mg/kg bw per day (GPx, GSR)	Oral gavage for 180 days (500, 1000 mg/kg bw per day)		Abhilash et al. (2011)
LPO/MDA, GPx, SOD, GST, GSH, CAT	Rat, Wistar albino (M)	Liver	↓: SOD, CAT, GPx, GSH, GST ↑: LPO/MDA	240 mg/kg bw per day	Oral gavage for 60 days	Single dose.	Lebda et al. (2017b)
LPO/MDA, GPx, SOD, GST, GSH, CAT	Rat, Wistar albino (M)	Brain	↓: SOD, CAT, GPx, GSH ↑: LPO/MDA, GST	240 mg/kg bw per day	Oral gavage for 60 days	Single dose.	Lebda et al. (2017a)
LPO/MDA, GPx, SOD, GST, GSH, CAT	Rat, Wistar (M)	Liver, kidney, brain	↓: SOD, CAT, GPx, GSH, GST ↑: LPO/MDA	35 mg/kg bw per day (SOD, CAT, GPx, GSH, GST), 15 mg/kg bw per day (LPO/MDA)	Oral gavage for 9 wk (15, 35, 70 mg/kg bw per day)		Adaramoye and Akanni (2016)
TBARS, vit-C, NPSH, TAC, GST, CAT	Rat, Wistar (M)	Liver Kidney	↓: vit-C, NPSH, TAC ↑: TBARS, GST, CAT ↑: GST, CAT No changes: TBARS, vit-C, NPSH, TAC	75 mg/kg bw per day	Oral gavage for 14 days	Single dose; vehicle, 2% DMSO; rats aged 21 days.	Souto et al. (2021)
MDA, GSH, GST, CAT	Rat, albino (M)	Liver, kidney, heart	↓: GST, GSH, CAT ↑: MDA	250 mg/kg bw per day	Oral gavage for 90 days	Used a commercial tablet; single dose.	Elballat and Abas (2020)
GSH, MDA, SOD, GPx	Rat, offspring	Kidney	↓: GSH, SOD, GPx ↑: MDA	40 mg/kg bw per day	Oral gavage from the first day of pregnancy until postnatal day 30	Single dose.	Fareed and Mostafa (2021)
LPO/TBARS, SOD, CAT, GPx, GSR, GST, LOOH	Rat, Wistar (M)	Brain	↓: SOD, CAT, GPx, GSR, GST ↑: LPO/TBARS, LOOH	40 mg/kg bw per day	Oral gavage for 6 wk (+ i.p. injection of NaCl from wk 5 for 2 wk)		Finamor et al. (2014)

Table 4.6 (continued)

End-point	Species, strain (sex)/cell line	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
LPO/MDA	Rat, SD (M)	Brain	↑: LPO/MDA	3 mg/kg bw per day	Drinking-water for 6 wk	Single dose.	Erbaş et al. (2018)
SOD, NO	Mouse, Swiss (M)	Brain (cerebral cortex)	↑: SOD, NO	40 mg/kg bw per day	Oral gavage for 28 days (20, 40, 80, 160 mg/kg bw per day)		Onaolapo et al. (2016)
SOD, NO	Mouse, Swiss (M)	Brain (hippocampal)	↑: SOD, NO	40 mg/kg bw per day (NO), 80 mg/kg bw per day (SOD)	Oral gavage for 28 days (20, 40, 80 160 mg/kg bw per day)		Onaolapo et al. (2017c)
LPO/MDA, SOD, GSH, NO	Mouse, Swiss (M)	Brain (cerebellum and pons)	↓: GSH ↑: SOD, NO, MDA	20 mg/kg bw per day (SOD, NO), 40 mg/kg bw per day (GSH, MDA)	Oral gavage for 28 days (20, 40, 80, 160 mg/kg bw per day)		Onaolapo et al. (2017b)
SOD, GPx, NO	Mouse, Swiss (M)	Brain	↓: GPx ↑: SOD, NO	160 mg/kg bw per day	Oral gavage for 21 days (160 and 320 mg/kg bw per day)		Onaolapo et al. (2017a)
LPO/MDA, GSH, NO	Mouse, Swiss albino (M)	Brain	↓: GSH ↑: MDA, NO	1.875 mg/kg bw per day	s.c. injection for 2 wk (0.625, 1.875, 5.625 mg/kg bw per day)		Abdel-Salam et al. (2012a)
LPO/MDA, GSH, NO	Mouse, Swiss albino (M)	Brain	↓: GSH ↑: MDA No changes: NO	11.25 mg/kg (GSH),22.5mg/kg bw (TBARS)	Single s.c. injection (11.25, 22.5, 45 mg/kg bw)		Abdel-Salam et al. (2012b)
		Liver	No changes: MDA, NO, GSH				
LPO/TBARS, GSH	Mouse, Swiss-Webster (M)	Brain (forebrain)	No changes: TBRAS, GSH	32 mg/kg bw per day	Drinking-water for 30 days	Single dose.	Abu-Taweel et al. (2014)
LPO/MDA, SOD, CAT	Rat, Wistar (M)	Spleen	↓: SOD, CAT ↑: LPO/MDA	500 mg/kg bw per day	Oral gavage for 1 wk	Single dose.	Ibrahim et al. (2022)
MPO, XO, LPO/MDA, SOD, CAT, GPx, GSH, total thiols	Rat, Wistar albino (M)	Heart	↓: MPO, SOD, CAT, GPx, thiols, GSH ↑: XO, LPO/MDA	75 mg/kg bw per day	Oral gavage for 4 wk (75, 150 mg/kg bw per day)		Al-Eisa et al. (2018)

Table 4.6   (continued)

End-point	Species, strain (sex)/cell line	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
TAC, CAT, SOD, GPx, LPO/MDA, NO	Mouse, NMRI (M)	Sperm, testis	↓: TAC, GPx, SOD, CAT ↑: MDA, NO	80 or 160 mg/kg bw per day	Oral gavage for 90 days (40, 80, and 160 mg/kg bw per day)		Anbara et al. (2021)
nNOS, NO	Rat, Wistar albino (M)	Brain	↑: NO	40 mg/kg bw per day	Oral gavage for 90 days (pre-treatment with MTX by s.c. injection, 0.2 mg/kg per day for 7 days)	Pre-treatment with MTX to mimic folate deficiency.	Iyaswamy et al. (2018)
LPO/MDA, GPx, SOD, GST, GSH, GSR	Rat, Wistar (M)	Sperm	↓: SOD, CAT, GPx, GSH, GSR ↑: LPO/MDA, GST	40 mg/kg bw per day	Oral gavage for 90 days (pre-treatment with MTX by s.c. injection, 0.2 mg/kg per day for 7 days)	Pre-treatment with MTX to mimic folate deficiency.	Ashok et al. (2017)
Hsp70	Rat, Wistar albino (M)	Spleen, thymus, lymph node	↑	40 mg/kg bw per day	Oral gavage for 90 days	Indirect measurement of oxidative stress.	Choudhary and Sheela Devi (2016)
LPO/MDA, SOD, GSH, CAT	Rat, Wistar albino (M)	Liver	↓: GSH ↑: SOD, CAT, LPO/MDA	40 mg/kg bw per day	Oral gavage for 90 days (pre-treatment with MTX by s.c. injection, 0.2 mg/kg per day for 7 days)	Pre-treatment with MTX to mimic folate deficiency.	Ashok et al. (2015)
LPO/MDA, SOD, GPx, GSH, CAT, protein thiols	Rat, Wistar albino (M)	Brain	↓: GSH, protein thiols ↑: SOD, CAT, GPx, LPO/MDA	75 mg/kg bw	Single oral gavage		
LPO/MDA, GSR, SOD, GPx, GSH, CAT, GST	Rat, Wistar albino (M)	Brain	↓: GSH, GSR ↑: LPO/MDA, SOD, CAT, GPx, GST	40 mg/kg bw per day	Oral gavage for 90 days (Pre-treatment with MTX by s.c. injection, 0.2 mg/kg per day for 7 days)	Pre-treatment with MTX to mimic folate deficiency.	Ashok and Sheeladevi (2014)

Table 4.6   (continued)

End-point	Species, strain (sex)/cell line	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
LPO, SOD, GPx, GSH, CAT, protein thiols	Rat, Wistar albino (M)	Brain	↓: GSH, protein thiols ↑: LPO, SOD, CAT, GPx	75 mg/kg bw per day	Oral gavage for 90 days (Pre-treatment with MTX by s.c. injection, 0.2 mg/kg per day for 7 days)	Pre-treatment with MTX to mimic folate deficiency.	Iyyaswamy and Rathinasamy (2012)

bw, body weight; CAT, catalase; DMSO, dimethyl sulfoxide; γ-GC, γ-glutamylcysteine; GPx, glutathione peroxidase; GSH, glutathione; GSH RI, glutathione redox index; GSR, glutathione reductase; GSSG, glutathione disulfide; GST, glutathione S-transferase; h, hour(s); HID, highest ineffective dose; H_2O_2, hydrogen peroxide; Hsp70, heat shock protein 70; i.p., intraperitoneal; LED, lowest effective dose; LOOH, lipid hydroperoxides; LPO, lipid peroxidation; M, male; MDA, malondialdehyde; MPO, myeloperoxidase; MTX, methotrexate; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; NO_2^-, nitrite; NPSH, non-protein thiol; O_2^-, superoxide anion; $ONOO^-$, peroxynitrite; s.c., subcutaneous; SOD, superoxide dismutase; TAC, total antioxidant capacity; TBARS, thiobarbituric acid reactive substances; vit-C, vitamin C; XO, xanthine oxidase.

[a] ↑, increase; ↓, decrease.

in erythrocytes, but SOD enzyme activity was unchanged (Prokić et al., 2014). In a subsequent study, the authors reported an increase in LPO and nitric oxide (NO) levels, together with significant alterations in GSH redox status in the blood of male Wistar albino rats (age 2 months) exposed to drinking-water containing aspartame at a dose of 40 mg/kg bw for 6 weeks (Prokić et al., 2015). In two groups of male Wistar albino rats (age 8 weeks) who were exposed to aspartame at daily doses equivalent to five times the ADI (250 mg/kg bw per day) or 10 times the ADI (1000 mg/kg bw per day) for 8 weeks, a decrease was observed in both CAT activity and TAC in blood plasma, when compared with the control group (Alkafafy et al., 2015). In two additional studies, adult male Wistar albino rats were exposed to aspartame at a dose of 40 mg/kg bw per day for 90 days. A significant increase in LPO and elevated levels of malondialdehyde (MDA) and NO were observed in the blood (Choudhary and Sheela Devi, 2015), as well as an increase in messenger RNA (mRNA) transcript levels of heat shock protein Hsp70 (an indirect marker of oxidative stress) in the spleen, thymus, and lymph nodes (Choudhary and Sheela Devi, 2016). In contrast, the activities of CAT and glutathione peroxidase (GPx) and levels of GSH and vitamin C were found to be reduced in the blood (Choudhary and Sheela Devi, 2015, 2016).

Markers of oxidative stress were also assessed in the blood of NMRI male mice (age 8–10 weeks) exposed to aspartame (40, 80, or 160 mg/kg bw per day) by gavage for 90 days. There was a significant increase in MDA levels at the highest dose and in nitric oxide (NO) levels at the intermediate and highest dose compared with the control group. Additionally, aspartame caused a reduction in TAC and CAT activity at the highest dose, resulting in a significant decrease in GPx and SOD activity at the intermediate and highest dose compared with the control group (Anbara et al., 2020). In a subsequent experiment by the same authors, NMRI male mice (age

10–12 weeks) were exposed to aspartame under the same conditions as in the previous study; the same biomarkers were studied and identical findings were reported (Anbara et al., 2022). In male Swiss mice exposed daily to aspartame at 80 mg/kg bw for 90 days, a 30% decrease in GSH levels in the liver was observed; there was no indication of GSH oxidation in the liver, despite the GSH reduction. Additionally, the administration of aspartame led to decreased levels of γ-glutamylcysteine in the liver and induced simultaneous depletion of glutathione disulfide (GSSG), maintaining the normal GSSG:GSH ratio (Finamor et al., 2017). In a later study, the same authors conducted a similar experiment, with the sole difference being an extended exposure duration of 12 weeks. Aspartame caused an increase in MDA levels, which was accompanied by a reduction in the activity of SOD, CAT, and GPx in the liver of exposed mice compared with the control group. Furthermore, mRNA levels of NAD(P)H quinone oxidoreductase 1 (Nqo1) and haem oxygenase 1 (Hmox1), which are known targets of Nrf2 (Nfe2 like bZIP transcription factor 2), were downregulated in the liver of aspartame-exposed mice compared with the control group (Finamor et al., 2021). Another experiment in male rats exposed to aspartame at low (75 mg/kg bw) or high (150 mg/kg bw) doses showed a reduction in the levels of SOD, CAT, GPx, and GSH in the liver (Hamza et al., 2019). In rats exposed to aspartame at doses of up to 1000 mg/kg bw per day by oral gavage for 180 days, tissue concentrations of GSH, GPx, and glutathione reductase (GSR) in the liver were decreased, but LPO/TBARS levels and SOD and CAT activities were unchanged (Abhilash et al., 2011). In a 2-month experiment, male Wistar strain albino rats (age 6–8 weeks) were exposed to aspartame at 240 mg/kg bw (equivalent to six times the ADI in humans). The aspartame-exposed rats exhibited a significant increase in MDA levels and a decrease in GSH levels, and GPx, CAT, and SOD activities in the liver, compared with the

control group (Lebda et al., 2017b). In another study by the same authors, which focused on the brain, it was reported that aspartame increased MDA levels and glutathione *S*-transferase (GST) enzymatic activity. Conversely, GPx, CAT, and SOD activity, and GSH levels were significantly reduced in the aspartame-exposed group (Lebda et al., 2017a).

In another experiment, adult male Wistar rats were exposed to aspartame at daily doses of 15, 35, or 75 mg/kg bw by oral gavage for 9 weeks. The administration of aspartame significantly increased levels of TBARS in the kidney, liver, and brain, and there was a concurrent decline in GSH levels. Additionally, exposure to aspartame at the intermediate and highest dose resulted in a significant decrease in the activities of SOD and GST in the kidney, liver, and brain. In the liver and brain, the activities of CAT and GPx were also adversely affected by aspartame, whereas no significant change was observed in the kidney (Adaramoye and Akanni, 2016).

Similar effects were observed in the kidney, liver, and brain of male Wistar rats (age 21 weeks) exposed to aspartame at 75 mg/kg bw by gavage for 14 days. The study reported an increase in TBARS in the liver, indicating elevated LPO. Consistent with these findings, non-enzymatic antioxidant defences were disrupted by aspartame exposure. Specifically, in the aspartame-exposed group, levels of vitamin C, non-protein thiol (NPSH), and TAC were reduced in the liver, although no significant changes in these biomarkers were reported in the kidney. There was an increase in the activities of hepatic and renal GST and CAT enzymes in rats exposed to aspartame compared with the control group (Souto et al., 2021).

Effects on oxidative stress markers in the liver, kidneys, and heart were assessed in male albino rats exposed daily to aspartame at 250 mg/kg bw by oral gavage for 90 days. The data consistently indicated that aspartame caused a significant rise in MDA levels and a significant decrease in GSH levels and GST and CAT activities across all tissues examined when compared with the control group (Elballat and Abas, 2020). Similarly, adult albino rats exposed to aspartame at 40 mg/kg bw both in utero and postnatally via oral gavage for 30 days after birth demonstrated a statistically significant rise in renal MDA levels associated with a significant decrease in GSH, SOD, and GPx levels compared with the control group (Fareed and Mostafa, 2021). Oral administration of aspartame at a daily dose of 40 mg/kg bw to adult male Wistar rats for 6 weeks concomitant with sodium chloride (NaCl) solution (154 mmol/L) from week 5 resulted in elevated levels of TBARS, lipid hydroperoxides (LOOH), and protein carbonyls in the brain compared with the control group. Additionally, brain CAT activity was lower in the aspartame-exposed group than in the control group. The same pattern was observed for GPx, glutathione reductase (GSR), and GST activities in the brain in rats exposed to aspartame. Moreover, brain non-protein thiol levels were also lower in the exposed group than in the control group. Conversely, brain levels of thrombin receptor-activating protein (TRAP) were higher in the exposed group than in the control group (Finamor et al., 2014). Male Sprague-Dawley albino mature rats exposed to drinking-water containing aspartame at a daily dose of 3 mg/kg bw displayed elevated levels of MDA in the brain (Erbaş et al., 2018). In a study in male Swiss mice (age 6 months), aspartame was administered orally at four different doses (20, 40, 80, and 160 mg/kg bw per day) for 28 days. In both the cerebral cortex and hippocampus of mice at 40, 80 and 160 mg/kg bw, SOD and NO levels showed a significant increase compared with levels in mice in the control group (Onaolapo et al., 2016, 2017c). In the pons and cerebellum of mice that underwent the same exposure paradigm, the same authors reported similar findings. Higher doses of aspartame led to an increase in the activity of SOD, and an elevation in MDA and NO levels. On the other hand, a decrease in

GSH levels was observed when compared with the control group (Onaolapo et al., 2017b). Lastly, Onaolapo et al. (2017a) conducted another study in male Swiss mice exposed to aspartame at 160 and 320 mg/kg bw by gavage for 21 days. The results were consistent with those of their previous studies with regard to SOD, GPx activity, and NO levels in the brain (Onaolapo et al., 2017a). Similar effects were observed in the brain of mice exposed to aspartame at doses up to 45 mg/kg bw or 5.625 mg/kg bw per day by single or multiple (for 2 weeks) subcutaneous injections, respectively. However, such changes were not observed in the liver (Abdel-Salam et al., 2012a, b). No changes in TBARS or GSH were seen in the brain of mice exposed to drinking-water containing aspartame at a dose of 32 mg/kg bw per day for 30 days (Abu-Taweel et al., 2014). Indications of aspartame-induced oxidative stress were also observed in the spleen of male Wistar albino rats (age 6–8 weeks) exposed to aspartame orally at 500 mg/kg bw per day for 1 week. In these rats, levels of LPO in the spleen tissues were increased, whereas the activities of SOD and CAT were significantly decreased compared with those in the control group (Ibrahim et al., 2022). Cardiac oxidative stress was assessed in adult male Wistar albino rats exposed orally to aspartame at 75 or 150 mg/kg bw per day for 4 weeks. The study revealed a significant increase in LPO levels and in the activities of MPO and xanthine oxidase (XO). Additionally, a substantial decrease in the activities of SOD, CAT, and GPx was detected, together with a reduction in levels of GSH and protein thiols. The effects of aspartame on the various markers were found to be dose-dependent when compared with the control group (Al-Eisa et al., 2018). Anbara et al. (2021) reported decreased TAC levels and SOD, CAT, and GPx activities in the sperm of mice exposed to aspartame at 40, 80, or 160 mg/kg bw per day by oral gavage for 90 days. Increased MDA and NO levels were also reported in the testis and sperm (Anbara et al., 2021).

[The Working Group identified several studies from the group of Ashok et al. (Ashok and Sheeladevi, 2014, 2015; Ashok et al., 2015, 2017). The authors, with the aim of mimicking physiological methanol toxicity in humans, and investigating the role of aspartame-derived methanol, investigated the effects of aspartame in conditions of folate deficiency (by pretreatment with methotrexate) in rodents. Long-term exposure to aspartame (40 mg/kg bw per day for 90 days) or a single dose of 75 mg/kg bw were both shown to induce LPO in the liver or in discrete regions of the brain, respectively. However, the lack of proper grouping, including the lack of a negative control group that was exposed to aspartame, limited the interpretation and use of the results.]

(ii)   Non-human mammalian cells in vitro

Aspartame was used to prevent the induction of LPO by ochratoxin A. When Vero (African green monkey kidney) cells were exposed to aspartame (0.5 mM [145 µg/mL]) for 24 hours, MDA production was not different from that in the controls (Baudrimont et al., 1997; Creppy et al., 1998).

4.2.4 Induces chronic inflammation

(a)   Humans

(i)   Exposed humans

See Table 4.7.

The potential for aspartame to exhibit the key characteristic "induces chronic inflammation", by eliciting changes in the key characteristic-associated end-points, was studied in humans in various settings, including consumption of aspartame-sweetened beverages (see Section 1.4.3).

Inflammatory markers

A large cross-sectional study investigated the associations between self-reported habitual intake of aspartame-sweetened beverages (using a food frequency questionnaire) and markers of inflammation in a cohort of women without

Table 4.7 End-points relevant to chronic inflammation in humans exposed to aspartame

End-point	Assay Biosample type	Location, setting, study design	Exposure level and no. of exposed and controls	Response[a] (significance)	Covariates controlled	Comments	Exposure assessment critique	Reference
Inflammatory markers								
CRP serum levels ICAM-1 serum levels Vascular adhesion protein 1 serum levels	Standard clinical chemistry Blood	USA, cross-sectional analysis on prospective cohort (Nurses' Health Study) Analysis: cycle 1, blood draw: 1989–1990; cycle 2, blood draw: 2000–2001	Aspartame-sweetened beverage intake among women Categories for selection: never or almost never; less than once per week; once to twice per week; three to six times per week; or one or more times per day	Frequency of intake associated with CRP serum levels: ($P < 0.048$) No association ($P > 0.05$) No association ($P > 0.05$)	Age, body weight, smoking status, physical activity, medication use and history of chronic diseases	Sample size for each biomarker varied: (CRP, $n = 5939$; ICAM-1, $n = 2290$; vascular adhesion protein 1, $n = 1205$).	Study population consisted of predominantly white health professionals; study authors noted possibility for residual confounding and reverse causation. There is a potential for measurement errors associated with the use of FFQs (frequency of exposure is self-reported). Furthermore, data on frequency of beverage consumption was used rather than actual intake to assess exposure.	Yu et al. (2018)

Table 4.7   (continued)

End-point	Assay Biosample type	Location, setting, study design	Exposure level and no. of exposed and controls	Response[a] (significance)	Covariates controlled	Comments	Exposure assessment critique	Reference
Coronary plaque characteristics: plaque segments, noncalcified, calcified, mixed Inflammatory markers: hsCRP, oxLDL, Lp-PLA2 Immune activation markers: sCD163, sCD14, MCP-1, CD4+, T-cell counts	CT angiography, physical activity questionnaire, standard blood clinical chemistry, immune markers. Blood	Boston, Massachusetts, USA, Matched control study	Dietary intake assessed using a 4-day food record. Cohorts: HIV-subjects (36) Non-HIV controls who consumed aspartame (15)	HIV cohort: ↑ number of coronary plaque segments ($P = 0.002$), noncalcified plaque segments ($P = 0.007$), and mixed segments ($P = 0.047$). Non-HIV cohort: ↑ number of calcified plaque segments ($P = 0.06$), no relationship with coronary plaque characteristics HIV cohort: ↑ MCP-1 ($P = 0.007$) and Lp-PLA2 ($P = 0.02$). ↓ hsCRP, oxLDL, and scCD163 and sCD14, although not significant ($P > 0.05$) Non-HIV cohort: no changes of any marker. ($P > 0.05$)	NA	Standardized 4-day food record based on self-reported dietary intake may not reflect chronic intake patterns.	No clear description of whether it was an open trial or blinded trial and time of sampling Study subjects consumed aspartame ~48 mg/day in 36 HIV-infected participants and ~24 mg/day in 15 non-infected controls.	Hall et al. (2017)

Table 4.7 (continued)

End-point	Assay Biosample type	Location, setting, study design	Exposure level and no. of exposed and controls	Response[a] (significance)	Covariates controlled	Comments	Exposure assessment critique	Reference
CRP serum levels	Standard clinical chemistry Blood	Mexico, 2007 Cross-sectional analysis of a prospective cohort study	Diet soda intake among women, divided into tertiles based on median intake: T1: 0 mL/day; (n = 540) T2: 11.8 mL/ day; (n = 122) T3: 50.7 mL/ day; (n = 163)	(–)	Age, biomarker batch, state of residence, socioeconomic status, family history of coronary heart disease, menopause, hormone use, smoking, alcohol use, physical activity, fruits and vegetables, and Western and modern Mexican dietary patterns	Intake of beverages containing sugar or artificial sweeteners served as a proxy for total dietary intakes.	Measurements limited to total beverages; aspartame exposure not determined.	Tamez et al. (2018)
CRP serum levels	Standard clinical chemistry Blood	Denmark, 2000 Two-arm-parallel randomized controlled trial, unblinded 10-week intervention	Sucrose group (n = 21) Sweetener group (n = 20)	No correlation was observed between dietary changes and changes in CRP	NA	Use of artificial sweetener mixture (54% aspartame, 23% cyclamate, 22% acesulfame-K, 1% saccharin).	Secondary analysis of an existing RCT. Not specific for aspartame.	Sørensen et al. (2005)

Table 4.7 (continued)

End-point	Assay Biosample type	Location, setting, study design	Exposure level and no. of exposed and controls	Response[a] (significance)	Covariates controlled	Comments	Exposure assessment critique	Reference
IL-6 TNFα Circulating neutrophils LPS-stimulated neutrophil degranulation	Standard clinical chemistry Blood	United Kingdom Randomized control study (counter balanced)	Fluid intake: 5 or 2 mL/kg body mass Healthy, trained soccer players ($n = 6$)	↑ Neutrophil count at 0 and 30 min post-exercise on both CHO ($P < 0.01$) and PLA ($P < 0.05$) trials ↑ IL-6 levels on CHO and PLA trials 0 and 30 min post-exercise ($P < 0.01$)	NA	In the PLA drink, ingredients were not defined. In the CHO drink, the presence of aspartame was not defined. Unclear sampling timing.	Artificially sweetened beverage. Type of beverage not reported. No information on type of beverage used for placebo. No information on background diet reported.	Bishop et al. (2002)
Allergic response								
Serum IgG, IgA, IgM, IgD, IgE C1q, C3, C4, factor B, glucose Histamine, epinephrine, and norepinephrine Histamine-induced cutaneous flare responsiveness and sensitivity	Cutaneous histamine reactivity. Blood (PBMCs)	Durham, North Caroline, USA, 1987 Double-blinded placebo-controlled crossover challenge study	30 mg/kg Overweight allergic subjects ($n = 40$)	Incidence of headache in exposed group was not different from incidence after placebo ($P = 0.5$)	NA	Abstract, lacked details.		Baraniuk et al. (1988)

Table 4.7   (continued)

End-point	Assay Biosample type	Location, setting, study design	Exposure level and no. of exposed and controls	Response[a] (significance)	Covariates controlled	Comments	Exposure assessment critique	Reference
Allergic response defined as urticaria or angioedema	Observation/ pathology Skin	USA and Canada, 1988–1991 Multicentre, randomized, double-blind crossover trial	50, 300, 600 mg, oral administration, three times daily for two alternate days Subjects (both sexes, children and adults, $n = 21$; 4 males, 17 females; age, 10–55 yr)	No statistically significant difference in the occurrence of allergic reactions after aspartame compared with placebo ($P = 1.00$).	Heart rate, respiratory rate, temperature, and blood pressure of individuals who had allergic reactions	Small sample size ($n = 21$). Findings limited to scoring allergic reactions.	Mixed group of children and adults, males and females. Recruitment: selection for those reporting adverse allergic reaction to aspartame-containing products. This affected sample size despite widespread recruitment over 4 yr.	EFSA UN07 (2011)

Table 4.7 (continued)

End-point	Assay Biosample type	Location, setting, study design	Exposure level and no. of exposed and controls	Response[a] (significance)	Covariates controlled	Comments	Exposure assessment critique	Reference
Reproduction of historical report of hypersensitivity reaction defined as nausea, difficulty swallowing, vomiting, abdominal pain, diarrhoea, abdominal distention, flatulence, urticaria. angioedema, pruritus, cutaneous flushing, eczema. rhinitis, asthma, hypotension, syncope, and/or headache Allergic response as defined by a positive skin test Decrease in lung function as defined by decrease in forced expiratory volume (FEV) > 20%	Observation/ pathology Skin test (prick skin method to various aeroallergens). Lung function tests, breaths.	Maryland, USA, 1986 Combined single-blind, double-blind, placebo-controlled study. Healthy subjects *n* = 5 (1 male, 4 females), Patients with allergic reactions atopic, *n* = 6 (2 males, 4 females) out of 61 referrals	Aspartame dose: 10, 100, 500, 1000, and 2000 mg oral administration	No subject with a clear and reproducible adverse reaction to aspartame was identified.	NA	Small sample size. Findings limited to scoring allergic reactions. No. of exposed and controls provided).	No demographic information provided on the participants. Recruitment: was selective selection for those reporting adverse allergic reaction to aspartame-containing products, which affected sample size.	Garriga et al. (1991)

acesulfame-K, acesulfame potassium; CHO, carbohydrate; CT, computed tomography; CRP, C-reactive protein; FEV, forced expiratory volume; FFQ, food frequency questionnaire; hsCRP, high-sensitivity C-reactive protein; Ig, immunoglobulin; LPS, lipopolysaccharide; min, minute(s); NA, not available; oxLDL, PLA, placebo; PBMC, peripheral blood mononuclear cells; RCT, randomized controlled trial; yr, year

↓ decrease; ↑ increase; (–), negative result in a study of limited quality.

diabetes or cardiovascular disease (n = 8492) in the USA (Yu et al., 2018). The study reported that more frequent intake of aspartame-sweetened beverages was associated with higher blood concentrations of C-reactive protein (CRP) (P for trend, 0.048) and with higher serum concentrations of adiponectin (P for trend, < 0.01). [The Working Group noted that adiponectin, the most abundant adipokine circulating in human blood, seems to play dual roles in both pro-inflammation and anti-inflammation in various chronic diseases in humans (Choi et al., 2020).] However, intake of aspartame-sweetened beverages did not appear to be associated (P > 0.05) with levels of the other markers of inflammation tested, such as intracellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion protein 1 (Yu et al., 2018). [The Working Group noted that the reported effects were adjusted for BMI.]

In another cross-sectional study that aimed to characterize the relationship between dietary sweeteners and metabolic dysregulation and cardiovascular disease in a cohort of people who were or were not infected with human immunodeficiency virus (HIV), aspartame consumption in the HIV-positive group (n = 36) was positively (but non-significantly) associated with the inflammatory markers lipoprotein-associated phospholipase A2 (Lp-PLA2; P = 0.02), high-sensitivity C-reactive protein (hsCRP; P = 0.06), and oxidized low-density lipoprotein (oxLDL; P = 0.07). Aspartame intake was associated with a significantly increased level of the immune activation marker monocyte chemoattractant protein 1 (MCP-1; P = 0.007), but there was no association with soluble cluster of differentiation 163 (sCD163) or 14 (sCD14), two other measured markers of monocyte or macrophage activation.

No associations between aspartame intake and markers of inflammation and immune activation were observed among the aspartame consumers in the HIV-negative group (n = 15) (Hall et al., 2017). [The Working Group noted that patterns of consumption of the dietary

sweetener in the cohort with 124 HIV-positive and 56 HIV-negative participants were determined on the basis of completion of a 4-day food record. Of these two groups of study participants, 36 HIV-positive and 15 HIV-negative participants were identified as aspartame consumers. The Working Group noted that aspartame intake in the HIV-negative group was much lower than in the HIV-positive group (89 ± 80 versus 164 ± 163 mg/day, P = 0.03) and the sample size was smaller (15 versus 36).]

In the HIV-positive participants in the same study, aspartame consumption was also positively associated with number of coronary plaque segments (P = 0.002), number of noncalcified plaque segments (P = 0.007), and number of mixed plaque segments (P = 0.047). Also, in the HIV-negative participants, aspartame intake appeared to be positively associated with number of calcified plaque segments (P = 0.06), although the findings were not statistically significant (Hall et al., 2017). [The Working Group noted that within this study, the aspartame consumption level was approximately twice as high in the HIV-positive group as in the HIV-negative group. Aspartame consumption was estimated to be 48 mg/day and 24 mg/day among HIV-positive participants and HIV-negative participants, respectively (P = 0.07). The 4-day dietary records provided limited insights into past dietary patterns and might not be reliable indicators of aspartame exposure. Also, the Working Group considered that this study was informative because chronic inflammation is a component of cardiovascular diseases often characterized by coronary plaques (Sorriento and Iaccarino, 2019), although some limitations of the study were identified. For example, no criteria for BMI, a major risk factor for cardiovascular events, were used in patient selection. Recruitment of HIV-positive and control study participants without regard to changes in body composition, weight, or metabolic criteria has been described by Lo et al. (2010); however,

they were considered to be a limitation of that study according to Hall et al. (2017). Exposure to aspartame was quantified from 4-day food records using a US database, although no details on the relevancy and history of the database were provided. The Working Group noted that information on food sources of the sweeteners and duration of aspartame intake was not provided.]

Several other studies investigated the effects of consumption of artificially sweetened food and/or artificially sweetened beverages (ASBs) on markers of inflammation in exposed humans; however, these studies were not particularly informative because the exposure was not clearly identified (Tamez et al., 2018), or because a mixture of artificial sweeteners was investigated (Bishop et al., 2002; Sørensen et al., 2005). For example, two studies in exposed humans reported no association between artificial sweetener consumption and increased levels of CRP. In a cross-sectional study of women from the Mexican Teachers' cohort, no association was reported between CRP and diet soda consumption (Tamez et al., 2018). [The Working Group noted that the focus of the exposure was total beverages, and thus exposure to aspartame was unclear.] Similarly, no correlation was observed between artificial sweetener intake and serum CRP in a study on a randomized controlled trial with a two-arm parallel, unblinded, design in Denmark (Sørensen et al., 2005). [The Working Group noted that an artificial sweetener mixture containing 54% aspartame (the other components being 23% cyclamate, 22% acesulfame-K, and 1% saccharin) was administered to the intervention diet group.]

Bishop et al. (2002) examined the effect of ingesting a carbohydrate beverage ("CHO" solution composed of 6.4% w/v glucose and maltodextrin) or an artificially sweetened placebo drink on plasma interleukin 6 (IL-6), tumour necrosis factor alpha (TNFα), and lipopolysaccharide (LPS)-stimulated neutrophil degranulation responses to high-intensity intermittent

running. There was a significant increase in neutrophil count, IL-6, and TNFα levels at both 0 and 30 minutes post-exercise in people who had consumed the CHO drink and in people who consumed the placebo, although changes in TNFα were non-significant. LPS-stimulated elastase release per neutrophil, an indicator of neutrophil degranulation, was lower than it was before exercise in individuals who had been received the CHO drink, although this was also non-significant. [The Working Group noted that this study was deemed less relevant to aspartame since no information on the presence of aspartame in the carbohydrate beverage or in the placebo beverage was provided, and information on the background diet of the study participants was not reported.]

Allergic response

A double-blind, placebo-controlled cross-over study reported that a 35% incidence of headache in aspartame consumers (30 mg/kg) was not significantly different from the 45% in the placebo group ($P = 0.5$). The analysis of the various parameters did not indicate any allergic association with aspartame consumption (Baraniuk et al., 1988). [The Working Group noted that this study summary was provided as an abstract only.]

In a regulatory study carried out in 1993 for marketing authorization, the authors reported that participants ($n = 21$) who had previously reported experiencing allergy (defined as urticaria or angioedema) to aspartame showed no difference in the occurrence of allergic reaction when challenged with aspartame or placebo in a controlled study. The study reported a non-statistically significant difference in the occurrence of allergic reactions after taking aspartame compared with placebo (EFSA_UN07, 2011). [The Working Group noted that the sample size was small ($n = 21$) and included male and female children and adults. Recruitment was selective for those reporting adverse allergic reaction to

Table 4.8 End-points relevant to chronic inflammation in human primary cells exposed to aspartame

End-point	Tissue, cell line	Results[a]	Concentration	Comments	Reference
IL-6 sIL-6r VEGF-A sVEGFR-2 secretion	HUVEC IMR-90 fibroblasts	HUVEC: ↑ IL-6 and sIL-6r (after 48 and 72 h) ($P < 0.05$); ↑ VEGF-A (dose-dependent) and sVEGFR-2 after 48 h and 72 h) ($P < 0.05$). IMR-90 fibroblasts: No changes on IL-6, sIL-6r, VEGF-A, or sVEGFR-2 ($P > 0.05$)	20, 40, 60, 80, 100 µM; up to 72 h		Alleva et al. (2011)
IL-6 IL-10 IFNγ	Lymphocytes (primary)	↓ IL-6 secretion ($P < 0.05$). No effect on levels of IL-10 or IFNγ ($P > 0.05$).	10 µg/mL (per well)	Only one dose tested	Rahiman and Pool (2014)

h, hour(s); HUVEC, human umbilical vein endothelial cells; IFNγ, interferon gamma; IL, interleukin; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

[a] +, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study); ↑, increase; ↓, decrease; (+) or (–), positive or negative result in a study of limited quality.

aspartame-containing products, which affected the sample size, despite widespread recruitment over 4 years.]

In a combined single-blind, double-blind, placebo-controlled study ($n = 12$), the capacity of aspartame to reproduce the historically reported hypersensitivity reaction, allergic response (indicated by a positive skin test result), or a decrease in lung function was investigated. Like the above study (EFSA_UN07, 2011), no participant with a clear and reproducible adverse reaction to aspartame was identified (Garriga et al., 1991).

(ii)   Human primary cells in vitro

See Table 4.8.

Two studies in primary human cells were relevant to chronic inflammation. In one study, HUVEC cells and IMR-90 fibroblasts were exposed to aspartame at concentrations ranging from 20 to 100 µM. In HUVEC (but not in IMR-90 fibroblasts), aspartame at 20 µM increased the secretion of IL-6 into the growth medium by twofold, with respect to controls, after 48 hours of incubation. IL-6 release was associated with an accompanying release of soluble interleukin-6

receptor (sIL-6r) after 24 hours of incubation, although these effects were not dose-dependent. Dose-dependent increases in cytokines/growth factors VEGF-A and sVEGFR-2 were reported in HUVEC but not in IMR-90 cells (Alleva et al., 2011). [The Working Group noted that Alleva et al. also showed that aspartame induced angiogenesis in HUVEC cells. At low doses, aspartame was a potential angiogenic agent that could induce regenerative cytokine production. It enhanced release of IL-6 and VEGF and their soluble receptors from endothelial cells and lead to the activation of mitogen-activated protein kinases (MAPKs) (extracellular signal-regulated kinase, ERK, and p38 phosphorylation), resulting in the formation of new blood vessels.]

Another study that investigated perturbations in cytokines IL-6, IL-10, and interferon gamma (IFNγ) in whole blood samples ($n = 21$) from human donors exposed to various artificial sweeteners (10 µg/mL), including two brands of commercial artificial sweetener containing aspartame, found no effects in unstimulated cells (Rahiman and Pool, 2014). [The Working Group noted that aspartame was one of the main

ingredients of one of the two aspartame-containing artificial sweeteners, although this branded sweetener also contained maltodextrin and acesulfame-K and may have contained dextrose, depending on the actual formulation applied. Similarly, although aspartame was an important component of the other branded sweetener, the product website indicated that this sweetener contained only 8% aspartame, the primary ingredient being lactose". This information was sought from external sources and was not provided or referenced in either of the primary studies.] After LPS stimulation, the two brands of aspartame-containing artificial sweetener blunted IL-6 secretion relative to the levels in the controls. There were no reported differences in IL-10 synthesis in phytohemagglutinin-stimulated and unstimulated whole blood cultures incubated with either branded aspartame-containing sweetener, relative to untreated controls. Similarly, the comparison of all sweeteners with the control cultures under both stimulated and unstimulated conditions showed that neither branded aspartame-containing sweetener had an effect on IFNγ synthesis, a biomarker of cell-mediated immunity ($P > 0.05$) (Rahiman and Pool, 2014).

(b) Experimental systems

See Table 4.9.

(i) Non-human mammals in vivo

In several studies in vivo, the inflammatory response and chronic inflammation were evaluated by means of measuring alterations in cytokine levels, tissue inflammation, and alterations in inflammation-related gene transcription.

Tissue inflammation

A histopathological analysis of the liver of rats exposed to aspartame at a dose of 240 mg/kg bw by intragastric intubation for 60 days showed severe hydropic degeneration of hepatocytes, which was characterized by swollen cells and clear fluids that replaced the cytoplasm, although nucleus, shape, and location were unaffected. Additionally, intense inflammatory cell aggregation in the portal area, congestion of the portal vein with focal hepatic necrosis, and infiltration of inflammatory cells were observed. Out of the five rats analysed, three showed inflammatory cell infiltration in the portal area (moderate in one rat and mild in two rats). In addition, four rats exhibited hepatic necrosis inflammatory cell infiltration (severe in one rat, moderate in two rats, and mild in one rat) (Lebda et al., 2017b). [The Working Group noted that this was a semi-quantitative histopathological analysis, that the descriptions of inflammation appeared to be inconsistent, and that portal vein congestion may be an artefact since this outcome is often seen in rats that have not been completely exsanguinated. Also, no statistical analysis was conducted for this study, and only five rats were examined microscopically.]

In another study, histopathological analysis of the livers of male Wistar rats exposed to aspartame at 1000 mg/kg bw for 180 days (by oral intubation) indicated a statistically significant increase in leukocyte infiltration in rats compared with the control group, but no pathological changes in the liver of rats exposed at 500 mg/kg bw (Abhilash et al., 2011).

Hamza et al. (2019) demonstrated that, together with altered liver function, alterations in oxidative biomarkers (see also Section 4.2.3), serum hepatic antioxidant enzyme and thiol levels, and lipid profile, and increases in inflammatory cytokines, rats exposed to aspartame at 75 mg/kg bw for 30 days exhibited major alterations in liver histology. Some hepatocytes displayed a large area of haemorrhage surrounded by necrotic and apoptotic hepatocytes. Hepatic lobules of rats exposed to aspartame at 150 mg/kg bw for 30 days exhibited a confused parenchymal architecture, some hepatocytes had infrequent nuclei and a vacuolated cytoplasm, and blood sinusoids were dilated and congested. Kupffer

Table 4.9 End-points relevant to chronic inflammation in non-human mammalian systems in vivo exposed to aspartame

End-point	Species, strain, (sex), tissue	Results	Dose (LED, HID)	Route, duration, dosing regimen	Comments	Reference
Tissue inflammation						
Inflammatory cell infiltration	Rat, Wistar albino (M), liver	Congestion of the portal vein and focal hepatic necrosis with inflammatory cell infiltration Fibrosis: not clearly identified	240 mg/kg bw per day aspartame	Control group: allowed to drink water ad libitum Aspartame group: oral intubation on dose-per-day basis Soft drink group: allowed to freely drink commercial cola beverages for 2 months; three groups (6 mice/group)	Only one dose group in which aspartame was administered.	Lebda et al. (2017b)
Leukocyte infiltration	Rat, adult Wistar, (M), liver	Increased leukocyte infiltration (1000 mg/kg) Fibrosis: not clearly identified	1000 mg/kg bw per day	Oral intubation, 0, 500, 1000 mg/kg bw per day for 180 days (6 rats/group)		Abhilash et al. (2011)
Inflammatory cell infiltration	Rat (M), liver	Accumulation of inflammatory cells in the portal tract (150 mg/kg) Fibrosis: not clearly identified	150 mg/kg bw per day	Oral administration, 0, 75, 150 mg/kg bw per day for 30 days (8 rats/group)	Hepatotoxicity was observed.	Hamza et al. (2019)
Inflammatory cell infiltration	Rat, Wistar albino (M) (age 7 wk), liver	Congestion of the portal vein and focal hepatic necrosis with inflammatory cell infiltration Fibrosis: not clearly identified	250 mg/kg bw per day	Oral administration, 0, 250, 1000 mg/kg bw per day for 8 wk (5 rats/group)		Alkafafy et al. (2015)

Table 4.9   (continued)

End-point	Species, strain, (sex), tissue	Results	Dose (LED, HID)	Route, duration, dosing regimen	Comments	Reference
Inflammatory cell infiltration Fibrosis	Rat, Sprague-Dawley albino (M), liver	Necrosis, disorganization of hepatic tissue, and mononuclear cell infiltration Fibrosis: increased deposition of collagen fibres around portal area in aspartame-only group Collagen fibres in congested blood vessel and bile ducts in diabetes-induced model	200 mg/kg bw per day	Oral administration, 0, 200 mg/kg bw per day for 4 wk (10 rats/group)	Only one dose tested. One additional dose group in which aspartame was administered in a streptozotocin-induced diabetes model.	Khidr et al. (2017)
Leukocyte infiltration	Mouse, Swiss (M), liver	Hepatocellular injury, leukocyte infiltration, reduction in nuclear area, and degeneration of hepatocytes with increased liver sinusoidal diameter in different areas of the liver Fibrosis: not identified	80 mg/kg bw per day	Oral gavage, 0, 80 mg/kg bw per day for 90 days (6 mice/group)	Only one dose tested. One additional dose group in which mice received i.p. NAC at 163 mg/kg on days 60–90 immediately after aspartame exposure.	Finamor et al. (2017)
Leukocyte infiltration	Mouse, Swiss (M), liver	Increased number of leukocytes per mm^2 of liver Fibrosis: increased deposition of collagen fibres	80 mg/kg bw per day	Oral gavage, 0, 80 mg/kg bw per day for 12 wk (6 mice/group)	Only one dose tested.	Finamor et al. (2021)
Fibrosis	Rat, Wistar Albino (M), spleen,	No inflammation recorded Fibrosis: thickening with fibrosis in splenic capsules, lymphoid depletion and necrosis in white pulp	500 mg/kg bw per day	Oral administration, 0, 500 mg/kg bw per day for 1 wk (8 rats/group)	Study assessed the protective effects of seaweed.	Ibrahim et al. (2022)

Table 4.9 (continued)

End-point	Species, strain, (sex), tissue	Results	Dose (LED, HID)	Route, duration, dosing regimen	Comments	Reference
Mechanical foot paw withdrawal thresholds Knee joint inflammation	Rat, Sprague-Dawley (M), foot/paw Knee joint	No effect on attenuating inflammatory response induced by carrageenan injection ↓ Mechanical hyperalgesia at 50 mg/kg Fibrosis: not assessed	50 mg/kg bw per day for hyperalgesia only	s.c. injection, 0, 25, 50, 100 mg/kg bw daily for 6 days; (8–10 rats/group) followed by intra-articular injection of carrageenan.	Aspartame co-administered with carrageenan.	LaBuda and Fuchs (2001)
Foot oedema	Rat, Sprague-Dawley (M), foot/paw	No effect Fibrosis: not assessed		i.g. administration, 0, 300 mg/kg, 1 h before injection of carrageenan) (8 rats/group)	Aspartame administered before carrageenan.	Aspinall et al. (1980)
Altered cytokine levels						
TNFα	Mouse, Swiss albino (M, F), peripheral blood	↑	22.5 mg/kg	i.p. injection 0.625, 1.875, 5.625, 11.25, 22.5 mg/kg (+ 100 µg/kg LPS) or 22.5, 45 mg/kg (no LPS) (single administration before euthanasia) (6 mice/group)		Abdel-Salam et al. (2012a)
IL-6 TNFα Serum CRP	Rat (M), liver	↑ (dose-dependent) ↑ ↑ (dose-dependent)	75 mg/kg bw per day	Oral administration, 0, 75, 150 mg/kg daily for 30 days (8 rats/group)		Hamza et al. (2019)
IL-2 IL-4 TNFα IFNγ Corticosteroid	Rat, adult Wistar (M), peripheral blood	↓ ↑ ↓ ↓ ↑	40 mg/kg bw per day	Oral gavage, 0, 40 mg/kg bw daily for 90 days (6 rats/group)	Only one dose group.	Choudhary and Sheela Devi (2015)
Phosphorylation of p65 subunit of NF-kB and NLRP3 inflammasome activation	Mouse, Swiss (M), liver	↑	80 mg/kg bw per day	Oral gavage, 0, 80 mg/kg bw daily for 12 wk (6 mice/group)	Only one dose group.	Finamor et al. (2021)

Table 4.9   (continued)

End-point	Species, strain, (sex), tissue	Results	Dose (LED, HID)	Route, duration, dosing regimen	Comments	Reference
CD11b+ cells	Mouse, C57BL/6J (age 3 wk) (M), peripheral blood	↑, slight increase	100 µM	Oral administration, 100 µM solution with equivalent sweetness of 30% w/v sucrose solution Fed ad libitum for 8 wk (8–9 mice/group)	Only one dose group.	Choi et al. (2017)
Altered inflammation-related gene transcription						
TNFa NF-κB	Rat, adult Wistar (M), liver	↑ ↑	40 mg/kg bw per day	Oral gavage, 0, 40 mg/kg bw daily for 90 days; folate-deficient diet was provided for 45 days before the experiment and MTX was administered for 1 wk before the experiment; three groups (6 rats/group)	Only one dose group in which MTX was administered; did not investigate effects of MTX isolation.	Ashok and Sheeladevi (2015)
TNFa, CXCL1, Il22ra2, Creb3I3, Gnb2I1, Pex13, Ptgs1	Mouse, C57BL/6J (age 3 wk) (M), brain	Unclear effects	100 µM	Oral administration, 100 µM solution with equivalent sweetness of 30% w/v sucrose solution Fed ad libitum for 8 wk (8–9 mice/group)	Only one dose group.	Choi et al. (2017)

Table 4.9   (continued)

End-point	Species, strain, (sex), tissue	Results	Dose (LED, HID)	Route, duration, dosing regimen	Comments	Reference
Adamts9, Fga, Fgb, Fgg, Plg, Hpx, Itih4, Ptgs/cox2, Tdo2, Kynu, Adipoq, Fabp4, Scarb1, Cd36 ALDH1	Mouse, C57BL/6J (M, F) Adrenal glands Brain Hypothalamus	↓ ↑ ↑	45.44 ± 1.16 mg/kg bw (aspartame) 46.59 ± 0.68 mg/kg bw (aspartame + developmental NMDAR antagonist CGP 39551)	Oral administration, mothers treated for 3 wk until breeding, then up to postnatal day 28; F₁ offspring treated up to week 21 Control group: ad libitum drinking-water NMDAR antagonist group: 5 mg/kg bw in drinking-water Aspartame: 0.25 g/L in drinking-water, equivalent to aspartame at 45.44 ± 1.16 mg/kg bw per day Aspartame + NMDAR antagonist: 0.25 g/L in drinking-water, equivalent to aspartame at 46.59 ± 0.68 mg/kg bw per day	Assessed gene transcription only using microarray and RT-PCR.	Collison et al. (2018)
Pro-fibrotic: *TGFβ, COL1A1, αSMA* Pro-inflammatory: *IL-1b, IL-6, CXCL1, IL-10, IL-18*	Mouse, Swiss (M) (*n* = 12), liver	↑ ↑	80 mg/kg bw per day	Oral gavage, 0, 80 mg/kg bw daily for 12 wk; two groups of mice (6 mice/group)	Only one dose group.	Finamor et al. (2021)

Table 4.9 (continued)

End-point	Species, strain, (sex), tissue	Results	Dose (LED, HID)	Route, duration, dosing regimen	Comments	Reference
TNFa, IL-6	Rat, Wistar albino (M) (*n* = 32), spleen	↑	500 mg/kg bw	Oral administration, 500 mg/kg bw daily for 1 wk; two additional dose groups, one group treated with *S. vulgare*-MeOH extract (150 mg/kg bw per day for 3 wk), the other group was treated with 500 mg/kg bw per day (aspartame) orally for 1 wk before *S. vulgare*-MeOH extract exposure		Ibrahim et al. (2022)
Leptin Adiponectin	Rat, Wistar albino (M) (*n* = 30), adipose tissue	↑ ↓	240 mg/kg bw per day aspartame	Three groups (5 rats/group) Control group: allowed to drink water ad libitum Aspartame group: i.g. intubation on dose-per-day basis Soft drink group: allowed to freely drink commercial cola beverages for 2 months (Alkhedaide et al., 2016)	Only one dose group in which aspartame was administered.	Lebda et al. (2017b)

CRP, C-reactive protein; F, female; h, hour(s); HID, highest ineffective dose; IFNγ, interferon gamma; i.g., intragastric; IL, interleukin; i.p., intraperitoneal; LED, lowest effective dose; LPS, lipopolysaccharide; M, male; MeOH, methanol; MTX, methotrexate; NA, not available; NAC, *N*-acetyl-ʟ-cysteine; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B-cells; RT-PCR, reverse transcription-polymerase chain reaction; s.c., subcutaneous; TNFα, tumour necrosis factor alpha; wk, week; w/v, weight per volume.

[a] +, positive; −, negative; +/−, equivocal (variable response in several experiments within an adequate study); ↑, increase; ↓, decrease; (+) or (−), positive or negative result in a study of limited quality.

cells became more prominent. The central vein was markedly dilated and congested and there was an accumulation of inflammatory cells in the portal tract. [The Working Group noted that the number of rats exhibiting these microscopic alterations was not reported.]

Similar results were reported with higher doses of aspartame: liver sections from male Wistar albino rats (age 7 weeks) (n = 25) who were treated with aspartame at 250 mg/kg bw or 1000 mg/kg bw showed various histological changes, including disorganized hepatic parenchyma, dilatation and congestion of the central vein and hepatic sinusoids, and proliferation of Kupffer cells. Some hepatocytes showed signs of degenerative changes in the form of cellular swelling and vacuolar cytoplasm, and others were necrotic. In rats at 250 mg/kg bw, liver sections presented focal areas of complete degeneration and diffuse and focal intralobular mononuclear cell infiltration; these changes were more pronounced in liver sections from rats at 1000 mg/kg bw (Alkafafy et al., 2015). [The Working Group noted that pathology descriptions were reported in the text but did not reflect the histopathology figures provided.]

Khidr et al. (2017) reported marked necrosis, disorganization of hepatic tissue, and mononuclear cell infiltration in the livers of adult male Sprague-Dawley rats exposed to aspartame at a dose of 200 mg/kg bw once daily for 4 weeks. In rats in which diabetes had been induced (via a single intraperitoneal dose of streptozotocin at 70 mg/kg bw) before exposure to aspartame, similar findings were reported, namely the presence of large necrotic area, loss of most of the architecture of the liver tissue, loss of cell boundaries, and degeneration in most of the hepatocytes.

Finamor et al. (2017) reported that administration of aspartame at a dose of 80 mg/kg bw by oral gavage to male Swiss mice for 90 days increased hepatocellular injury, triggered leukocyte infiltration, reduced nuclear volume, and

contributed to the degeneration of hepatocytes with increased sinusoidal diameter in different areas of the liver. [The Working Group noted that these results were described by the study authors and that the changes described were not discernible in the figures presented in the manuscript.] In a subsequent publication, Finamor et al. (2021) showed that administration of aspartame at a dose of 80 mg/kg bw by oral gavage to male Swiss mice for 12 weeks induced statistically significant inflammatory infiltration, calculated as number of leukocytes per mm^2 of liver tissue. Evidence of fibrosis, as demonstrated by the elevated deposition of collagen fibres in the mouse liver, was also reported in aspartame-exposed mice. [The Working Group noted that the microscopic changes observed appeared to be nonspecific.] These changes were accompanied by the upregulation of several pro-fibrotic markers (e.g. transforming growth factor beta, TGFβ, collagen type 1 alpha 1, and alpha smooth muscle actin), and other pro-inflammatory transcripts (Il6, Cxcl1, Il1b, and Il18) (Finamor et al., 2021). Similarly, rats exposed orally to aspartame (500 mg/kg bw per day for 1 week) showed fibrosis in splenic capsules, lymphoid depletion, and necrosis in the white pulp, accompanied by elevated levels of pro-inflammatory splenic transcripts of TNFα and IL-6 (Ibrahim et al., 2022).

Another study indicated that the subcutaneous administration of aspartame (at concentrations of 2.5, 5, and 10 mg/mL in distilled water, to reach final doses of 25, 50, and 100 mg/kg, delivered in an injection volume of 10 mL/kg) to male Sprague-Dawley rats could attenuate mechanical hyperalgesia (heightened sensitivity to pain) but did not decrease carrageenan-induced inflammation (LaBuda and Fuchs, 2001). Similarly, Aspinall et al. (1980) reported that aspartame (300 mg/kg) administered intragastrically before carrageenan injection did not have any effect on the development of arthritic swelling in male rats with adjuvant arthritis. [The Working Group noted that these studies may be of limited

relevance to the key characteristic of carcinogens "induces chronic inflammation".]

Altered cytokine levels

TNFα levels, measured via ELISA, in the brain tissue of Swiss albino mice exposed subcutaneously to aspartame in saline solution at doses of 22.5 or 45 mg/kg were elevated relative to levels in controls exposed intraperitoneally to saline solution at 0.1 mL/mouse (Abdel-Salam et al., 2012a). Dose-dependent increases in levels of hepatic pro-inflammatory cytokines, TNFα, and IL-6, and serum CRP were observed in male rats exposed orally to aspartame (75 or 150 mg/kg) for 30 days (Hamza et al., 2019). In a 90-day study in which male Wistar albino rats were exposed orally to aspartame at 40 mg/kg bw per day, there was a marked increase in plasma corticosteroid levels, and an increase in circulatory IL-4 levels, whereas a significant decrease in levels of the cytokines IL-2, TNFα, and IFNγ was reported (Choudhary and Sheela Devi, 2015). These effects were reported in aspartame-exposed rats that had or had not been intraperitoneally immunized with sheep erythrocytes, and in groups of rats that had been fed either a normal diet or a folate-deficient diet.

Finamor et al. (2021) reported increased levels of p65 protein and phospho-p65 (Ser536) of nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB) in the livers of aspartame-exposed male Swiss mice relative to study controls. This study also reported higher levels of NLR family pyrin domain containing 3 (NLRP3) and cleaved caspase-1 protein levels in the livers of exposed rats, suggesting a role for aspartame in inflammasome activation.

Altered inflammation-related gene transcription

As described above, levels of mRNA expression of inflammation-related transcripts were upregulated by aspartame in the liver of male Swiss mice (*Il6*, *Cxcl1*, *Il1b*, and *Il18*) (Finamor et al., 2021) and in the spleen of exposed male

Wistar albino rats (*TNFα* and *Il6*) (Ibrahim et al., 2022). Several studies in vivo solely examined the effects of aspartame exposure on mRNA expression of inflammation-related genes. Wistar albino rats (pre-treated with methotrexate to produce folate deficiency similar to in humans exposed to methanol) then exposed to aspartame (40 mg/kg) showed a marked increase in the *TNF* gene expression and a decrease in *NF-κB* gene expression in the liver relative to the liver of unexposed study controls ($P < 0.05$) (Ashok and Sheeladevi, 2015). [The Working Group considered the study to be of low informativeness because of the experimental design in which the pre-treatment with methotrexate was used to induce folate deficiency and lack of proper negative control with aspartame.]

Increases in the expression of neuroinflammatory genes (*Adipoq*, *Fabp4*, *Scarb1*, *Cd36*) have been reported in the brain tissue of exposed C57BL/6J mice (at least fivefold), whereas decreases were shown in *Adamts9*, *Fga*, *Fgb*, *Fgg*, *Plg*, *Hpx*, *Itih4*, *Ptgs2/cox2*, *Tdo2*, and *Kynu* (two- to threefold) (Collison et al., 2018). In a study that assessed the effects of sugar-sweetened beverages in weaned male C57BL/6J mice (age 3 weeks) provided drinks of 30 w/v% sucrose solution (S30), an aspartame solution with sweetness equivalent to the sucrose solution (A30), or plain water (CT) until age 11 weeks, unclear effects were reported on mRNA expression of inflammation-related transcripts (*TNFα*, *Cxcl1*, *Il22ra2*, *Creb3I3*, *Gnb2I1*, *Pex13*, *Ptgs1*) in the brain tissue. The authors reported that the frequency of CD11b+ myeloid lineage cells was slightly higher in the peripheral blood of rats exposed to S30 compared with rats exposed to aspartame or plain water, and a pattern of positive correlation was reported between the frequency of CD11b+ cells and serum levels of corticosterone (Choi et al., 2017).

Increased mRNA expression of leptin (a pro-inflammatory agent and a potential mediator of hepatic fibrosis) and decreased mRNA

Table 4.10 End-points relevant to chronic inflammation in primary non-mammalian cells in vitro exposed to aspartame

End-point	Cells and/or tissue	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Leukotriene C4	Macrophages derived from rats	↑	NT	0, 10^{-6} M	Cells pre-treated for 6 h with 0.5 μCi/mL of ^{14}C-arachidonic acid. Only one dose group.	Hardcastle and Bruch (1997)
Leukotriene B4		↑	NT			
15-Hydroxyeico-satetraenoic acid		↑	NT			

h, hour(s); HIC, highest ineffective concentration; LEC, lowest effective concentration; NT, not tested.

[a] +, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study); (+) or (–), positive or negative result in a study of limited quality.

expression of adiponectin (an anti-inflammatory adipocytokine) were also observed in the adipose tissue of male Wistar albino rats exposed to aspartame by intragastric intubation (Lebda et al., 2017b).

(ii) Non-human mammalian cells in vitro

See Table 4.10.

A large increase in the production of arachidonic acid metabolites and leukotriene C_4 (LTC$_4$) levels was reported in aspartame-exposed rat macrophage cell cultures compared with control cell cultures (Hardcastle and Bruch, 1997). [The Working Group noted that the cysteinyl leukotrienes and other lipoxygenase products of arachidonic acid are important mediators in both intestinal inflammation and in the inflammatory response in general.]

4.2.5 Is immunosuppressive

(a) Humans

(i) Exposed humans

Bishop et al. (2002) compared the effects of ingesting a carbohydrate beverage ("CHO" solution composed of 6.4% w/v glucose and maltodextrin), or an artificially sweetened placebo drink that may have contained aspartame, on plasma levels of IL-6 and TNFα, and LPS-stimulated neutrophil degranulation responses to high-intensity intermittent running. There was a significant increase in neutrophil count in those who had consumed the placebo drink. In the CHO and the placebo groups, plasma levels of IL-6 ($P < 0.01$) were increased at both right the after the exercise (time 0) and 30 minutes post-exercise compared with pre-exercise levels; however, IL-6 levels were higher in participants ingesting placebo than those ingesting CHO at 30 minutes post-exercise ($P < 0.02$). LPS-stimulated elastase release per neutrophil (an indicator of neutrophil degranulation) was lower after exercise than before exercise in participants who were given the placebo drink ($P < 0.06$). [The Working Group noted that the study was not informative because the type of sweetener in the placebo beverage was not reported. Additionally, no information on the participants' diet was reported.]

(ii) Human primary cells in vitro

Two commercial brands of aspartame-containing artificial sweeteners (diluted to 10 μg/mL), among others, were shown to suppress the secretion of IL-6 in LPS-stimulated (10 ng/mL) whole blood cells from human donors when compared with control cells exposed to cell medium or natural sweeteners, whereas no effect was reported on levels of IL-10. The comparison of all sweeteners with the control cultures under

stimulated and unstimulated conditions showed that none of the sweeteners had an effect on IFNγ synthesis ($P > 0.05$) (Rahiman and Pool, 2014). [The Working Group noted that the sweeteners used in this study contained aspartame, but that the other sweetener constituents were not described. In this study, none of the sweeteners applied to the human lymphocytes had any effect on cell-mediated immunity.]

(b) Experimental systems

Non-human mammals in vivo

In NC/Nga mice in which atopic dermatitis had been induced by dinitrofluorobenzene, aspartame (0.5 µg/kg or 0.5 mg/kg) administered daily for 9 days (from day 9 to day 18) inhibited inflammatory cells (including eosinophils, mast cells, and CD4+ T-cells) and suppressed the expression of cytokines (IL-4 and IFNγ) and total serum IgE levels relative to mice exposed to dinitrofluorobenzene only (Kim et al., 2015a).

4.2.6 Modulates receptor-mediated effects

(a) Humans

(i) Exposed humans

No data on exposed humans were available to the Working Group.

(ii) Human primary cells in vitro

Aspartame (0.4–410 µM) had no effect on the transcriptional activity of the genes encoding the aryl hydrocarbon receptor (AhR) and glucocorticoid receptor (GR), or on AhR-dependent or glucocorticoid receptor-dependent expression of cytochrome P450, family 1, subfamily A, polypeptide 1 (CYP1A1) in human primary hepatocytes (Kamenickova et al., 2013).

(iii) Human cell lines

In human granulosa-like tumour (KGN) cells, Chen et al. (2022) assessed the effects of exposure to aspartame (5 and 20 mM) for 48 hours. The higher concentration of aspartame (20 mM) was observed to significantly decrease progesterone secretion, which is normally correlated with adenosine triphosphate (ATP) production. The observed effects were consistent with mitochondrial respiratory dysfunction resulting from oxidative stress caused by aspartame (see Section 4.2.3).

In contrast, Kamenickova et al. (2013) reported that aspartame, and other non-nutritive sweeteners, had no effect on AhR-dependent or glucocorticoid receptor-dependent expression of CYP1A1 in human intestinal cancer cell lines.

(b) Experimental systems

(i) Non-human mammals in vivo

Steroid hormones and receptors

In a study of reproductive toxicity, the effects of aspartame were investigated in mature male NMRI mice exposed to aspartame (40, 80, and 160 mg/kg bw) for 90 days (Anbara et al., 2020). A dose-dependent reduction in serum concentrations of testosterone and pituitary–testicular axis hormones (follicle-stimulating hormone, FSH, and luteinizing hormone, LH) was observed. Aspartame also decreased sperm and testicular functionality indices. [The Working Group noted that the decrease in in serum concentrations of pituitary–testis axis hormones could indicate male reproductive toxicity. The Working Group also noted that the same group had subsequently published a similar study that also reported decreases in testosterone identical to those in the study in 2020 (Anbara et al., 2021).]

In studies by Lebda et al. (2017a, b), rats exposed to drinking-water containing aspartame at a dose of 240 mg/kg for 2 months showed a decrease in triiodothyronine (T3) levels and an increase in thyroxine (T4) and parathyroid hormone levels (Lebda et al., 2017a), as well as upregulation of leptin, adiponectin, and peroxisome proliferator-activated receptor gamma (PPARγ) mRNA gene expression in adipose tissue (Lebda et al., 2017b). In a similar study by

Ibrahim et al. (2022), exposure to aspartame at 500 mg/kg bw per day for 1 week caused increases in markers of apoptosis and inflammation in the spleen of exposed rats, and decreases in thyroid hormones T3 and T4, and antioxidant defence enzymes CAT, and SOD (see also Section 4.2.3).

In male rats, aspartame caused an increase in FSH levels when administered at doses of > 100 mg/kg bw per day but not at doses of > 300 mg/kg bw per day. Levels of LH and prolactin were unaltered (EFSA_E103, 2011).

In a series of regulatory studies carried out in mice, rabbits, and rats in the 1970s (EFSA_E19, 2011), the administration of aspartame was shown not to exert effects on the activities of estrogen, progesterone, or androgen. Estrogenic agonist and antagonist activities were measured by evaluating uterine weight after the administration of aspartame to female albino mice. No significant alterations in uterine weight were observed after the oral administration of 1350 mg of aspartame for three consecutive days. Saunders et al. (1980) showed that aspartame at up to 300 mg/kg bw per day did not have a hormone mimetic response in the endocrine target organs evaluated in mice, rats, and rabbits. [The Working Group noted that the duration of treatment could not be identified.] Progesterone-like activity and progesterone antagonism were measured in female rabbits primed for 6 days with 17β-estradiol after intragastric administration of 300 mg of aspartame. No increase in the concentration of carbonic anhydrase in the uterus (as a measure of the degree of glandular proliferation in the endometrium) was observed compared with controls (EFSA_E19, 2011). Androgenic and myotrophic activity and androgen antagonism were measured in male castrated Sprague-Dawley rats after intragastric administration of 50 or 350 mg of aspartame for 7 days. No significant alterations in the seminal vesicles, ventral prostate gland, or in the elevator ani muscle were observed compared with controls (EFSA_E19, 2011).

[The Working Group noted that these were studies of acute toxicity and would not have identified any effects on receptor systems that may occur after exposure to aspartame for longer periods. In addition, the Working Group noted that the study did not investigate potential interactions of aspartame with hormone receptors and only documented changes in hormone activities.]

Glutamate receptors

The observation of reduced levels of glutamic acid and aspartic acid in the brains of weanling rats exposed perinatally to aspartame led Reilly and Lajtha (1995) to investigate the effects of aspartame on glutamatergic receptor kinetics. Aspartame (500 mg/kg bw per day in drinking-water) was administered to Sprague-Dawley rats throughout gestation and lactation for 30 days. The kinetics of the *N*-methyl-D-aspartate receptor and total glutamatergic binding in the cerebral cortex and hippocampus were found to be unaffected by perinatal exposure to aspartame. However, Pan-Hou et al. (1990) previously showed that aspartame inhibited L-[³H]glutamate binding to its *N*-methyl-D-aspartate (NMDA)-specific receptors in a dose-dependent manner in ex vivo isolated rat synaptic membranes. More recently, Arcego et al. (2020) reported decreased immunocontent of the GluN2A subunit of the *N*-methyl-D-aspartate receptor (NMDAR) in the hippocampus in adolescent male and female rats given unlimited access to aspartame (2 g/L) on postnatal days 21–55. [The Working Group noted that the study (Arcego et al., 2020) was not informative since no evidence linking alterations of the glutamate receptor to carcinogenesis was reported.]

In a binding assay (Monastyrskaia et al., 1999), aspartame (up to 1 mM) did not bind to the rat brain mGlu4 receptor, a G-protein-coupled metabotropic glutamate receptor.

4.2.7 Causes immortalization

Humans

(i)   Exposed humans

No relevant data were available in exposed humans.

(ii)   Human cells in vitro

Gezginci-Oktayoglu et al. (2021) investigated the effects of aspartame exposure compared with glucose-exposed and non-exposed controls in human pancreatic adenocarcinoma cells (PANC-1). In a first range-finding study, the authors assessed cell viability after 24 hours exposure to aspartame or glucose at increasing concentrations (1 ng/mL to 100 mg/mL and from 5 mM to 25 mM, respectively). Cell viability decreased with aspartame at concentrations > 1 ng/mL and with glucose at > 15 mM. The authors used this information to select high and low doses (aspartame, 1 ng/mL and 100 ng/mL; glucose, 5 mM and 25 mM) for 7 days or 14 days, to verify effects in a cancer stem cell population. Exposure to aspartame at the lower dose (1 ng/mL) for 7 days significantly increased the percentage of CD24+/CD44+ cells and the overall cancer stem cell population, as assessed by flow cytometric analysis, but this effect was not observed for glucose. In contrast, at the higher dose of aspartame (100 ng/mL) there was a reduction in cell viability and in the percentage of CD24+/CD44+ cells at 7 and 14 days. After a 7-day exposure, aspartame (1 ng/L) or glucose (25 mM) did not induce colony formation and did not alter the cell cycle status of the population.

Aspartame at (1 ng/mL) or glucose (25 mM) altered the protein levels of epithelial–mesenchymal transition (EMT) markers of invasion and migration – E-cadherin, vimentin, and N-cadherin. Aspartame induced a significant decrease in levels of the epithelial marker E-cadherin, as did glucose, compared with non-exposed controls, but a significant increase in levels of the mesenchymal marker vimentin and count of cells expressing N-cadherin, compared with both glucose exposure and non-exposed controls.

In addition, aspartame, but not glucose, was shown to increase protein levels of the membrane sweet taste receptor 1 (T1R1) and to decrease protein levels of sweet taste receptor 3 (T1R3), but aspartame did not alter protein levels of sweet taste receptor 2 (T1R2) after exposure at 1 ng/mL for 7 days. Binding to the T1R2/T1R3 complex in PANC-1 cells increased in cells exposed to aspartame or glucose, but more so in aspartame-exposed cells. It is noteworthy that T1R1/T1R3 binding was not present in PANC-1 cells in the absence of a sweet taste stimulus, but it did occur at a low level in PANC-1 cells exposed to glucose or aspartame. The T1R2/T1R3-mediated effects of aspartame were transduced to downstream effectors: levels of Ca^{2+}, ROS, and AKT/GSk3β were observed to increase, leading to activation of Notch intracellular domain (NICD) and GLI family zinc finger 1 (GLI1), nuclear translocation of p21, and expression of octameric transcription factor 3/4 (OCT3/4) and c-Myc transcription factors (Gezginci-Oktayoglu et al., 2021).

4.2.8 Alters cell proliferation, cell death, or nutrient supply

(a)   Humans

(i)   Exposed humans

A few studies were available that investigated the safety of long-term administration of aspartame in exposed humans, mainly in healthy participants or in participants with diabetes. These studies included biomarker measurements, such as routine haematology analysis, that could be relevant to the key characteristic of carcinogens "alters cell proliferation, cell death, or nutrient supply".

The effects of long-term administration of aspartame at a dose of 75 mg/kg in capsules three times per day with meals for 24 weeks

were investigated in a randomized, double-blind placebo-controlled study in volunteers. Approximately 54 participants per group completed the study (EFSA UN08, 2011; and reported in Leon et al., 1989). Capsules containing aspartame were administered to 23 men and 30 women, and capsules containing the placebo were administered to 28 men and 27 women. The study incorporated several end-points specific to aspartame metabolism (e.g. serum methanol and amino acids), routine haematology analysis (complete blood count), blood chemistry (collected at baseline and every 3 weeks for the first 12 weeks then at weeks 18 and 24; included alkaline phosphatase, alanine and aspartate aminotransferases, LDH, total bilirubin, creatinine, creatinine phosphokinase, and total blood urea nitrogen, BUN), and urine analysis (collected at baseline and at weeks 6, 12, 18, and 24; included 24-hour creatinine). At each time point, the results for each test with aspartame were compared with those with placebo, using a two-tailed Student t-test without correction for multiple comparisons. All tests were done at the 5%, two-sided level of significance. The data did not indicate any toxicologically significant clinical findings. [The Working Group noted that alteration in haematopoietic parameters would be relevant as an indicator of cell proliferation; however, no changes in routine clinical tests were observed during the study.] In two additional placebo-controlled double-blind studies, aspartame was administered in capsule form three times per day with meals (EFSA E60, 2011; EFSA E65, 2011). In a long-term tolerability study in healthy adults with normal phenylalanine metabolism, aspartame was initially administered to 11 men and 19 women for 6 weeks, after which the study duration was extended for an additional 21 weeks and the study population was expanded to include 12 additional men and 5 additional women (EFSA E60, 2011). A placebo was administered to 7 men and 13 women for the entire 27-week duration of the study. Participants took two capsules containing 300 mg of aspartame (or placebo) three times per day with their normal meals (1.8 g/person per day). Blood was drawn and analysed at baseline and at weeks 6, 12, 18 (original participants), 20 (or 26), and 21 (or 27). Haematological parameters were measured: complete blood count, hepatic function (serum glutamic-oxaloacetic transaminase, (SGOT), alkaline phosphatase, direct and indirect bilirubin) or kidney function (creatinine, uric acid, BUN). At 12, 16, 20, and 21 weeks, serum glucose and insulin were measured after a 4-hour fast and again 30 minutes later after oral challenge with 100 g of glucose. The data were evaluated by comparing the mean values for the groups exposed to aspartame versus placebo using the Student t-test and by linear regression for each of the parameters as a function of time. No changes were observed in any of the clinical tests in the group receiving aspartame compared with the group receiving the placebo (EFSA E60, 2011). [The Working Group noted that the small sample size, particularly of the control group, limited the sensitivity of the study to detect changes.]

In a long-term tolerability study in adults with insulin-dependent diabetes, 25 women and 14 men consumed aspartame capsules for 13 weeks (EFSA E65, 2011). Some study participants had poor insulin control and abnormal kidney function results at baseline but were not excluded from the study. Blood was drawn at baseline and at the conclusion of the study. Haematological parameters included complete blood count; liver function tests included total bilirubin, alkaline phosphatase, serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and LDH; and kidney function was monitored via levels of uric acid, creatinine, and BUN. No changes were reported in the parameters of liver or haematological function. The kidney function test results were mixed since eight aspartame consumers had elevated BUN and creatine levels at entry, and these levels did not change significantly at the end of the

study. BUN levels increased in two participants during the study and decreased in three. The kidney function test results did not change in the other participants receiving aspartame, or in the participants receiving the placebo (EFSA_E65, 2011). [The Working Group noted that most of the studies in exposed humans had limitations in study design, including sample size.]

In one study that directly evaluated a relevant end-point in humans, aspartame was administered as a single skin prick or intradermal injection of up to 1 mM in three healthy volunteers. The authors concluded that there was no induration or erythema due to histamine release from either route of administration, attributing erythema observed at the highest dose in two volunteers receiving intradermal injection to irritation. Associated experiments using a mouse mast cell line did not elicit histamine release, as noted in Section 4.2.8(b) (Szucs et al., 1986). [The Working Group considered the study to be of low relevance.]

(ii)   Human primary cells

See Table 4.11.

In HUVEC cells exposed to aspartame at 20 or 100 µM for 14 days, stimulation of angiogenesis was observed via microscopic examination of the cultures for tubule formation followed by quantification of absorption of a soluble colourimetric marker dye. The time course of growth factor expression was examined on days 0, 4, 7, and 14 using ELISA (also discussed in Section 4.2.4). VEGF levels increased about fourfold on days 7 and 14 at both concentrations; levels of its soluble receptor also increased on day 7 but fell to baseline levels on day 14 (Alleva et al., 2011). [The Working Group noted that the numbers of replicate plates and experiments were not reported.]

Aspartame-induced angiogenesis was also reported in HUVECs co-cultured with primary human adipose stromal cells. The co-cultured cells were exposed to aspartame at about 0.3–100 µM for 6 days, after which angiogenesis

was measured by computational evaluation of tubule length in photomicrographs. There was a significant increase ($P < 0.05$, Student t-test) at the lowest concentration and at about 3 µM, but not at two intermediate or four higher concentrations. There were no statistically significant changes in metabolic activity measured using a water-soluble tetrazolium salt (Hautanen et al., 2023). [The Working Group estimated the concentrations from a graph and noted that the statistical test did not correct for multiple comparisons, in contrast to the authors' original version of the Angio Kit model assay (Huttala et al., 2015) in which results were analysed using more appropriate methods: a one-way analysis of variance with post-test application of the Dunnett test, where applicable.]

Angiogenesis in HUVECs was also examined by Yesildal et al. (2015). The cells were exposed to aspartame at 20–100 µM. In this study, a shorter time of exposure (16–18 hours) to aspartame at 20 µM did not induce angiogenesis in HUVECs on the basis of evaluation of the ratio of tubule length to area measured in photomicrographs (Yesildal et al., 2015). Cell viability, as measured by a tetrazolium dye (sodium 3′-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis (4-methoxy-6-nitro) benzene sulfonic acid hydrate; XTT), was not reduced after 24 or 48 hours of exposure. [The Working Group noted that the number of replicates for the tubule formation assay was not provided.] However, in the same study, the authors reported angiogenesis in chicken ova and wound healing in rat skin (the results are described in Section 4.2.8(b)). In chicken ova, the stimulation of angiogenesis was observed with aspartame at doses of 15 and 30 mM for 3 days but not at shorter exposures or at lower concentrations (described in more detail in Section 4.2.8(b)).

In HUVEC cells, exposure to aspartame at 1 mM for 48 hours did not affect angiogenesis, as evaluated after exposure of a single plate to a single concentration and by measuring tubule

Table 4.11 End-points relevant to alterations in cell proliferation, cell death, or nutrient supply in human cells in vitro exposed to aspartame

End-point	Assay	Cell or tissue type	Results[a]	Concentration	Comments	Reference
Angiogenesis	Tubule length measurement	HUVEC	No differences	1 mM, 48 h		Schiano et al. (2020)
Angiogenesis	Tubule length measurement	HUVEC	No differences	20 µM, 16–18 h		Yesildal et al. (2015)
Angiogenesis	Tubule length measurement	HUVEC	↑	20 µM, 14 days		Alleva et al. (2011)
Angiogenesis	Membrane ATPase activity	Erythrocytes from healthy donors	↓	0.14 mM methanol 2.8 mM aspartate 0.14 mM phenylalanine		Schulpis et al. (2006)
Cell migration	Wound healing Migration of cells into a "wound" scratched in the monolayer	HT-29	↓	6 mM		Maghiari et al. (2020)
Cell proliferation	³H-thymidine incorporation	HTR8 SV neo	↓	1 µM	Aspartic acid and phenylalanine had the same antiproliferative effect.	Rodrigues et al. (2022)
Apoptosis	Caspase-3 cleavage Phosphorylation of γH2AX	HepG2	↑	680 µM		Qu et al. (2019)
Apoptosis	Downregulation of mRNA expression of tumour suppressor gene *TP53*, and pro-apoptotic gene *BAX*	HeLa	↑	34–102 µM		Pandurangan et al. (2016b)
Cell proliferation	PCNA; Ki-67	HeLa	↑	34–102 µM		Pandurangan et al. (2016b)

BAX, BCL2 associated X, apoptosis regulator; γH2AX, phosphorylated histone H2AX; h, hour(s); HeLa, human cervical adenocarcinoma cell line; HIC, highest ineffective concentration; HT29, human colon carcinoma cell line; HTR-8 SVneo, SV-40-transformed human extra-villous trophoblast cell line; HUVEC, primary human endothelial umbilical vein cells; LEC, lowest effective concentration; PCNA, proliferating cell nuclear antigen; TP53, tumour protein 53.

[a] ↓, decrease; ↑, increase.

length in photomicrographs. Cell viability, measured by the trypan blue exclusion test, was slightly reduced after exposure to aspartame at 1 mM for 96 hours, but not at intermediate time points. Also, exposure to aspartame at 100 µM did not induce cytotoxicity, measured as LDH leakage. Moreover, aspartame did not alter the cell cycle (as measured by cell cycle distribution and blockage in G0/G1 phase) or apoptosis (as measured by annexin-V/propidium iodide staining, compared with controls) (Schiano et al., 2020).

Inhibition of membrane ATPase activity was noted when erythrocytes were isolated, lysed from healthy donors, and the membrane fraction incubated with aspartic acid, phenylalanine, or methanol. An additive effect was observed in mixtures of the three aspartame metabolites at concentrations modelled on literature reports of maximum concentrations (C_{max}) in the plasma of volunteers ingesting aspartame at doses of 34 mg/kg bw and above (Schulpis et al., 2006).

(iii) Human cell lines

See Table 4.11.

Exposure of HT-29 cells (a human colon carcinoma cell line) to aspartame at up to 50 mM had little impact on overall cell viability, measured as oxidation of alamar blue after 72 hours. After creation of a "wound" by scratching a gap in the cell monolayer, a significant reduction in the ability of cells to migrate into the gap was observed with aspartame at non-cytotoxic concentrations of up to 3 mM, but not at 6 mM (Maghiari et al., 2020).

Rodrigues et al. (2022) studied cell proliferative capability in an SV40-transformed human extra-villous trophoblast cell line (HTR-8 SVneo) exposed to aspartame. After exposure to aspartame at concentrations of 1–1000 µM for 24 hours, metabolic activity (as measured by MTT) was reduced to about 85% of the control value at each of the five concentrations tested; however, there was no increase in LDH release into the culture medium or reduction in total cell protein content (as measured by sulforhodamine B staining). Aspartame (500 and 1000 µM) significantly decreased the uptake of glucose ([³H]deoxy-D-glucose, ³H-DG), as well as [³H]thymidine incorporation, as observed after exposure to aspartame at increasing concentrations (1–1000 µM). In addition, aspartame at 500 µM did not affect cell migration (measured by wound-healing assay) or alter the level of lipid peroxidation (LPO/TBARS). Cell cycle analysis indicated an accumulation of cells in S-phase, without any indication of apoptosis, as measured using the TUNEL assay (terminal deoxynucleotidyl transferase dUTP nick end labelling). The aspartame metabolites aspartic acid and methanol, but not phenylalanine, induced reduction of metabolic activity of about 25–30%. There were smaller or no changes in LDH release and in cell protein levels, and no effects on cell proliferation after exposure to phenylalanine aspartic acid, and methanol; only the latter metabolite induced oxidative stress and reduced the migration rate, and the number of cells in G2/M.

Qu et al. (2019) evaluated the toxicity of aspartame in HepG2 cells (a human liver carcinoma cell line). Concentrations between 0.68 and 3.4 mM caused a steep, concentration-dependent decline in metabolic activity, measured using the MTT assay. This toxicity was accompanied by an increase in the proportion of cells exhibiting apoptosis, indicated by cleavage of caspase-3 and phosphorylation of γH2AX, and by an increase in intracellular ROS (discussed in Section 4.2.5) (Qu et al., 2019).

Exposure of HeLa cells (a human cervical carcinoma cell line) to aspartame at millimolar concentrations of 10–20 mM for 48 hours induced cytotoxicity, generation of intracellular ROS, DNA strand breakage (comet assay), and apoptosis at the highest concentrations (Pandurangan et al., 2016a). In a follow-up study in the same cell line, the same laboratory evaluated exposure to aspartame at low concentrations of 34, 68, and 102 µM (10, 20, and

30 μg/mL) ([Pandurangan et al., 2016b](#)). At these concentrations, aspartame did not induce cytotoxicity but suppressed apoptosis, as described by downregulation of mRNA expression of the tumour suppressor gene *TP53* and the pro-apoptotic gene *BAX*, and upregulation of mRNA expression of the anti-apoptotic gene *BCL2*. For cell proliferation marker Ki-67 and proliferating cell nuclear antigen (PCNA), mRNA and protein expression increased in a dose-dependent manner ([Pandurangan et al., 2016b](#)). [The Working Group noted that the results from studies in human cell lines did not indicate alteration of cell proliferation, cell death, or nutrient supply. Discrimination between mechanisms of necrosis, apoptosis, and cytotoxicity is a notable data gap for aspartame.]

(b) Experimental systems

(i) Non-human mammals in vivo

Cell proliferation

Repeated-dose toxicity studies (with durations of 14 days, 28 days, 90 days, 562 days, and up to 2 years) evaluating the effects of aspartame administered orally in the feed or drinking-water in several non-human mammalian species were available to the Working Group. The studies reported on end-points related to altered morphology, cell proliferation, and hyperplasia, which are relevant to the key characteristic "alters cell proliferation, cell death, or nutrient supply".

[Sun et al. (2019)](#) investigated the gastrointestinal response in pre-weaned Hu sheep (age 14 days) fed with starter diet supplemented with aspartame at a concentration of 200 mg/kg for 35 days. When compared with the control group that was fed with starter diet alone, exposure to aspartame for 35 days did not induce body-weight gain but significantly increased small intestine weight/live body weight, jejunum weight/live body weight, and jejunum weight. However, aspartame did not affect small intestine weight (P = 0.208), duodenum weight,

duodenum weight/live body weight), ileum weight, or ileum weight/live body weight. These effects were accompanied by an increase in crypt depth and villus height in the jejunum, and there was a similar but not significant trend in the ileum but not in the duodenum. Aspartame significantly increased the plasma concentrations of gastrointestinal hormone glucagon-like peptide-2 (GLP-2); however, there were no significant effects on plasma glucose concentrations. After aspartame exposure, differential upregulation of mRNA expression levels of cyclin D1, cyclin A2, cyclin E1, cyclin-dependent kinases CDK2, CDK4, and CDK6, proglucagon (GCG), glucagon-like peptide-2 receptor (GLP-2R), insulin-like growth factor 1 (IGF-1), and insulin-like growth factor 1 receptor (IGF-1R) were observed in the different regions of the intestine. In the duodenum, there was upregulation of cyclin D1 but no changes in the mRNA expression of GCG, GLP-2R, IGF-1, and IGF-1R. In the jejunum, there was upregulation of mRNA expression of cyclin A2, cyclin D1, CDK6, GCG, GLP-2R, and IGF-1. In the ileum, there was upregulation of mRNA expression of cyclin A2, cyclin D1, CDK4, GCG, and IGF-1. [The Working Group noted that the findings did not directly explain the relationship between the changes in villus height and crypt depth in the small intestine and GLP-2 release; however, in experiments in vitro in epithelial cells isolated from the jejunum tissue of four healthy Hu lambs aged 56 days, GLP-2 indirectly promoted cell proliferation mainly through the IGF-1 pathway.]

In Swiss mice exposed to aspartame (20, 40, 80, and 160 mg/kg bw per day) by gavage for 28 days, alterations in cell morphology were observed in various areas of the hippocampus. Lower doses of aspartame induced significant increases in cell density. At the lower doses of aspartame, the number of neurons that were positive for neuron-specific enolase significantly increased within the dentate gyrus, but at the highest dose (160 mg/kg bw per day)

there was marked neuron loss and reduction in neuron-specific enolase-positive neurons. A parallel increase in several oxidative biomarkers was observed, starting at 40 mg/kg bw per day (Onaolapo et al., 2017b). [The Working Group noted that, despite the differential effects on the hippocampal regions and decrease in antioxidant status, it was suggested that aspartame exerts neurogenesis.] In contrast, in an earlier study (see also Section 4.2.3b), Ashok and Sheeladevi (2014) reported neurodegenerative effects mediated by oxidative stress-induced apoptosis in discrete brain regions of Wistar albino male rats exposed to aspartame at 40 mg/kg bw per day for 90 days under conditions of folate deficiency (pre-treatment with methotrexate). [However, as mentioned in Section 4.2.3(b), the Working Group considered this study to be of low relevance because of the lack of proper grouping including negative control group with aspartame exposure that limited the interpretation of the results.]

In Wistar albino rats, aspartame was administered at doses of 250 and 1000 mg/kg bw per day in the drinking-water for 56 days (8-week study) (Alkafafy et al., 2015). Aspartame increased mRNA expression of the oncogene *ha-Ras* [*HRas*] and decreased mRNA expression of the tumour suppressor gene *p27* [*Cdkn1b*]. Liver sections from rats receiving aspartame showed histological changes, including disorganized hepatic parenchyma, dilatation, and congestion of the central vein and hepatic sinusoids with proliferation of Kupffer cells. Some hepatocytes showed signs of degenerative changes as cellular swelling and vacuolar cytoplasm, and others were necrotic. Some sections showed focal areas of complete degeneration and diffuse and focal intralobular mononuclear cell infiltration. Hyperplasia of the bile duct was also observed. Collagen deposition was more pronounced in the liver, indicating periportal fibrosis (Alkafafy et al., 2015). [The Working Group found several limitations in the histopathological part of the study; therefore, some of the findings were considered to be of limited relevance.]

In a subacute toxicity study, groups of 30 male and 30 female young adult albino rats were exposed to feed containing aspartame at a dose of 0, 5, or 125 mg/kg bw per day for 56 days (8-week study). Findings of bile duct hyperplasia were reported at the lower dose of 5 mg/kg bw in both male and female rats; however, these findings were considered incidental. Similarly, an increase in peribronchial lymphoid and reticular hyperplasia was observed in the lung; however, this was diagnosed as chronic murine pneumonia (EFSA_E20, 2011). [The Working Group noted that the purity of the agent was "assumed to be 100% pure".]

Groups of two male and two female young adult purebred Beagle dogs were exposed orally (by capsule) with aspartame at a dose of 0, 5, or 125 mg/kg bw per day for 56 days (8-week study). No cell proliferation or hyperplasia was observed in various tissues. Slight changes in the adrenal gland, spleen, and mesenteric lymph node, including the presence of a moderate to largely moderate amount of reticuloendothelial tissue in males and females, were observed at the lower and higher dose; however, the changes were similar to those in the controls (EFSA_E21, 2011). [The Working Group considered that the study was limited by the small number of animals. In addition, the purity of the agent was "assumed to be 100% active".]

In a 90-day toxicity study in 36 mature male NMRI mice, aspartame was administered at a dose of 0, 40, 80, and 160 mg/kg bw per day by gavage. Aspartame reduced sperm count and spermiogenesis in a dose-dependent manner, and induced reduction of seminiferous tubules and damage to Leydig cells in the testis. These effects were highly significant at the two higher doses, at which cytotoxicity and apoptosis were also observed (Anbara et al., 2021).

In rats, oral administration of aspartame at a dose of 40 mg/kg bw per day for 90 days did not cause alterations in levels of mRNA transcripts or protein for Bcl-2 and Bax in the spleen, thymus, and lymph nodes (Choudhary and Sheela Devi, 2016).

In a 2-year carcinogenicity study in groups of 100–150 male and 100–150 female Sprague-Dawley rats (age 8 weeks), hyperplasia was observed in the renal pelvis and olfactory epithelium in females Sprague-Dawley rats after administration of feed containing aspartame at increasing concentrations up to 100 000 ppm until natural death (Soffritti et al., 2006; reviewed in Section 3.2).

In male and female Slc Wistar rats exposed to feed containing aspartame at a dose of 1000, 2000, or 4000 mg/kg bw per day for 104 weeks, an increased incidence of transitional cell hyperplasia (synonymous with the more recent term, urothelial cell hyperplasia) of the renal pelvis was observed in males at the highest dose (Ishii, 1981). [The Working Group noted that the study was re-analysed by Shibui et al. (2019), who reported that renal pelvis mineralization was increased in males in a dose-dependent manner (with the incidence being significantly increased at 2000 and 4000 mg/kg bw per day), and in females (with the incidence being significantly increased at the highest dose) (see more details in Section 3.1); thus the Working Group considered that the observed transitional cell hyperplasia was likely to be a response to mechanical irritation induced by the mineral deposition with no evidence of cellular atypia.]

In a study in groups of 20–30 male Fischer 344 rats (age 6 weeks), effects on tumour promotion were determined after administration of 0.05% or 0.01% N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) in the drinking-water for 4 weeks and feed containing 5% aspartame for 32 weeks. Aspartame did not induce promoting effects, measured as preneoplastic lesions (papillary or nodular hyperplasia). At necropsy, there was no evidence of increased proliferative urothelial lesions of the urinary bladder that could be attributed to aspartame exposure (Ito et al., 1984).

In a long-term study (reviewed in detail in Section 3.2) in Charles River albino rats exposed to feed containing aspartame at a dose of 0, 1000, 2000, 4000, or 8000 mg/kg bw per day for 104 weeks, increased nodular hyperplasia in the pancreas (a non-neoplastic proliferative lesion) was reported in female rats – but the increases in male rats were unremarkable. There were increases in the incidence of renal pelvis epithelial hyperplasia in males – but the increases in female rats were unremarkable (EFSA_E33, 2011; EFSA_E34, 2011). [The Working Group considered that the study was limited by the lack of information on the purity of the agent, the low survival rate, and an infection (murine pneumonia) observed in controls and exposed animals.]

In three different cancer models in genetically modified mice (reviewed in Section 3.2) (NTP, 2005), male and female Tg.AC, p53-haploinsufficient, and Cdkn2a-deficient mice were exposed to feed containing aspartame (purity, > 98%) at doses ranging from 0 to 50 000 ppm (approximately 0–7660 or to 7400 mg/kg bw in males; 0–8180 or to 9560 mg/kg bw in females, in the respective deficient mouse strains). In the p53-haploinsufficient B6.129-Trp53^{tm1Brd} (N5) female mice, histopathology reported hyperplasia in the adrenal cortex, endometrium, and mild haematopoietic cell proliferation although not related to aspartame exposure. No other non-neoplastic lesions were observed. In male mice of the same strain, no alteration in any cell growth/proliferation-associated end-points was observed. In B6.129-Cdkn2a^{tm1Rdp} (N2) deficient mice, minimal to mild cytoplastic vacuolization of periportal hepatocytes was found in control and exposed male mice, with an increased incidence in groups at 6250 ppm, 25 000 ppm, and 50 000 ppm. In the FVB/N-TgN(v-Ha-ras)Led (Tg.AC) hemizygous male and female mice,

histopathology did not show cell proliferation or hyperplasia in any of the tissues evaluated. [The Working Group noted that the authors stated that this study was done in a new test system and there was uncertainty as to whether it possessed sufficient sensitivity; therefore, the Working Group concluded that these findings were of limited informativeness.]

In a long-term study in groups of five male and five female Beagle dogs (age 150–160 days), randomized and housed individually, aspartame was administered at a dose of 0, 1, 2, or 4 g/kg bw per day in the diet for 106 weeks. Microscopic observation of the brain showed ependymal proliferation (focal) and glial cell proliferation (minimal, focal subependymal) (EFSA_E28, 2011; EFSA_E86, 2011). [The Working Group noted that the study had some limitations since the purity of the aspartame was not clearly reported; it was reported that from week 20 until the end of the 106-week feeding period, the test material contained 0.1–1.0% DKP (by weight). In addition, the stability and homogeneity of aspartame in the diet was not determined.]

Gombos et al. (2007) examined the effects of aspartame consumption on the expression of key oncogenes and a tumour suppressor gene. Female CBA/CA mice were exposed to aspartame at a dose of 40, 200, and 2500 mg/kg by gavage twice daily for 1 week, and the expression of *Tp53*, *Myc*, and *Hras* genes was assessed in the bone marrow, liver, spleen, thymus, lymph nodes, lung, and kidney. An increase in the expression of all the investigated genes was observed, especially in organs with a high proliferation rate, that is, the lymphoreticular organs, including bone marrow, and the kidney. [The Working Group noted that the study was limited by the lack of statistical analysis on pooled tissue samples. No dose–response relationship was observed in the gene comparisons.]

Dooley et al. (2017) investigated the effects of lifelong exposure to aspartame on the development of spontaneous pancreatic acinar carcinoma in C57BL/6 Ela1-Tag mice. These mice express the SV40 large T antigen (TAg) under the control of the elastase-1 acinar cell promoter, driving spontaneous formation of pancreatic cancers of acinar origin. Aspartame was administered at 0.035% w/v in the drinking-water, starting from birth. No change in the growth rate of pancreatic acinar carcinomas, as measured by histopathology, was observed in aspartame-exposed mice compared with the controls.

Angiogenesis

Shalaby et al. (2019) observed histopathological changes in the placenta (excised on day 19 of gestation) of pregnant female albino rats exposed to aspartame at a dose of 14 mg/kg bw by oral gavage for three consecutive days (days 9, 10, and 11) during gestation. Histopathology and morphometric analysis showed a significant decrease in placenta weight and mean thickness of the labyrinth and basal zones, with areas of degeneration and vacuolization in exposed animals compared with non-exposed controls. In addition, placenta sections from aspartame-exposed rats, compared with the non-exposed controls, showed extended areas in which there was a significant increase in the expression of VEGF) (as measured by immunostaining) in trophoblasts in the labyrinth and in the cytoplasm of spongiotrophoblasts and giant cell basal zones, indicating angiogenesis.

(ii)   Non-human mammalian cells ex vivo and in vitro

Yesildal et al. (2015) investigated the effects of aspartame on angiogenesis in various test models (see also Section 4.2.8(a)). In a model of skin wound healing in rats, full thickness round portions of skin (diameter, 5 mm) excised from the dorsal area of male Sprague-Dawley rats (age 8 weeks) were exposed to 50 µL of a 50 mM solution of aspartame in phosphate-buffered saline daily for 7 days. Histopathological examination was performed on the treated fixed

specimens. The wound-healing process was scored according to the following parameters: wound surface area, re-epithelization, granulation tissue formation, collagen deposition, presence of inflammatory cells, and angiogenesis. The aspartame-exposed skin showed enhanced cellularity and increased angiogenesis when compared with non-exposed skin. [The Working Group noted that, despite quantification of the wound-healing surface area, the effects on cellularity and angiogenesis were qualitative.] In the same study, angiogenesis was also explored in a chick chorioallantoic membrane (CAM) model using 6-day Atak-S fertilized chicken eggs. A dose-dependent increase in the vascular area of the CAM was observed on day 7 after a single exposure to 50 μL of a solution containing aspartame at concentrations of 6, 15, 30, and 60 mM. The effect, measured using a computer-aided stereomicroscope, was significant at the highest concentration (60 mM).

Szucs et al. (1986) observed a significant increase in cell proliferation rate in the growth factor-dependent mouse PT18 mast cell line after exposure to medium containing aspartame at 2×10^{-3} M for 9 days. Cells were fed by replacing 50% of the supernatant medium with fresh aspartame-containing medium on day 4 or 5. The increase in proliferation rate started as earlier as day 5. To determine whether the increased proliferation rate was related to the additional nutrients provided by the component amino acids of aspartame, the authors performed experiments enriching the controls with free amino acids at concentrations that would be expected after aspartame exposure. The proliferation rate was higher in cells provided with free amino acids than in cells exposed to aspartame. [The Working Group noted that the authors indicated that the observed effect was not related to aspartame itself but to an increase in nutrients in the cell culture.]

Horio et al. (2014) investigated the effects of aspartame in rat adrenal pheochromocytoma (PC12) cells. Cells were incubated with medium containing aspartame at 0–8 μg/L for 72 hours. Cell toxicity was measured by trypan blue exclusion and LDH, and apoptosis was evaluated by DNA fragmentation, changes in apoptotic factors as cytochrome c in the cytosol, and apoptosis-inducing factor (AIF) by western blot. Caspase-8 and caspase-9 mRNA was detected using reverse transcription-polymerase chain reaction (RT-PCR). Cell viability decreased in a concentration-dependent manner, and a significant decrease was observed at concentrations > 1 μg/mL when trypan blue was used, but the relative activity of LDH increased significantly even at the lowest concentration tested (0.001 μg/mL). DNA fragmentation was observed and visualized by electrophoresis as fragments of DNA of 180 to 200 base-pairs that increased in a concentration-dependent manner. Fragmentation was quantified by the TUNEL method and confirmed the results obtained in the electrophoresis, indicating induction of apoptosis. Caspase-8 mRNA increased significantly at aspartame concentrations of > 1 μg/mL, and caspase-9 mRNA started to increase significantly at > 0.01 μg/mL. Cytochrome c and AIF also increased in the cytosol, starting at 0.001 μg/mL, but the increased level of AIF in the exposed cells was not significantly different from that in the controls.

4.2.9 Data relevant to multiple key characteristics

See Table 4.12.

(a) Results from omics

Choi et al. (2017) examined the long-term effects of sugar-sweetened beverages on social aggression in mice. Weaned mice (age 3 weeks) were exposed to plain water (control), a solution of sucrose 30% w/v, or a solution of aspartame

Table 4.12 Microarray and omics results for multiple key characteristics in experimental systems in vivo or in vitro after exposure to aspartame

End-point	Normalization and statistics	Curation	Results	Relevant KCs[a]	Tissue, cell type or cell line	Exposure concentration or range and duration	Comments	Reference
Transcriptomics Illumina MouseWG-6 v2 Expression BeadChip arrays containing > 45 200 transcripts	Background correction was performed, the data were log-transformed to log2 scale and normalized by quantile normalization method implemented in the Genome Studio software (Illumina Inc.). Significant differences among three groups were identified using ANOVA test ($P < 0.05$) on log2-transformed normalized intensities by Partek Genomics Suite software version 6.3 (Partek, Missouri, USA). Transcripts with more than a twofold differential were selected for each specific comparison analysed. Used IPA for pathway analysis.	None noted	The aspartame solution changed the expression of genes in categories related to development and growth, such as cellular function and maintenance, nervous system development and function, cellular development, and cellular growth and proliferation	KC10	Hypothalamus	Male C57BL/6J mice (age 3.5 wk). Treatment groups were composed of 30 w/v% sucrose solution, aspartame solution with equivalent sweetness of 30 w/v% sucrose solution or plain water ad libitum for 8 wk. Mice were killed on day 56.	Aspartame was used as a sweetness control	Choi et al. (2017)

Table 4.12 (continued)

End-point	Normalization and statistics	Curation	Results	Relevant KCs[a]	Tissue, cell type or cell line	Exposure concentration or range and duration	Comments	Reference
Transcriptomics Affymetrix Mouse Gene 1.0 ST arrays containing 28 853 genes	Analysis performed with Partek Genomics Suite software version 6.15. Probe set data summarized and robust multiarray average algorithm used for background correction as implemented in the microarray analysis software. Genes that were regulated in response to the treatments were identified using the false discovery rate method, in which *P* values were adjusted simultaneously across multiple subgroup comparisons. IPA software used for pathway analysis.	GSE100325; GSE100324	Identified 189 aspartame-responsive differentially expressed genes (DEGs) in the adult male hypothalamus and 2188 in the adrenals. In the hypothalamus, alteration in several pathways including mineral corticoid and glucocorticoid biosynthesis, triacylglycerol degradation, retinol biosynthesis, FXR/RXR activation and many others. In the adrenal gland, there were changes in glutamate receptor signalling, FXR/RXR activation, axonal guidance signalling, GABA receptor signalling and many others. Elevated the expression of genes involved in hypothalamic neuro-steroidogenesis, together with cell stress and inflammatory genes	KC8	Hypothalamus and adrenals	Mice were exposed in utero and through weaning to aspartame (0.25 g/L); a group of these mice were administered NMDAR competitive antagonist CGP 39551 in utero only. Control mice were not given aspartame or CGP 39551.	No information about what the control mice were exposed to; unconventional pooling of the samples before microarray analysis which resulted in technical replicates and not biological replicates.	Collison et al. (2018)

Table 4.12 (continued)

End-point	Normalization and statistics	Curation	Results	Relevant KCs[a]	Tissue, cell type or cell line	Exposure concentration or range and duration	Comments	Reference
Transcriptomics Affymetrix Mouse Gene 1.0 ST arrays containing 28 853 genes	Microarray analysis was performed using Partek Genomics Suite software version 6.3 (Partek, Missouri, USA). Probe set data were summarized and background adjusted using the GC-Robust MultiArray (GCRMA) algorithm. All data normalized using nonlinear transformation quantile normalization. Used a one-way ANOVA to test for diet interactions without correction for multiple testing. IPA was used for detection of gene ontology and canonical pathway analysis.	GSE38444; GSE38445	Microarray analysis of liver and adipose tissue of mice treated with aspartame and *trans* fat-enriched diet showed a pattern of gene expression with the significant upregulation of genes involved in the organization of the cytoplasm, morphology of blood vessels, and angiogenesis, and the downregulation of genes with ontologies relating to hepatic protein metabolism, organization of peroxisomes and expression of RNA	KC10	Liver, adipose tissue	The four diet regimens used in this study were: (1) TFA control diet: consisting of 20% (w/w) partially hydrogenated vegetable shortening; (2) MSG + TFA diet: TFA diet with ad libitum drinking-water containing 0.75 g/L of MSG-hydrate; (3) aspartame + TFA diet: TFA diet with ad libutum drinking-water containing 0.25 g/L Asp-Phe methyl ester; (4) aspartame + MSG + TFA diet: TFA diet with ad libitum drinking-water containing 0.25 g/L aspartame and 0.75 g/L MSG. The four groups of breeder dams were maintained on their respective diets throughout gestation and lactation. The offspring used in the experiments were weaned onto the same maternal dietary regimen at age 4 wk and maintained on their respective diets for the duration of the study.	Analysis performed under specific dietary conditions. Gene ontology analysis not focused on canonical pathways or upstream regulators.	Collison et al. (2013)

ANOVA, analysis of variance; FXR/RXR, farnesoid X receptor/retinoid X receptor; GABA, gamma-aminobutyric acid; IPA, Ingenuity Pathway Analysis; KC, key characteristic of carcinogens; MSG, monosodium glutamate; NMDAR, *N*-methyl-ᴅ-aspartate receptor; TFA, *trans* fat diet; wk, week(s).

[a] KC10, "alters cell proliferation, cell death, or nutrient supply"; KC8, "modulates receptor-mediated effects".

with sweetness equivalent to the sucrose solution, until they were adults (age 11 weeks). Sucrose solution, but not aspartame solution, promoted social aggression, which was accompanied by increased serum corticosterone levels and reduced body weight. Transcriptome analyses of the hypothalamus indicated that the profiles for mice exposed to sucrose solution were dramatically different from those for mice in the control group or exposed to aspartame. Aspartame dysregulated genes involved in the development and functioning of the nervous system. Aspartame did not mimic the effects of sugar on social aggression and inflammatory responses. [The Working Group noted that aspartame was used as a sweetness control; the dose of aspartame administered was not provided.]

Collison et al. (2018) examined the effects of aspartame on the hypothalamic–pituitary–adrenal axis in the presence or absence of CGP 39551, a competitive antagonist of developmental NMDAR. This receptor was shown previously to modulate the effects of aspartame on several metabolic parameters (Collison et al., 2016). Mice were exposed in utero and through weaning to aspartame (0.25 g/L); a group of these mice were exposed to CGP 39551 in utero only. Control mice were not exposed to aspartame or CGP 39 551. The authors identified 189 aspartame-responsive differentially expressed genes (DEGs) in the adult male hypothalamus and 2188 in the adrenals. Aspartame exposure elevated the expression of a network of genes involved in hypothalamic neurosteroidogenesis, as well as genes involved in cellular stress and inflammation. The changes in these genes were not seen in aspartame-exposed mice that had received the antagonist CGP 39551. In the adrenal glands of aspartame-exposed mice, gamma-aminobutyric acid (GABA) and glutamate receptor subunit genes were among those that were most highly upregulated. In summary, aspartame exposure increased the expression of functional networks of genes involved in hypothalamic

neurosteroidogenesis and adrenal catecholamine synthesis; these patterns of expression were not present in aspartame-exposed mice with developmental NMDAR antagonism. [The Working Group could not determine what the control mice were exposed to. Unconventional pooling of samples was carried out. The authors stated that "to minimize the differences of individual variability and increase the statistical power for the identification of potential biomarkers, microarray analysis was performed using equal amounts of purified RNA pooled from all of the study subjects ($n = 18$ per treatment group) and applied in triplicate". Thus, the authors examined technical replicates, not biological replicates.]

Collison et al. (2013) investigated the effects of changes in hepatic and adipose tissue gene expression induced by the food additives aspartame or monosodium glutamate (MSG), or a combination of both, in C57Bl/6 J mice fed a *trans* fat-enriched diet. Microarray analysis of liver and adipose tissue of mice exposed to aspartame and the *trans* fat-enriched diet showed significant upregulation of genes involved in the organization of the cytoplasm, morphology of blood vessels, and angiogenesis, and downregulation of genes with ontologies relating to hepatic protein metabolism, organization of peroxisomes, and expression of RNA. Aspartame-induced genes relating to cell proliferation and the development of blood vessels were upregulated in the adipose tissue of mice fed a *trans* fat-enriched diet, whereas downregulated ontologies included adipogenesis and the proliferation of immune cells. [The Working Group considered this study less informative, since the effects of aspartame were observed under specific dietary conditions. In addition, the gene ontology (GO) analysis did not focus on canonical pathways of upstream regulators; a global view of the impact of exposure on such pathways was not provided.]

(b) Evaluation of high-throughput in vitro toxicity screening data

The analysis of the in vitro bioactivity of the agents reviewed in *IARC Monographs* Volume 134 was informed by data from high-throughput screening assays generated by the Toxicology in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the government of the USA (Thomas et al., 2018). Aspartame was one of thousands of chemicals tested across the large assay battery of the Tox21 and ToxCast research programmes. Detailed information about the chemicals tested, assays used, and associated procedures for data analysis is publicly available (US EPA, 2023).

The ToxCast/Tox21 high-throughput screening results are presented according to the assays that have been mapped to the key characteristics of carcinogens (Reisfeld et al., 2022). The detailed results are available in the supplementary material for this volume (Annex 4, Supplementary material for Section 4, Mechanistic Evidence, online only, available from: https://publications.iarc.who.int/627). Here, for brevity, assays for which there was a positive "hit call" are referred to as "active" assays. A summary of these results is given below as the number of active assays (without any caution flags) divided by the total number of key characteristic-related assays for the chemical.

Among the 192 assays in which aspartame was tested, it was found to be active and without caution flags in only one assay relevant to the key characteristics of carcinogens, that is, one of the 95 assays mapped for key characteristic 8, "modulates receptor-mediated effects" (Reisfeld et al., 2022). This assay, ATG_ERE_CIS_up, is a cell-based, multiplexed-readout assay in HepG2 (a human liver cell line) with measurements taken 24 hours after chemical dosing in a 24-well plate. The assay measures the ability of estrogen receptor to bind to the estrogen receptor binding element (ERE) and activate a reporter gene; the AC_{50} (50% of maximum activity) for aspartame was 39.46 µM. The assay is also one of 17 assays that measure the ability of a chemical to perturb the estrogen receptor pathway (Reisfeld et al., 2022) and have been used to assess estrogen receptor agonism or antagonism described in previous studies (e.g. Judson et al., 2015). [The Working Group noted that the fact that aspartame gave positive results in only one of the 17 assays and the relatively high AC_{50} compared with 17β-estradiol itself makes it unlikely that the compound has any estrogen receptor activity.]

4.3 Other relevant evidence

4.3.1 Alterations in glucose and lipid metabolism

Hyperinsulinaemia (a hallmark of insulin resistance) and dyslipidaemia can play relevant roles in carcinogenesis (Tumminia et al., 2019; Neshat et al., 2022; Rojas et al., 2023). In the past two decades, an increasing number of studies (either cross-sectional or intervention studies) have investigated the effects of various non-nutritive sweeteners, including aspartame (single or repeated dosing), on glucose or lipid metabolism under various conditions, i.e. in participants who were healthy, obese, diabetic, or pregnant, and before, after, or during exercise. Numerous investigations have also been conducted in experimental systems, especially in vivo. The findings are reported in the present section, and several end-points are also relevant to the 10 key characteristics of carcinogens. Although many of the studies in humans reported conflicting results, there was consistent evidence that aspartame increased serum insulin levels in lambs and in several studies in rodents, indicating alterations in insulin sensitivity, including changes to lipid metabolism.

(a) Humans

See Table 4.13.

(i) Cross-sectional studies

Liu et al. (2022b) investigated associations between maternal serum aspartame (and sucralose) levels and metabolic health during pregnancy in a nested population-based case–control study. The study recruited 632 women in early pregnancy (weeks 9–14 of gestation) and undertook detailed biochemical and metabolic assessments at 24–32 weeks (as close to 28 weeks as possible). The 109 women diagnosed with gestational diabetes mellitus at this visit were compared with 109 controls, matched on age and self-reported pre-pregnancy BMI. Serum levels of aspartame and sucralose measured at the same visit were quantified using "ultra-performance liquid chromatography coupled to a tandem mass spectrometer", a method previously used for urine specimens. [The Working Group noted that this was the only study to detect aspartame in the blood. Although advances in the sensitivity of analytical methods may explain the detection of aspartame in this recent study compared with previous studies, concerns about the study methodology limited the informativeness of this study (see Section 1.3).] Other parameters, including those used for the diagnosis of gestational diabetes mellitus, were measured by standard methods. There were no significant differences between cases with gestational diabetes and matched controls with regard to serum levels of aspartame or sucralose. Further analyses of the data from all participants combined examined relations between levels of each of the two non-nutritive sweeteners (both as categorical and continuous variables) and the various metabolic and biochemical markers measured at 28 weeks. The higher serum levels of aspartame levels were positively associated with elevated haemoglobin A1C (HbA1c), insulin resistance, hypercholesterolaemia, and hyper-low-density lipoprotein (LDL) cholesterolaemia. These associations were identified in separate analyses of the cases with gestational diabetes mellitus and the controls.

Hess et al. (2018) reported a cross-sectional study of non-nutritive sweetener consumption and metabolic syndrome in 123 adults. Participants completed three 24-hour dietary recall questionnaires over a 2-week period and had a fasting blood draw for glucose and lipids analyses at the time of the second dietary recall. The dietary data were used to generate estimates of the amount of specific non-nutritive sweeteners consumed and whether specified levels of consumptions had been met. On the basis of the information collected, 18 participants met the criteria for metabolic syndrome, and 63 participants were categorized as consumers of non-nutritive sweeteners, including 33 who were consumers of aspartame. Data were adjusted for age, sex, total caloric intake, dietary quality, physical activity, and non-nutritive sweetener consumption, and a significant positive association was observed between aspartame consumption and levels of blood glucose and triglycerides, but not waist circumference. The latter parameter was, however, statistically significantly associated with consumption of the other non-nutritive sweeteners considered. [The Working Group noted that the exposure assessment had some limitations (see Table 4.13).]

Kuk and Brown (2016) reported analyses of data from a subset of 2856 participants aged 40–74 years in the Third National Health and Nutrition Examination Survey (NHANES III) who had, in addition to the standard survey measures, undergone an oral glucose tolerance test (OGTT) and were not taking medication for diabetes. A 24-dietary recall questionnaire was used to classify participants as either high- or low-level consumers of specific sugars and as consumers versus non-consumers of specific non-nutritive sweeteners (saccharin and aspartame). Consumers of non-nutritive sweeteners had a higher average BMI than did non-consumers (28 versus 27 for both saccharin and

Table 4.13 Effects of aspartame on glucose and lipid metabolism in exposed humans

End-point	Location, setting, study design, study population	Results	Exposure level and no. of exposed and controls	Comments	Exposure assessment critique	Reference
Cross-sectional studies						
Insulin HbA1c Total cholesterol LDL	Shanghai, China, nested case–control study Women with gestational diabetes mellitus	Positive associations of plasma aspartame with cholesterol (total and LDL) levels, and insulin resistance indices	Serum levels of aspartame quantified in 109 women with gestational diabetes mellitus compared with 109 controls	The study measured aspartame in the blood, but the detection method was not standardized for blood samples (see also Section 1.3).		Liu et al. (2022b)
Fasting glucose TG HDL-C	South-western Virginia, USA, cross-sectional study Adults with metabolic syndrome	Fasting glucose and triglyceride values were positively associated with aspartame consumption	24-hour dietary recall questionnaire 33 consumers of aspartame out of 125 study participants	Linear regression models, adjusted for age, sex, caloric intake, dietary quality, and physical activity, examined associations between total and individual types of NNS with metabolic syndrome indices.	Small sample size Limited description on the source of composition data, aspartame amounts consumed, and use of medicines or supplements.	Hess et al. (2018)
Glucose Insulin	USA, cross-sectional study NHANES III cohort	Aspartame intake significantly influenced the association between BMI and glucose tolerance	24-hour dietary recall questionnaire 2856 participants	Single 24-hour dietary recall questionnaire could miss less frequent use	Limited description of how items with non-nutritive sweeteners were classified as containing aspartame or other sweeteners.	Kuk and Brown (2016)
Insulin GLP-1 Leptin	Canada, randomized, controlled, double-blinded, crossover design Healthy subjects, *n* = 17 (10 women and 7 men)	No changes in insulin, glucose, active GLP-1, and leptin levels between the baseline visit and the visit after the 2-wk washout period	Repeated dose, 425 mg/day for 2 wk Measurement at baseline and after 2 wk	No effect of aspartame on insulin sensitivity in normoglycaemic subjects. The dose used was reflective of common consumption. Sucralose was also tested. Well-conducted study	Quantitative aspartame exposure was assessed. Comprehensive measurement of background diet conducted. Compliance with treatment assessed.	Ahmad et al. (2020)

Table 4.13   (continued)

End-point	Location, setting, study design, study population	Results	Exposure level and no. of exposed and controls	Comments	Exposure assessment critique	Reference
Insulin Leptin GLP-1 GIP	USA, randomized, controlled trial Healthy male subjects ($n = 100$)	No changes in glucose, insulin, resting leptin, GLP-1, or gastric inhibitory peptide	Repeated dose, 0, 350, or 1050 mg of aspartame in a beverage for 12 wk. Measurement at baseline and after 12 wk (about 30 subjects/dose group).	There were no effects of aspartame ingestion on appetite, body weight, or body composition. Well-conducted study.	No record of intakes from other sources. A screening questionnaire was used to select participants who were low or non-consumers of low-calorie sweeteners.	Higgins et al. (2018)
Insulin GLP-1	Türkiye, single-blinded randomized trial Healthy males ($n = 8$), and participants with diabetes type 2 ($n = 8$)	No changes of insulin, C-peptide and GLP-1 in both healthy and subjects with diabetes type 2	72 mg of aspartame 15 min before oral glucose tolerance test Control was non-sweetened water.	Also tested was sucralose, which enhances GLP-1 release in healthy subjects. Well-conducted study.		Temizkan et al. (2015)
Intervention studies						
Insulin Glucagon Cholesterol TG HDL	Japan, intervention trial Healthy participants ($n = 7$) and untreated diabetic patients ($n = 22$), (gender not reported)	A small but significant decrease in blood glucose. No changes in insulin or glucagon levels.	Single dose, 500 mg of aspartame. Measurements at baseline and after 3 hours.	Well-conducted study.		Okuno et al. (1986)
	Hospitalized diabetic patients ($n = 9$) (6 women, 3 men)	No changes in glucose, cholesterol, TG, or HDL levels.	Repeated dose, 125 mg of aspartame, 2 wk, to hospitalized diabetics with steady-state glycaemic control. Measurements at the beginning and end of aspartame administration.			Okuno et al. (1986)

Table 4.13 (continued)

End-point	Location, setting, study design, study population	Results	Exposure level and no. of exposed and controls	Comments	Exposure assessment critique	Reference
Glucose Insulin	USA, interventional trial Healthy participants ($n = 7$) (5 women and 2 men), and PKU patients ($n = 7$) (4 women and 3 men)	No changes in glucose and insulin levels	(1) 12 oz of unsweetened cherry-flavoured beverage; (2) 12 oz of cherry-flavoured beverage providing 60 g of carbohydrates; (3) 12 oz of cherry-flavoured beverage providing 200 mg of aspartame; (4) 12 oz of cherry-flavoured beverage providing 200 mg of aspartame and 60 g of carbohydrates.	Each participant (healthy and PKU) was studied on four different days after an overnight fast with different treatments. Well-conducted study.		Wolf-Novak et al. (1990)
Prolactin Growth hormone, cortisol Insulin	USA, intervention trial Healthy subjects, ($n = 16$) (8 women and 8 men)	No changes in prolactin, growth hormone or cortisol levels. Serum insulin decreased slightly, and serum glucose increased slightly over the course of all test procedures.	Single dose of 0.534 g of aspartame, acute administration. Measurements at baseline and after 150 min	Well-conducted study.		Carlson and Shah (1989)
Glucose Insulin CCK GLP-1 GIP	Surrey, United Kingdom, intervention study, triple crossover design Healthy participants ($n = 6$) (4 women, 2 men)	GLP-1 concentrations decreased after a liquid meal (60–120 min) after aspartame treatment. No changes in CCK, GIP, insulin, and glucose levels.	Single dose, capsules consisting of either aspartame (400 mg), 176 mg aspartic acid + 224 mg phenylalanine, or 400 mg of corn flour (control) in 450 mL of water			Hall et al. (2003)

Table 4.13 (continued)

End-point	Location, setting, study design, study population	Results	Exposure level and no. of exposed and controls	Comments	Exposure assessment critique	Reference
Insulin	Australia, intervention trial Healthy participants (*n* = 17) in three different studies: study 1 (4 women, 2 men), study 2 and 3 (7 and 5 men, respectively)	No changes in insulin levels, or in cephalic response	Each subject was studied twice (a food stimulus study and a control study) in the morning after an overnight fast. Aspartame dose, 19 mg.	Well-conducted study.		Bruce et al. (1987)
Glucose Insulin	Japan, randomized crossover design Healthy participants (*n* = 9) (7 women and 2 men)	No changes in glucose or insulin levels.	Participants rinsed their mouths with either 25 mL of water (control) or a 2.5% *Gymnema sylvestre* extract solution, and then ingested 200 g (50 g × 4) of 0.09% aspartame.	*Gymnema sylvestre* is a plant that selectively suppresses sweet taste sensation in humans.	Quantitative aspartame exposure specifically assessed. No measurement of background or usual diet conducted.	Kashima et al. (2019)
Insulin	USA, intervention trial Healthy participants (*n* = 15) (all men)	No changes.	Participants sipped and spit solutions of water or aspartame. Measurement at baseline and then at 1 min post-stimulus, followed by every 2 min for 15 min and then every 5 min for 15 min.	The study aimed to describe cephalic response. Well-conducted study.		Teff et al. (1995)
Glucose Insulin	France, intervention trial Healthy participants (*n* = 7) (3 women and 4 men)	No changes in insulin levels.	Single dose, 250 mg of aspartame dissolved in 250 mL of water. Measurements at baseline and 30, 60, 90, 120, 150, and 180 min after the ingestion of aspartame.	The study was designed to measure calcium and oxalate excretion in the urine, which was not affected by aspartame. Well-conducted study.		Nguyen et al. (1998)

Table 4.13   (continued)

End-point	Location, setting, study design, study population	Results	Exposure level and no. of exposed and controls	Comments	Exposure assessment critique	Reference
Glucose Insulin	Denmark, intervention trial Healthy participants (*n* = 6) (all men)	Decrease in glucose levels compared with control group (water). No changes in insulin levels.	Single dose of 1.0 g of aspartame dissolved in 200 mL of water, or water alone. Measurements at baseline and after 4 h.	The study showed that the intake of aspartame at the high dose produced a marked and persistent increase in the availability of phenylalanine, which was not observed after protein intake.		Møller (1991)
Glucose Glycated haemoglobin (HbA1c) Insulin TG HDL LDL	USA, randomized controlled trial Overweight or obese adult participants (*n* = 154; total *n* = 30) (gender not specified), randomly assigned to aspartame consumption.	No changes	Repeated dose of beverages containing 0.73, 0.58, or 0.66 g of aspartame for 12 wk; 123 subjects completed the study. Measurements at baseline and after 12 wk.	No effect on body composition. Glucose and lipid metabolism were included with body weight and related parameters measurements. Aspartame used as positive control in comparison with other sweeteners.	Quantitative aspartame exposure specifically assessed. Measurement of background diet included.	Higgins and Mattes (2019)
Glucose Insulin Glucagon Cholesterol TG	USA, double-blind randomized intervention trial Overweight participants (age 10–21 yr) (*n* = 59; total, *n* = 55) completed the study.	No changes	Repeated dose, 2.7 g of aspartame per day in capsules for 7 wk.			Knopp et al. (1976)
Glucose Insulin	Overweight participants (*n* = 12) (6 men and 6 women), and normal weight participants (*n* = 12) (6 men and 6 women)	No changes	Single dose, beverage containing 0.25 g of aspartame in 500 mL of deionized distilled water. Blood samples were taken at 10, 18, 25, 33, 40, and 48 mm after the start of ingestion preload			Rodin (1990)

Table 4.13 (continued)

End-point	Location, setting, study design, study population	Results	Exposure level and no. of exposed and controls	Comments	Exposure assessment critique	Reference
Insulin Glucagon	USA, intervention trial Healthy participants ($n = 12$) (all women), and patients with type 2 diabetes ($n = 10$) (5 women and 5 men)	No changes in peak insulin and glucagon levels, and glucose in subjects with or without diabetes. AUC for insulin was significantly higher after aspartame ingestion in normal participants, but not in participants with type 2 diabetes.	Single dose of an unsweetened beverage, or a sweetened beverage with 400 mg of aspartame, or a sweetened beverage with 135 mg of saccharin. Measurements at baseline and 15, 30, 45, 60, 75, 90, 120, 150, and 180 min after consuming the beverage.	Glucagon levels showed time-to-time variation but no overall differences, but the magnitude of the difference was small. The amount of sweetener approximated to that in 1 L of sugar-free soft drink. Well-conducted study.		Horwitz et al. (1988)
Insulin Glucagon	USA, open-label crossover study Healthy subjects, $n = 12$ (all females), and type 2 diabetes patients, $n = 10$ (5 women and 5 men)	AUC for insulin was significantly higher after aspartame ingestion in normal subjects, but not in subjects with type 2 diabetes.	Single dose 400 mg aspartame. Measurements at baseline and after 3 hours.	Part of the data was published in Horwitz et al. (1988), who prepared a regulatory study report.		EFSA_UN01 (2011)
Glucose	Chicago, USA, randomized controlled trial Diabetic patients, $n = 62$ (all with both types of diabetes)	No changes	Repeated dose, 2.7 g of aspartame per day for 18 days or placebo.			Nehrling et al. (1985)
Glucose Insulin Cholesterol HDL TG	Australia, double-blind crossover study Type 2 diabetes patients, taking medications ($n = 9$) (1 woman and 8 men)	No changes	Repeated dose, 162 mg of aspartame for 6 wk.	Well-conducted study. Compared the effects of adding sucrose and aspartame to the usual diet of participants with type 2 diabetes.		Colagiuri et al. (1989)

Table 4.13 (continued)

End-point	Location, setting, study design, study population	Results	Exposure level and no. of exposed and controls	Comments	Exposure assessment critique	Reference
Glucose Insulin	Japan, two groups: (1) type 2 diabetes patients, *n* = 15	No changes	75 g oral glucose tolerance test and 5 days after the test, a 225 mg oral aspartame loading test. Plasma glucose and insulin levels were measured at 0, 30, 60, 90, 120, and 180 min.	Patients were receiving different therapies for diabetes.		Shigeta et al. (1985)
	(2) type 2 diabetes patients, *n* = 20	No changes	Consumed jellies sweetened with 240 mg of aspartame. The fasting plasma glucose was measured in the morning before and after aspartame loading.			
De novo lipogenesis-derived fatty acids Stearoyl-CoA desaturase activity Phospholipids Cholesteryl esters TG	Denmark, Copenhagen, randomized parallel study Healthy subjects, *n* = 41 (11 out of 41 consuming aspartame)	No changes	Consumption of aspartame-sweetened soda for 24 wk.	The study compared the effects of sugar-sweetened soda, semi-skimmed milk, aspartame-sweetened soda, or water.		Bajahzer et al. (2022)
Glucose Insulin	Australia Healthy subjects, *n* = 9 (all men)	No changes	Dosing: (1) carbohydrate 2% maltodextrin and 5% sucrose; (2) 0.04% aspartame, 2% maltodextrin and 5% sucrose; (3) water; and (4) aspartame (0.04% aspartame with 2% maltodextrin).	Well-conducted study. Each participant completed four trials under the same conditions (45 min rest + 60 min self-paced intense exercise) differing only in their fluid intake.		Siegler et al. (2012)

AUC, area under the curve; BMI, body mass index; CCK, cholecystokinin; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; HDL, high-density lipoprotein; HDL-C, high-density lipoprotein cholesterol; LDL, low-density lipoprotein; min, minute(s); NHANES, National Health and Nutrition Examination Survey; NNS, non-nutritive sweetener; PKU, phenylketonuria; TG, triglycerides; wk, week(s).

aspartame). There were no significant differences in markers of glucose tolerance and insulin resistance according to level of consumption of either sugars or non-nutritive sweeteners. In participants classified as aspartame consumers, the association between BMI and the 2-hour glucose test was significantly higher than among non-consumers. There was no similar interaction for higher levels of sucrose, fructose, or saccharin. [The Working Group noted that a limitation of this study was that a single 24-dietary recall questionnaire could fail to detect infrequent use. Additionally, it was unclear how non-nutritive sweetener items were classified as containing aspartame or other sweeteners.]

[The Working Group reviewed articles in which aspartame was used as a control to test the effect of other sweeteners and deemed these studies uninformative since the study design precluded any interpretation related to the effects of aspartame (Anton et al., 2010; Maersk et al., 2012; Kimura et al., 2017; Harder et al., 2020; Hieronimus et al., 2020; Sorrentino et al., 2020; Sigala et al., 2021, 2022).]

(ii)   Intervention studies

The effects of single or repeated doses of aspartame have been assessed in healthy, obese, and diabetic participants, during pregnancy, and before, after, or during exercise.

No significant effects on glucose metabolism, including insulin levels, were observed in several intervention studies in which aspartame was administered either as a single dose or as repeated doses for up to 12 weeks to healthy participants (Okuno et al., 1986; Bruce et al., 1987; Carlson and Shah, 1989; Wolf-Novak et al., 1990; Møller, 1991; Teff et al., 1995; Nguyen et al., 1998; Hall et al., 2003; Temizkan et al.; 2015; Higgins et al., 2018; Kashima et al., 2019; Ahmad et al., 2020). Similarly, in the few studies conducted in participants who were overweight or obese and were given a single dose of aspartame or repeated doses for up to 12 weeks (Knopp et al., 1976; Rodin,

1990; Higgins and Mattes, 2019), no alterations in blood concentrations of glucose, insulin, leptin, or glucagon were observed. The effect of aspartame in people with type 2 diabetes was assessed in several single-dose studies (Okuno et al., 1986; Horwitz et al., 1988; EFSA_UN01, 2011; Temizkan et al., 2015) and in a few short-term studies (up to 6 weeks) (Nehrling et al., 1985; Shigeta et al., 1985; Colagiuri et al., 1989). In general, no significant changes in plasma glucose, insulin, glucagon, C-peptide, or glucagon-like peptide-1 (GLP-1) were observed. [The Working Group noted that these studies, although well conducted, presented several limitations. The studies were not designed to evaluate the effects of insulin after long-term ingestion of aspartame. Some of these studies measured the cephalic response to food and were therefore not specifically designed to observe effects on insulin. Some studies administered aspartame in capsules that impaired the sweet sensor in the mouth. In addition, the study participants were not clearly screened for past use of artificial sweeteners to determine whether they were non-consumers or light-consumers, or whether they had a history of prolonged use.]

In a randomized parallel study, Bajahzer et al. (2022) did not observe alterations in de novo lipogenesis fatty acids in 11 individuals who consumed aspartame-sweetened soda for 24 weeks. [The Working Group noted that the study aimed to compare the effects of sugar-sweetened soda, semi-skimmed milk, aspartame-sweetened soda, and water, and the change at 24 weeks was assessed and compared across the groups using analysis of variance (ANOVA) and mixed-effects models.]

There were two studies that evaluated glucose metabolism after exposure to aspartame combined with exercise (Ferland et al., 2007; Siegler et al., 2012). Ferland et al. (2007) investigated plasma glucose and insulin levels during an acute bout of exercise in 14 men with type 2 diabetes. In the group that received aspartame, there was a 34%

reduction in glucose and 73% reduction in insulin levels after exercise, in contrast to the group in the fasting state for which there were no changes after exercise. [The Working Group noted that this was a short report and did not report many details on the study design and results.] Siegler et al. (2012) studied the effects of aspartame in nine healthy subjects at rest and during endurance exercise. No differences between pre- and post-exercise levels of insulin and blood glucose were observed after consumption of aspartame. [The Working Group noted that aspartame may affect the release of insulin during exercise, or at least that exercise may interfere with the possible effects of aspartame; however, there are few studies exploring this association.]

In a study by Singleton et al. (1999), 12 male and 10 female healthy adults ingested a milkshake containing 108 g dairy cream alone, or supplemented with 30 g of fructose, 17.5 g of glucose, or 1 g of aspartame. Blood samples were collected at baseline, and 2, 4, 6, and 8 hours after ingestion. All milkshakes resulted in significant elevations of triglycerides at the 2- and 4-hour time points. At 6 hours, only the groups that were given glucose and fructose had significantly higher values of triacylglycerol than at baseline. No significant effects or interactions were noted related to concentrations of insulin, glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol, or low-density lipoprotein (LDL) cholesterol.

[The Working Group noted that studies in which aspartame was co-administered with other non-nutritive sweeteners during exercise (Sylvetsky et al., 2016; Kim et al., 2020), or in which the group receiving aspartame was used as a control (Wouassi et al., 1997; Sigala et al., 2020) were reviewed but considered to be uninformative.]

(b) Experimental systems

See Table 4.14.

Several studies that examined the effects of aspartame on glucose metabolism and associated hormones in non-human mammals in vivo (including lambs, rats, and mice) were available to the Working Group.

The studies measured glucose and insulin under several conditions. Glucose levels were measured using standard techniques, such as fasting blood glucose levels, blood glucose levels after a bolus of glucose, by using the glucose tolerance test (GTT), or blood glucose levels after an insulin tolerance test (ITT) (designed to determine whole-body sensitivity of insulin receptors by measuring blood glucose level changes before and after insulin administration). In some studies, the homeostatic assessment insulin resistance (HOMA-IR) computational model was used, measuring glucose and insulin after aspartame exposure. Several observed end-points also linked to the key characteristics of carcinogens "induces oxidative stress", "induces chronic inflammation", and "alters cell proliferation, cell death, or nutrient supply".

In a study in lambs, Sun et al. (2019) observed that, as well as histological alterations in the small intestine, aspartame exposure caused a significant increase in plasma concentrations of gastrointestinal hormone glucagon-like peptide-2 (GLP-2) and induced upregulation of GCG, GLP-2R, IGF-1, and IGF-1R mRNA expression levels in the different intestine regions. No effects on plasma glucose levels were observed (see also Section 4.2.8(b)).

Increases in blood levels of insulin or glucose after the administration of aspartame were observed in several studies in rats (Palmnäs et al., 2014; Janssens et al., 2017, Lebda et al., 2017b; Ryuk et al., 2019; Mbambo et al., 2020). When administered in the drinking-water for approximately 7 weeks, aspartame increased fasting insulin levels without changes in lipid

Table 4.14 Effects of aspartame on glucose and lipid metabolism in experimental systems

End-point	Species, strain (sex)	Tissue	Results	Route, duration, dosing regimen, dose	Comments	Reference
Glucose GLP-2	Sheep, Hu lambs	Blood	No change in glucose Increase in GLP-2	Starter diet by itself or supplemented with aspartame at 200 mg/kg for 35 days ($n = 6$ per group).	Aspartame supplementation in starter diet accelerates small intestinal cell cycle (see Section 4.2.9).	Sun et al. (2019)
Glucose Insulin Hepatic dietary lipid uptake De novo lipogenesis	Rat, Wistar (M)	Blood	Increase in fasting insulin. No changes in glucose or in lipid metabolism	There were four treatment groups: control group: normal drinking-water; glucose group: 13% (w/v) glucose; fructose group: 13% (w/v) fructose; and aspartame group: 0.4% (w/v) aspartame in drinking-water for 7 wk.	Fructose stimulates hepatic de novo lipogenesis.	Janssens et al. (2017)
Glucose	Rat, Sprague-Dawley	Blood	Increase in glucose after 60 and 120 min of glucose ingestion	Rats had access ad libitum to 10% sucrose solution, while rats belonging to the aspartame, sucralose, cyclamate, saccharin, and stevia groups had ad libitum access ad libitum to their respective solutions (dissolved in drinking-water) at concentrations equivalent to the sweetness of 10% sucrose. Treatment continued for a 5-wk period, during which the normal control rats received normal water ad libitum.	Sex not indicated. No information on the concentration of aspartame administered.	Mbambo et al. (2020)
Glucose Cholesterol TG HDL LDL VLDL mRNA leptin Adiponectin PPARγ	Rat, Wistar (M)	Blood	Increase in glucose. Increase in TG, LDL, and VLDL No changes in cholesterol and HDL Upregulation of mRNA leptin, and downregulation of mRNA of adiponectin and PPARγ in adipose tissues	There were three treatment groups: group I was allowed to drink water ad libitum; group II was intragastrically intubated with aspartame at 240 mg/kg bw; and group III was allowed to freely drink commercial cola beverages for 2 mo.		Lebda et al. (2017a)

Table 4.14 (continued)

End-point	Species, strain (sex)	Tissue	Results	Route, duration, dosing regimen, dose	Comments	Reference
Glucose Insulin GLP-1 TG	Rat, ovariectomized Sprague-Dawley (F)	Blood	Increase in HOMA-IR, HOMA-B, serum insulin; increases in IGF-1 mRNA in jejunum. Increase serum GLP-1. Decrease in TG.	Rats were fed five high-fat diets (45% fat) containing: (1) 10% corn starch; (2) 10% sucrose; (3) 10% fructose; (4) 0.05% aspartame + 9.95% starch; or (5) 0.05% sucralose + 9.95% starch (for 8 wk).		Ryuk et al. (2019)
Glucose Insulin	Rat, Wistar (M)	Blood	Increase in glucose at 104 and 197 days. Decrease in glucose at 288 days, and insulin at 9 mo.	Nutritive sweeteners (fructose 7% and sucrose 10%) and non-nutritive or low-calorie sweeteners (acesulfame, 0.015%; aspartame, 0.3%; aspartame/acesulfame mixture, 0.04%; saccharin, 0.3%; and sucralose, 0.19%) in drinking-water, as well as a control group with no sweeteners; treated for up to 288 days.		Mendoza-Pérez et al. (2021)
Glucose Insulin TG	Rat, Sprague-Dawley (M)	Blood	On regular chow diet: increase of fasting blood glucose; increase blood glucose in the insulin tolerance test. On high-fat diet: increase in fasting blood glucose, increase in blood glucose in the insulin tolerance test, decrease in plasma insulin. No changes in TG.	Adult rats were exposed to standard chow diet (12% kcal fat) or high-fat diet (60% kcal fat) and further divided into ad libitum water control or low-dose aspartame (5–7 mg/kg per day in drinking-water) for 8 wk.	Faecal analysis of gut bacterial composition showed that aspartame increased total bacteria, and the abundance of *Enterobacteriaceae* and *Clostridium leptum*. Aspartame increased levels of acetate and butyrate in chow diet groups, while formate and isobutyrate levels remained unchanged.	Palmnäs et al. (2014)
Glucose Insulin Cholesterol LDL HDL	Rat, Sprague-Dawley (M)	Blood	Increase in fasting glucose; increase in HOMA-IR strongly related to the amount of ingested aspartame; no changes in fasting insulin.	Aspartame (0·05%) was added to the diet or drinking-water or both diet and water, and a control group was given a non-sweetened diet with plain water for 7 wk.		Ragi et al. (2021)
Glucose Insulin	Rat, Charles River	Blood	No changes	7 days of exposure to diet containing aspartame (0.2% or 2%).		EFSA_E1 (2011)

Table 4.14 (continued)

End-point	Species, strain (sex)	Tissue	Results	Route, duration, dosing regimen, dose	Comments	Reference
Glucose Insulin	Rat, Sprague-Dawley (M)	Blood	No changes	Rats were treated with water as a control or aspartame (6–8 mg/kg bw per day in week 1 to 15–21 mg/kg bw per day in week 6). Rats were evaluated after 6 wk of treatment.		Tovar et al. (2017)
Glucose Insulin Leptin	Rat, Long–Evans	Blood	No changes in glucose or insulin levels Decreased levels of leptin	Tap water controls and aspartame group (1 g/L) made with tap water for 14 wk (starting at weaning).		Beck et al. (2002)
Glucose Insulin	Rat, STZ-induced Sprague-Dawley diabetic (F)	Blood	No changes	After 18 h of fasting, glucose (150 mg per 100 g bw) or aspartame (0.45 mg per 100 g bw) dissolved in 2 mL of saline was administered through a stomach tube to the STZ-induced diabetic and control rats, respectively. Blood samples were obtained through a polyethylene catheter inserted into the femoral vein for the measurement of plasma glucose and insulin levels at appropriate intervals.		Shigeta et al. (1985)
Glucose Insulin	Rat, STZ-induced Wistar (M)	Blood	No changes	Diabetic aspartame group: rats that were given aspartame (50 mg/kg) intragastrically and daily for 6 wk starting from wk 6 after developing diabetes. Diabetic group treated with insulin and aspartame (D-insulin + aspartame): rats that were given NPH insulin (12 UI/kg) daily for 11 wk from the first week of having developed diabetes mellitus. Starting on the fifth week of insulin administration, all the rats in this group were also given aspartame (50 mg/kg bw per day intragastrically) for 6 wk. Control groups were compared to diabetic rats		Nosti-Palacios et al. (2014)

Table 4.14   (continued)

End-point	Species, strain (sex)	Tissue	Results	Route, duration, dosing regimen, dose	Comments	Reference
Glucose Insulin	Mouse, C57BL/6J (M, F)	Blood	Increase of insulin sensitivity in male offspring	Dams were randomly assigned to drinking-water; sucrose (45 g/L, ~7.2 g/kg bw per day anticipating 4 mL water intake, and 25 g bw); aspartame (0.2 g/L, ~32 mg/kg bw per day); or sucralose (0.04 g/L, ~6.4 mg/kg bw per day) throughout pregnancy and lactation. Mice were evaluated at age 4–12 wk.	Maternal aspartame consumption under all conditions increased body weight in male offspring by age 7 wk compared with the male offspring of control dams.	Azad et al. (2020)
Glucose Insulin	Mouse, C57BL/6J mice (M, F)	Blood	Aspartame affects visceral fat deposition and glucose homeostasis particularly in males, and to a lesser extent in females.	Mice were exposed starting in utero up to age 17 wk. Treatment groups were: (1) ad libitum standard chow with ad libitum drinking-water; (2) ad libitum standard chow with ad libitum drinking-water containing aspartame at 0.25 g/L as the only source of drinking-water.		Collison et al. (2012a)
Glucose Insulin	Mouse, C57BL/6J (M, F)	Blood	Increase in fasting glucose in both sexes, and in glucose in insulin tolerance test in males.	Mice were exposed starting in utero up to age 17 wk. Treatment groups were: (1) ad libitum standard chow with ad libitum drinking-water; (2) ad libitum standard chow with ad libitum drinking-water containing MSG at 0.75 g/L; (3) ad libitum standard chow with ad libitum drinking-water containing aspartame at 0.25 g/L; (4) ad libitum standard chow with ad libitum drinking-water containing aspartame at 0.25 g/L and MSG at 0.75 g/L.		Collison et al. (2012b)

Table 4.14 (continued)

End-point	Species, strain (sex)	Tissue	Results	Route, duration, dosing regimen, dose	Comments	Reference
Glucose Insulin	Mouse, C57BL/6J (M)	Blood	Increase in serum glucose and insulin.	Mice were exposed in utero and during weaning to aspartame (0.25 g/L); a group of these mice were treated with the NMDAR antagonist CGP 39551 in utero only. Control mice were not given aspartame or CGP 39551. Dams continued to receive the treatment in the drinking-water until weaning was completed at day 28, whereupon male F_1 offspring (no more than 3 animals per litter) in the control and control + CGP 39551 group were given ad libitum plain drinking-water and standard chow, and the offspring in the aspartame and the aspartame + CGP 39551 group were given ad libitum drinking-water containing aspartame at 46 mg/kg bw together with standard chow for the duration of the study.	Examined effects in males only.	Collison et al. (2016)
Glucose Insulin	Mouse, C57BL/6J (M)	Blood	Increase in fasting glucose levels at 6 wk but decrease at 17 wk. No changes in insulin or HOMA-IR.	The four diet regimens used in this study were: (1) TFA control diet consisting of 20% (w/w) partially hydrogenated vegetable shortening; (2). MSG + TFA diet: TFA diet with ad libitum drinking-water containing 0.75 g/L of L-glutamic acid monosodium salt hydrate; (3) aspartame + TFA diet: TFA diet with ad libitum drinking-water containing 0.25 g/L aspartame; or (4) aspartame + MSG + TFA diet: TFA diet with ad lib drinking-water containing 0.25 g/L aspartame and 0.75 g MSG. After mating, the breeder dams were maintained on their respective diets throughout gestation, birth, and nursing. The offspring used in the following experiments were weaned onto the same maternal dietary regimen at age 4 wk and maintained on their respective diets for the duration of the study. Measurements in males were carried out at age 6 and 17 wk.	The study was performed under specific diet conditions.	Collison et al. (2013)

Aspartame

Table 4.14 (continued)

End-point	Species, strain (sex)	Tissue	Results	Route, duration, dosing regimen, dose	Comments	Reference
Glucose Cholesterol LDL HDL	Mouse, Swiss (M)	Blood	Decrease in fasting glucose levels. Increase in cholesterol, LDL, and TG levels.	Aspartame at 80 mg/kg (2.5 mL/kg, prepared in 0.9% saline solution) or vehicle, administered daily by gavage for 12 wk.		Finamor et al. (2021)
Glucose Insulin	Mouse, C57BL/6	Blood	Increase in fasting glucose.	Four groups: control high-fat diet, aspartame-treated high-fat diet, control normal diet, and aspartame-treated normal diet. Mice received either regular autoclaved drinking-water or an aspartame-containing solution (0.96 mg/mL). Mice were treated for 18 wk.		Gul et al. (2017)
Glucose Insulin	Mouse, C57BL/6 pregnant dams	Blood	Decrease in glucose plasma levels. No change in insulin plasma levels.	Pregnant mice were treated on days 10–17 of gestation in four groups: control (vehicle); low-dose aspartame (3.5 mg/kg); medium-dose aspartame (7 mg/kg); and high-dose aspartame (14 mg/kg).		Huang et al. (2023b)
Glucose Insulin	Mouse, C57BL/6J (M)	Blood	Increase in glucose, insulin, HOMA-IR; decrease in glucose tolerance, and insulin tolerance.	The control group was fed standard chow, and the experimental group was fed standard chow containing 1% aspartame for 12 wk.		Zhou et al. (2022)
Glucose Cholesterol HDL LDL TG	Rat, Sprague-Dawley (M)	Blood	No changes in glucose levels. Increase in total cholesterol, LDL-cholesterol, and TG.	Control (water) or aspartame (4.1 mg/kg bw per day) for 18 wk.		Morales-Ríos et al. (2022)
Cholesterol TG VLDL LDL HDL	Rat, Wistar (M)		Increase in cholesterol, TG, and LDL at the intermediate and highest dose of aspartame. Decrease in HDL at the highest dose.	Control (water); aspartame at a dose of 15 mg/kg; aspartame at a dose of 35 mg/kg; aspartame at a dose of 70 mg/kg for 9 wk		Adaramoye and Akanni (2016)

Table 4.14   (continued)

End-point	Species, strain (sex)	Tissue	Results	Route, duration, dosing regimen, dose	Comments	Reference
Glucose Cholesterol LDL TG	Rat, Wistar (M, F)	Blood	Increase in glucose in male and female offspring. Increase in cholesterol and LDL in males and females. Increase in TG in males.	Female rats were divided into four groups: control (receiving water); sucrose (45 g/L); saccharin (1.35 g/L); and aspartame (2 g/L), and treated for 30 days. The rats were mated and maintained on the diet until birth. The pups were assessed at 21 and 112 days.		von Poser Toigo et al. (2015)

bw, body weight; F, female; GLP-1, glucagon-like peptide-1; HDL, high-density lipoprotein; HOMA-B, homeostatic model assessment for insulin secretion; HOMA-IR, homeostatic model assessment for insulin resistance; IGF-1, insulin-like growth factor-1; LDL, low-density lipoprotein; M, male; mo, month(s); MSG, monosodium glutamate; NMDAR, *N*-methyl-ᴅ-aspartic acid receptor; NPH, neutral protamine Hagedorn; PPARγ, peroxisome proliferator-activated receptor gamma; STZ, streptozotocin; TFA, *trans* fat; TG, triglycerides; UI, International Units; VLDL, very low-density lipoprotein; wk, week(s); w/v, weight per volume.

metabolism (Janssens et al., 2017) or it increased glucose levels with alteration in lipid metabolism (Lebda et al., 2017b). In the study by Ryuk et al. (2019), ovariectomized Sprague-Dawley rats fed high-fat diets containing 0.05% aspartame for 8 weeks showed increases in insulin resistance HOMA-IR, HOMA for insulin secretion (HOMA-B), serum insulin, and GLP-1, and decreases in serum triglycerides. In addition, there were increases in IGF-1 mRNA expression levels in the jejunum.

Palmnäs et al. (2014) observed that aspartame alters the gut microbiota and serum metabolome. Groups of adult male Sprague-Dawley rats were fed a standard chow diet or high-fat diet for 2 weeks, then exposed ad libitum to drinking-water with or without aspartame at a low dose (5–7 mg/kg bw per day) for 8 weeks. Aspartame consumption was associated with fasting hyperglycaemia and impaired insulin tolerance in both groups (standard chow diet and high-fat diet). The same study reported distinctive changes in the gut microbiota of the rats consuming aspartame (see Section 4.3.2). In addition, aspartame increased levels of acetate and butyrate in the group receiving the standard diet, whereas levels of formate and isobutyrate remained unchanged. In the study by Ragi et al. (2021), male Sprague-Dawley rats (age 7 weeks) were exposed to aspartame (0.05%) in the diet or the drinking-water, or both. A control group was exposed to a non-sweetened diet and plain drinking-water for 7 weeks. Aspartame ingestion was associated with glucose intolerance. There were no changes in fasting insulin levels; however, there were increases in fasting glucose and HOMA-IR in the aspartame-exposed groups, and these were strongly related to the amount of ingested aspartame ($r = 0.518$, $P = 0.006$).

Mbambo et al. (2020) compared the effects of commonly used commercially available non-nutritive sweeteners, including aspartame, on diabetes-related parameters in non-diabetic rats. Experimental animals exposed to aspartame consistently showed higher blood glucose levels during an OGTT test, particularly at 60 and 120 minutes after glucose ingestion, when compared with other groups [The Working Group noted that the limitations of the study were that there was no indication of the sex used, and no information on the dose administered.]

In contrast, no changes in insulin or glucose metabolism were observed after aspartame administration in some studies in rats. After 7 days of exposure to diet containing aspartame (0.2% or 2%), serum levels of glucose and insulin were not altered in male and female rats (EFSA_E1, 2011). [The Working Group noted that the percentages of aspartame in the diet were estimated to yield an average daily aspartame consumption of 0.2 g and 2 g/kg bw for males and 0.15 g and 1.48 g/kg bw for females.] In the study by Tovar et al. (2017), male Sprague-Dawley rats were exposed to drinking-water containing aspartame at 6–8 mg/kg bw per day in week 1 and 15–21 mg/kg bw per day in week 6. Rats were evaluated after 6 weeks of exposure. No changes in glucose or insulin levels were observed after the OGTT. In the study by Beck et al. (2002), male Long-Evans rats were exposed to tap water (control) or tap water containing aspartame (1 g/L) for 14 weeks, starting at weaning. No changes in glucose or insulin levels were noted after aspartame exposure. There were two studies in streptozotocin-induced diabetic rats. In the study by Shigeta et al. (1985), streptozotocin-induced female Sprague-Dawley diabetic rats were exposed to aspartame (0.45 mg/100 g bw), after 18 hours of fasting, through a stomach tube. The administration of aspartame had no effect on plasma glucose and insulin levels. In the second study carried out by Nosti-Palacios et al. (2014), streptozotocin-induced male Wistar rats were exposed to aspartame (50 mg/kg) intragastrically daily for 6 weeks, starting from the sixth week after developing diabetes. Blood glucose levels did not change in the group exposed to aspartame compared with the diabetic controls.

Some studies examined C57BL/6J mice exposed to aspartame either in utero until lactation or in utero until adulthood. In the study by Azad et al. (2020), dams were randomly assigned to receive drinking-water with or without aspartame (0.2 g/L, ~32 mg/kg bw per day) throughout pregnancy and lactation. Mice were evaluated at age 4–12 weeks. Maternal consumption of aspartame had no effect on glucose intolerance in male and female offspring. In contrast, male but not female offspring exhibited insulin resistance. There were no changes in the area under the curve (AUC) for glucose in the GTT after aspartame exposure in males or females. In addition, there was upregulated expression of the adipocyte differentiation genes *Cebpa* and *Fabp4* in the adipose tissue of male offspring.

Mice were exposed to drinking-water containing aspartame (0.25 g/L, 50 mg/kg bw per day) as the only source of drinking-water, starting in utero until up to age 17 weeks (Collison et al., 2012a, b, 2016). Aspartame-exposed male and female mice had elevated fasting blood glucose levels and aspartame-exposed males presented decreases in insulin sensitivity. Collison et al. (2016) investigated the involvement of the gut–brain axis in regulating glucose homeostasis by administering CGP 39551, an NMDAR-antagonist in utero to one group of mice exposed to aspartame. Alterations in blood glucose and impaired insulin sensitivity caused by aspartame were normalized by NMDAR antagonism. Collison et al. (2013) investigated the effects of changes in hepatic and adipose tissue gene expression induced by aspartame (0.25 g/L) in the offspring of C57Bl/6 J mice fed a *trans*-fat-enriched diet (see Section 4.2.9). The offspring used in the experiments were weaned onto the respective maternal dietary regimens at age 4 weeks and maintained for the duration of the study. Fasting glucose levels were elevated at 6 weeks but decreased at 17 weeks. There were no changes in insulin or HOMA-IR after exposure to aspartame.

There were few studies examining the effects of aspartame in adult mice. Finamor et al. (2021) investigated the effects of aspartame on oxidative stress and inflammatory mechanisms associated with liver fibrosis progression in mice (see also Sections 4.2.3 and 4.2.4). Male Swiss mice were exposed to aspartame at a dose of 80 mg/kg bw (2.5 mL/kg, prepared in 0.9% saline solution) by gavage for 12 weeks. Aspartame decreased fasting induced glucose levels and impaired gluconeogenesis possibly through decreased peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*Ppargc1a*) mRNA expression levels and PGC-1α protein levels. In addition, aspartame decreased HDL cholesterol and increased total cholesterol, LDL cholesterol, triglycerides, and total lipids. Gul et al. (2017) investigated C57BL/6 mice fed a standard or high-fat diet and drinking-water with or without aspartame (0.96 mg/mL) for 18 weeks. Mice receiving aspartame-infused drinking-water developed glucose intolerance. Fasting blood glucose levels at 16 hours were significantly higher in groups that were exposed to aspartame than in the controls. In pregnant mice exposed to aspartame at a dose of 3.5, 7 or, 14 mg/kg per day by oral gavage for 17 days, there was a significant decrease in fasting plasma glucose levels in aspartame-exposed groups compared with vehicle controls, but no significant differences in plasma insulin levels (Huang et al., 2023b).

More recently, Zhou et al. (2022) found that aspartame, through its metabolite, phenylalanine, modifies insulin receptor β (IRβ) and inactivates insulin signalling and glucose uptake. Male C57BL/6J mice were exposed to diet containing 1% aspartame for 12 weeks. Aspartame increased levels of blood glucose and insulin, and HOMA-IR, and decreased glucose tolerance and insulin tolerance. In addition, C57BL/6J mice fed a diet that was enriched in phenylalanine or phenylalanine-producing aspartame, and C57BL/6J mice overexpressing human phenylalanyl-tRNA synthetase α subunit (hFARSA), which induces

phenylalanylation of lysine, were shown to develop insulin resistance and symptoms of type 2 diabetes. Specifically, FARS catalyses the phenylalanylation of insulin receptor β (IRβ) on lysine residues 1057 and 1079, thus inactivating the receptor. The inactivation of IRβ inhibits insulin signalling and, finally, glucose uptake by cells. The authors showed also that the inhibition of insulin signalling could be abrogated by de-phenylalanylating lysine residues F-K1057/1079 by overexpressing sirtuin 1 (SIRT1) in hepatocytes from C57BL/6J mice. On the other hand, in the hepatocytes of *Sirt1*-knockout C57BL/6J mice, levels of F-K1057/1079 are higher and insulin signalling is inhibited. In another experiment, the authors showed that blocking the activity of F-K1057/1079, using phenylalaninol, induces insulin sensitivity and relieves the symptoms of type 2 diabetes in C57BL/6J mice overexpressing hFARSA or in C57BL/KsJ-db/db diabetic mice (db/db).

Malaisse et al. (1998) observed that a single acute exposure to aspartame (1–10 mM), in the presence or absence of D-glucose, did not elicit insulin secretion from pancreatic islets isolated from female Wistar rats (ex vivo) (Malaisse et al., 1998).

Increases in circulating total cholesterol and LDL cholesterol were observed in many studies (Collison et al., 2012a, b, 2013; von Poser Toigo et al., 2015; Lebda et al., 2017b; Mbambo et al., 2020; Finamor et al., 2021; Mendoza-Pérez et al., 2021; Morales-Ríos et al., 2022). Decreases in HDL cholesterol were observed by Collison et al. (2012a, b) and Mbambo et al. (2020), whereas an HDL increase was observed by Finamor et al. (2021). [The Working Group noted that, overall, exposure to aspartame led to alterations in lipid metabolism in experimental systems.]

Adaramoye and Akanni (2016) investigated the effects of long-term administration of aspartame on redox status, lipid profiles, and biochemical indices in male Wistar rats exposed to distilled water (control) or aspartame at a dose of 15, 35, or 70 mg/kg, respectively, daily by oral gavage for nine consecutive weeks. There were significant increases in concentrations of LPO products in the kidney, liver, and brain at all doses of aspartame in parallel with decreases in antioxidant defences, including GST, GPx, SOD, CAT, and GSH (see also Section 4.2.3). At 35 and 70 mg/kg, aspartame increased the levels of total cholesterol, triglycerides, and LDL cholesterol. Histopathological analysis showed degeneration, monocyte infiltration, and necrotic lesions in the brain, kidney, and liver. [The Working Group noted that aspartame may induce redox and lipid imbalance in rats via a mechanism that involves oxidative stress and depletion of the GSH-dependent system. No statistical analysis was carried out on the histopathological analyses.]

Pandurangan et al. (2014) investigated the effects of aspartame on obesity, focusing on differentiation in the 3T3-L1 preadipocyte cell line. The 3T3-L1 adipocytes were differentiated in culture for 6 days in the absence and presence of aspartame (10 μg/mL). Aspartame reduced lipid accumulation in differentiated adipocytes (as measured by Oil Red O staining) and significantly reduced the gene expression (as measured by quantitative reverse transcription-polymerase chain reaction, qRT-PCR) of PPARγ, FABP4, C/EBPα, and SREBP, which are involved in adipogenesis. In addition, aspartame reduced the protein expression of p-PPARγ, PPARγ, SREBP1, and adiposin, which are normally induced in differentiating cells.

4.3.2 Microbiome

(a) Humans

Exposed humans

In a cross-sectional study, Frankenfeld et al. (2015) investigated bacterial abundance (according to phylogenetical class or order) in consumers and non-consumers of aspartame

(*n* = 7) or acesulfame-K (*n* = 7). Thirty-one healthy adults completed a 4-day food record and provided a faecal sample on day 5. No differences in median bacterial abundance were observed between consumers and non-consumers of either sweetener; however, the overall bacterial population composition was different between non-consumers and consumers of aspartame and acesulfame-K, assessed as separate groups.

Suez et al. (2022) assessed the effects of supplementation with aspartame (240 mg/day) for 2 weeks in a randomized controlled trial in 120 healthy adults. Aspartame affected the faecal and oral microbiomes (according to metagenomic analysis) and the plasma metabolome, but had no significant effect on the glycaemic response, (which was the case for other sweeteners). In addition, aspartame was shown to modify oral and faecal microbiome composition and key functions, such as purine or pyrimidine metabolism, glycolysis, and amino acid metabolism. In gnotobiotic mice that were not exposed to aspartame and that were transplanted with the microbiomes from multiple human donors with either a high or a low response to aspartame supplementation, murine glycaemic responses reflected those reported in the respective human donors.

In a study that gave results contrary to those of the above studies, Ahmad et al. (2020) conducted a 12-week crossover clinical trial in 17 healthy participants who were exposed to 425 mg of aspartame or 136 mg of sucralose. Aspartame (*n* = 9) or sucralose (*n* = 8) was administered during weeks 5 and 6, after a 4-week period without any artificial sweeteners. In weeks 7 through 10, all participants underwent a washout period during which no artificial sweeteners were consumed. During weeks 11 and 12, all participants who had previously consumed aspartame were exposed to sucralose, and vice versa. No changes in faecal microbiota or faecal short chain fatty acid concentrations were observed before and after exposure.

(b)   Experimental systems

Palmnäs et al. (2014) demonstrated that low doses of aspartame (5–7 mg/kg per day) administered for 8 weeks affected gut microbiota in obese rats. Compared with animals receiving water, rats exposed to aspartame consumed fewer calories and gained less weight. However, aspartame elevated fasting glucose levels and impaired insulin-stimulated glucose disposal in rats receiving control and high-fat diets. Faecal analysis of gut bacterial composition showed that aspartame increased total bacteria and the abundance of Enterobacteriaceae and *Clostridium leptum*. Serum metabolomics analysis revealed aspartame to be associated with elevations in butyrate and propionate, a bacterial metabolite and highly gluconeogenic substrate, potentially explaining the negative effects of aspartame on insulin tolerance. [The Working Group noted that modifications of the gut microbiota could be caused by aspartame metabolites (Oppermann et al., 1973a, b; Lipton et al., 1991) undergoing fermentation by the gut microbiota at the colon level, thus inducing the observed modifications in the bacterial population.] Suez et al. (2014) reported that, in mice, aspartame caused glucose intolerance through induction of compositional and functional alterations to the intestinal microbiota and that this derangement was abrogated by antibiotic treatment.

Nettleton et al. (2020) showed that low doses of aspartame (5–7 mg/kg) altered gut microbiota as well as altering the metabolism and mesolimbic reward system, in rat dams (in which obesity had been induced by diet) and their offspring. In line with this evidence, germ-free mice receiving a faecal microbiota transplant from obese aspartame-exposed offspring showed greater gain in body weight and body fat and impaired glucose tolerance than did the obese controls.

In a subsequent study, the same group (Wang et al., 2021) characterized the major alterations in the caecal microbiome of the offspring of obese

dams consuming a high-fat/sucrose diet with or without aspartame (5–7 mg/kg). Maternal consumption of aspartame altered caecal microbial composition and metabolism of propionate/lactate in the offspring. In the offspring, daily body-weight gain, liver weight, and body fat were positively correlated with the relative abundance of key microbes and enzymes involved in succinate/propionate production and negatively correlated to those involved in lactose degradation and lactate production.

Shil and Chichger (2021) investigated the pathogenicity of two model strains of microbiota (*E. coli* NCTC10418 and *Enterococcus faecalis* ATCC19433) exposed to aspartame at different concentrations in vitro and their effects in a co-culture of human colon adenocarcinoma (Caco-2) cells. The ability of bacteria to form a biofilm increased significantly after exposure to aspartame (100 μM) for 24 hours. In the co-culture experiments, aspartame also increased the ability of gut bacteria to adhere to, invade, and kill Caco-2 cells. In addition, the aspartame-mediated increase in the ability of *E. coli* to invade Caco-2 cells was attenuated by the presence of 100 μM zinc sulfate (a potent inhibitor of sweet taste sensing), as was the ability of *E. faecalis* to form biofilm, adhere and invade Caco-2 cells (see also Shil et al. (2020), described in Section 4.2.8(b) and 4.3.3).

In another recent study investigating bacteria quorum sensing (a function that enables microorganisms to communicate within their community and regulate group behaviours) showed that aspartame has inhibitory actions on the Gram-negative bacteria *N*-acyl homoserine lactone-based communication system involved in the quorum sensing process (Markus et al., 2021).

4.3.3 Sweet taste receptors

There is evidence in humans that aspartame binds to the sweet taste receptor, a heterodimeric G protein-coupled receptor consisting of two subunits (T1R2 and T1R3) (Pin et al., 2003). Activation of the lingual sweet taste receptors triggers the release of α-gustducin, which activates phospholipase C, producing inositol phosphate and resulting in intracellular calcium release. In the mouth, this cascade induces neuronal signals to convey the sensation of sweetness to the brain. In addition, sweet taste receptors have also been located outside of the oral cavity, including in the intestine and in pancreatic β cells, where a signalling cascade analogous to that in the oral cavity elicits GLP-1 and insulin secretion, respectively (Jang et al., 2007; Nakagawa et al., 2009).

Some studies in which the interaction of aspartame with the sweet taste receptors can be linked to downstream effects relevant to the key characteristic of carcinogens are reported below.

(a) Human cell lines

Shil et al. (2020) reported that aspartame at high concentrations (1000 μM) induces apoptosis and cell death in intestinal Caco-2 cells, whereas lower concentrations (100 μM) increase epithelial barrier permeability, downregulate claudin-3 expression, and induce ROS production; these effects were attenuated by knockdown of the sweet taste receptor gene *T1R3* (Shil et al., 2020). Gezginci-Oktayoglu et al. (2021) investigated the effects of aspartame in human pancreatic adenocarcinoma cells (PANC-1) (see Section 4.2.8). Aspartame significantly increased the expression of markers of epithelial–mesenchymal transition (EMT) such as vimentin and N-cadherin, activated AKT, and deactivated GSK3β, by increasing levels of ROS and cytoplasmic Ca^{2+}, respectively, through T1R2/T1R3 stimulation.

(b) Experimental systems

In a study on the effects of aspartame exposure on placental growth, Huang et al. (2023b) exposed pregnant mice to aspartame by oral gavage. Aspartame impaired placenta growth, induced ROS levels, hyper-activated Akt, and downregulated manganese superoxide dismutase (MnSOD) expression (see also Section 4.2.5). Importantly, pre-treatment with antioxidants or sweet taste receptor inhibitors reversed the effects of aspartame on trophoblast function. In addition, the aspartame metabolite phenylalanine similarly induced ROS production and affected proliferation of trophoblasts. [The Working Group noted that these data suggested that aspartame consumption during pregnancy may have an impact on the structure, growth, and function of the placenta via sweet taste receptor-mediated stimulation of oxidative stress.]

Several studies evaluated the effects of aspartame on glucose metabolism in healthy, diabetic, or overweight participants. Several intervention trials of up to 12 weeks gave largely negative results. A small number of cross-sectional studies were uninformative with regard to the effects of aspartame on glucose metabolism. These studies presented several limitations associated with difficulty in controlling for confounding variables in a human population, and the complexity of the different study designs and relative protocols. Often unusual regimens or routes of administration (i.e. sweeteners in juice or lemonade) were considered. Additionally, in many studies aspartame was considered as the reference positive control in comparisons with other sweeteners; thus, the lack of an appropriate background control limited informativeness or invalidated the studies (Ahmad et al., 2020). However, there was consistent evidence that exposure to aspartame induced insulin increase in rodents. Recent studies have suggested that aspartame has effects on metabolism and gut microbiota. The interactions of aspartame with sweet taste receptors,

which have been implicated in the signalling cascade that activates metabolism in the body, and with the gut microbiota were identified by the Working Group as notable research gaps.

5. Summary of Data Reported

5.1 Exposure characterization

Aspartame is an odourless white crystalline powder that is added as a low-calorie artificial sweetener to foods and beverages. It is also used in cosmetics, medicines, and tobacco products. Aspartame has a sweetness that is 200 times that of sucrose.

Aspartame is measured in consumer products mostly by chromatographic methodologies. It is reported to be present in food in the milligrams-per-kilogram range. Aspartame has been detected in wastewater samples in different regions of the world.

Aspartame was first authorized for food use in most countries in the 1980s. Artificially sweetened beverages are an important source of aspartame exposure, although aspartame is present in a more concentrated form in tabletop sweeteners, chewing gums, and food supplements. In the USA from the mid-1980s until the beginning of the 2000s, artificially sweetened beverages almost exclusively contained aspartame as the sweetener but, more recently, it has been partly or completely replaced by other low-calorie sweeteners. Trends are likely to be similar in Europe and other regions, but data on exact timing are lacking for many countries.

There are no validated biomarkers of dietary intake of aspartame because, upon consumption, aspartame is rapidly metabolized to endogenous human metabolites. Therefore, assessments of human exposure via ingestion are reliant upon self-reported methods for estimating dietary intake, which have limitations relating to accuracy, recall bias, and lack of comprehensive

food composition data. Exposure assessments have typically shown that daily exposure of the general population is in the low milligrams-per-kilogram-body-weight range but can be higher in certain consumer groups.

Occupational exposure via inhalation during the synthesis of aspartame and the production of aspartame-containing products has been reported in the past, but no recent data on occupational exposure are available.

5.2 Cancer in humans

The association between the consumption of aspartame and/or artificially sweetened beverages and cancer was investigated in 12 prospective cohort studies and 13 retrospective case–control studies (two of which were considered to be not informative).

The majority of these studies assessed consumption of artificially sweetened beverages and/or tabletop sweeteners likely or known to contain aspartame as the exposure of interest, and the Working Group decided to use these assessments as a proxy for aspartame exposure. The quality of the proxy varied across studies according to country- and time-period-specific evidence on aspartame use. Only one study, the NutriNet-Santé cohort, provided information on exposure to aspartame across the entire spectrum of dietary sources and reported brand-specific information (the main analysis considered intake over the first 2 years of follow-up and additional analyses using repeated assessments throughout the follow-up period). The aspartame assessment in this study was based on repeated 24-hour dietary records with: (1) brand-specific information (allowing specific assessment of intakes of aspartame and other artificial sweeteners); (2) all dietary sources accounted for (artificially sweetened beverages, tabletop sweeteners, but also dairy and other products); and (3) a dynamic matching of consumption and composition data accounting for the date when the food or beverage

was consumed. Several other cohort studies were also considered particularly informative because of the close overlap of the follow-up period with time periods during which aspartame was the prevailing artificial sweetener used in the respective country: the Nurses' Health Study (NHS), the Health Professionals Follow-up Study (HPFS), the European Prospective Investigation on Cancer (EPIC), the Cancer Prevention Study II (CPS-II), the National Institutes of Health-American Association of Retired Persons (NIH-AARP) Diet and Health Study, and the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial. In addition, three of these studies had exposure assessments that were repeated over time and were therefore more comprehensive.

Three informative studies consistently described positive associations for liver cancer overall or in subgroups of the study population (i.e. grouped according to sex, diabetes, and smoking status). No other studies investigated associations with liver cancer. The available studies included one from EPIC (including populations from ten European countries) and two studies from the USA (the pooled NIH-AARP and PLCO cohorts, and the CPS-II cohort). All three cohort studies controlled for potential confounding by diabetes status at baseline through stratification, model adjustment, or restriction. New-onset diabetes after exposure to aspartame could be one among many potential pathways for liver cancer. In the EPIC cohort, an overall positive association was observed for incidence of hepatocellular cancer (HCC) per each increment in 330 mL servings per week of artificially sweetened beverage consumption (hazard ratio, HR, 1.06; 95% confidence interval, CI, 1.03–1.09; 151 cases). In one of the other two studies (the pooled NIH-AARP and PLCO), positive associations between artificially sweetened beverage consumption and incidence of primary cancer of the liver and intrahepatic biliary duct were observed among individuals with diabetes,

in whom consumption of artificially sweetened beverages was higher than in individuals without diabetes. These positive findings were observed in the first 12 years of follow-up, which coincided with the years in which aspartame was used as the predominant artificial sweetener in the USA. Another study in the USA (CPS-II) found a positive association between consumption of artificially sweetened beverages and mortality from cancer of the liver and intrahepatic biliary duct only among men who were never-smokers. This association was attenuated after adjustment for body mass index (BMI); however, this finding was not considered to refute the positive association seen in other studies, which also adjusted for BMI. Other informative studies, including the NutriNet-Santé study, did not investigate liver cancer separately.

The main limitations of these studies related to the potential for non-differential exposure misclassification, lack of repeat exposure assessments in all studies (although in a subgroup of the CPS-II in the USA, it was shown that high consumption of artificially sweetened beverages was stable over 17 years), and lack of detailed information on aspartame consumption from sources other than artificial beverages (although artificially sweetened beverages are estimated to capture most of the aspartame consumption in the USA during the time periods when the studies were conducted). Despite probable bias towards the null, these studies observed positive associations between aspartame consumption and liver cancer; however, the possibility of confounding by exposure to other known liver carcinogens could not be excluded.

For other cancer sites, the restricted epidemiological data available were not sufficiently informative or consistent to permit a conclusion to be drawn about the presence or absence of a causal association. However, some positive findings from single informative studies suggesting a potential link between aspartame exposure and cancer risk were observed. In particular, the NutriNet-Santé cohort observed positive associations between aspartame exposure and increased risk of all cancer sites combined, breast cancer, and obesity-related cancers combined. Other studies on these cancers did not find evidence of positive associations. Additional findings that emerged included positive associations with some cancers of the lymphatic system or tissue (NHS and HPFS studies, assessing artificially sweetened beverages and tabletop sweeteners containing aspartame), as well as pancreatic cancer (CPS-II and EPIC studies, using baseline consumption of artificially sweetened beverages).

5.3 Cancer in experimental animals

Treatment with aspartame caused an increase in the incidence of malignant neoplasms or an appropriate combination of benign and malignant neoplasms in two species (mouse and rat) and both sexes, in three studies that did not comply with Good Laboratory Practice (GLP). During the evaluation of the available studies, the Working Group noted concerns regarding diagnoses for lymphomas predominantly, but not exclusively, located in the lung. Therefore, the synthesis of evidence of carcinogenicity in experimental animals focused on all neoplastic lesions except lymphoid tumours and related combinations.

Aspartame was administered by transplacental and perinatal exposure followed by oral administration (feed) in male and female Swiss mice. In males, aspartame caused hepatocellular carcinoma, hepatocellular adenoma or carcinoma (combined), bronchioloalveolar carcinoma, bronchioloalveolar adenoma or carcinoma (combined), and a significant increase in the incidence of lymphoblastic leukaemia, of monocytic leukaemia, and of total myeloid tumours, was also reported. In females, aspartame caused lymphoblastic leukaemia.

Aspartame administered by oral administration (feed) in Sprague-Dawley rats caused carcinoma and papilloma and carcinoma (combined) of the renal pelvis and ureter in males and females, and mammary gland carcinoma in females. In males, a re-analysis of the above data indicated a significant increase in the incidence of monocytic leukaemia, histiocytic sarcoma, and a positive trend in the incidence of total myeloid tumours at all doses.

Aspartame administered by transplacental and perinatal exposure followed by oral administration (feed) in male Sprague-Dawley rats caused malignant schwannoma and in female rats aspartame caused mammary gland carcinoma, and renal pelvis papilloma. Another re-analysis of these data reported a positive trend for myeloid leukaemia and total myeloid tumours in females.

Although data from the studies on transplacental and perinatal exposure followed by oral administration (feed) in mice and rats and the study on oral administration (feed) in rats indicated that aspartame had carcinogenic activity, overall, the Working Group had serious questions about the adequacy of the design, conduct, interpretation, and reporting of each of the studies. For example, no adjustments were made for litter effects, which can lead to false-positive results regarding increases in incidence and trends. A minority of the Working Group did not consider that these concerns would substantially change the interpretation of the evidence of carcinogenicity in experimental animals.

5.4 Mechanistic evidence

All available studies in humans consistently indicated that ingested aspartame is hydrolysed by esterases and peptidases in the gastrointestinal tract yielding three components – the amino acids aspartic acid and phenylalanine, and methanol. These components undergo absorption from the intestinal lumen, reaching the systemic circulation, and appear to enter normal endogenous metabolic pathways. Alternatively, ester hydrolysis in the lumen may yield methanol and the dipeptide, aspartylphenylalanine, which is absorbed into intestinal mucosal cells via a peptide-transport mechanism and subsequently undergoes efficient hydrolysis to aspartic acid and phenylalanine in the enterocytes. No studies were available that investigated routes of exposure other than ingestion. Upon absorption, phenylalanine enters the plasma pool of free amino acids from the portal blood, after partial conversion to tyrosine in the liver. Aspartic acid undergoes transamination in the enterocytes, producing oxaloacetate, an intermediate in the citric acid cycle. Methanol does not undergo metabolism in the enterocytes, rapidly enters the portal circulation, and is oxidized to formaldehyde. Formaldehyde quickly undergoes further oxidation to formic acid, which is ultimately converted to carbon dioxide (CO_2). Exposure to methanol from aspartame consumption is estimated to account for 1–10% of overall exposure to methanol, whereas endogenous methanol accounts for more than 80%. It is not known whether exposure to formaldehyde from aspartame consumption would significantly affect concentrations of endogenous formaldehyde.

The metabolism of ingested aspartame and the kinetics for each of its components have been studied in multiple settings, including in healthy adults, healthy infants, lactating women, adults who are heterozygous or homozygous for phenylketonuria, and people with diabetes. These studies have included single, repeated, or long-term dosing, and co-administration with meals. Across subpopulations, the concentration of plasma aspartic acid typically remained within the normal range after intake of high doses of aspartame. Plasma phenylalanine concentrations, measured after single or repeated doses of aspartame, were within the normal postprandial levels in normal subjects, and higher in individuals heterozygous for phenylketonuria, but declined in the hours following exposure.

Blood formate concentrations did not differ from baseline levels after high doses of aspartame, and urinary formate excretion was significantly increased.

Similar to the results reported in humans, aspartame appears to be completely hydrolysed in the small intestine of experimental animals, producing methanol, aspartic acid, and phenylalanine. Most of the absorbed methanol and aspartic acid from aspartame are excreted as CO_2 in experimental animals. Phenylalanine is also excreted as CO_2 in experimental animals; however, a greater proportion of phenylalanine is retained. Because of the greater activity of phenylalanine hydroxylase in rats, plasma levels of phenylalanine in rats increase at a much slower rate than in humans, primates, and pigs after aspartame or phenylalanine exposure. Evidence in rats suggests that the effects of aspartame exposure on phenylalanine hydroxylase activity are similar to those of phenylalanine at equimolar doses, and that aspartame exposure has no effects on gastric juice secretion, concentration of gastric acid, acid output, and proteolytic activity. It is reported that aspartame exposure may significantly increase cytochrome P450 (CYP) protein levels (i.e. 1A1, 1A2, 2B, 3A2) and enzymatic activities (i.e. ethoxyresorufin O-deethylase, methoxyresorufin O-demethylase, pentoxyresorufin O-depentylase, benzyloxyresorufin O-debenzylase, 4-nitrophenol hydroxylase, and erythromycin-N-demethylase) in the cerebrum and cerebellum of rats after 30 days of aspartame treatment.

Data were available for aspartame for the following key characteristics of carcinogens: "is electrophilic or can be metabolized to an electrophile", "is genotoxic", "induces oxidative stress", "induces chronic inflammation", "is immunosuppressive", "modulates receptor-mediated effects", "causes immortalization", and "alters cell proliferation, cell death, or nutrient supply".

Overall, the mechanistic evidence for aspartame regarding the key characteristics of carcinogens is suggestive. There is consistent and coherent evidence that aspartame induces oxidative stress in experimental systems. There were no studies in exposed humans. Three studies in human primary cells observed alterations in biomarkers of oxidative stress; however, two studies were of limited quality. Aspartame also altered biomarkers of oxidative stress in a variety of human cell lines; however, these biomarker changes were accompanied by significant cytotoxicity or mitochondrial toxicity, and there was no determination of whether those toxicities caused or were caused by oxidative stress. One study in a human intestinal cell line presented clear evidence that, after binding to the sweet taste receptor, aspartame increased reactive oxygen species (ROS) and disrupted cell-membrane barrier function. Consistent and coherent evidence for oxidative stress derives from several studies in rodents. Aspartame induced lipid peroxidation in a wide variety of tissues in rats. There were also many studies in rats, and a few in mice, that showed the increased presence of ROS or activation of biomarkers of cellular responses to ROS, such as increased expression or activity of catalase, superoxide dismutase, or glutathione transferases in several tissues, including the liver, blood, spleen, kidney, and brain.

There is suggestive evidence that aspartame induces chronic inflammation. Several studies in exposed humans were deemed uninformative because it was not possible to determine whether the exposure was to aspartame only. However, aspartame exposure has been confirmed in two cross-sectional studies. One of the studies in exposed humans reported that intake of aspartame-sweetened beverages was associated with a higher concentration of serum C-reactive protein (CRP) but not with two other markers of inflammation, intracellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion protein 1 (VCAM-1). Another study evaluated the effects

of aspartame consumption in small groups of HIV-positive and HIV-negative individuals; in the HIV-positive group, aspartame consumption was significantly positively associated with monocyte chemoattractant protein 1 (MCP-1), lipoprotein-associated phospholipase A2 (Lp-PLA2), and coronary plaque segments. Levels of two other markers of inflammation, high-sensitivity CRP and oxidized low-density lipoprotein, were increased but not statistically significantly. The informativeness of this study was limited because of the lack of clear group-selection criteria. In addition, information on food sources of the sweeteners and duration of aspartame intake was not provided. In one study in primary human umbilical vein endothelial cells, an increase in the pro-inflammatory cytokine interleukin-6 (IL-6) was observed; however, no alteration in inflammatory cytokines (IL-6, IL-10, and interferon gamma, IFNγ) was reported in human fibroblast cell lines. Dose-dependent increases in levels of pro-inflammatory cytokines – tumour necrosis factor alpha (TNFα) and IL-6 in the liver, and CRP in the serum – were observed in rats treated orally with aspartame for 30 days. An increase in levels of circulatory IL-4 and a decrease in levels of cytokines IL-2, TNFα, and IFNγ were also observed in rats after intraperitoneal injection of aspartame for 90 days. Similarly, TNFα levels were elevated in the brain tissue of mice exposed to aspartame by intraperitoneal injection. Signs of tissue inflammation in various organs were also reported in several repeated-dose toxicity studies of various durations; however, the studies were considered of limited informativeness on the basis of limitations in the design of the study and accuracy of the data analysis.

There is suggestive evidence that aspartame exhibits the key characteristic of carcinogens "alters cell proliferation, cell death, or nutrient supply". Multiple tolerance studies in exposed humans, which reported on standard clinical chemistry and urine analysis were considered not informative to this key characteristic. Regarding cell proliferation, the evidence for this end-point was inconsistent. There were no studies in exposed humans or in human primary cells. In human cell lines, aspartame decreased markers of cell proliferation in one study and increased markers of apoptosis in two studies. In experimental systems in vivo, aspartame promoted cell proliferation via the insulin-like growth factor 1 (IGF-1) pathway in the jejunum (measured as crypt depth and villus height) in lambs fed aspartame for 35 days. In mice, it caused neurogenesis in the dentate gyrus subgranular zone of the hippocampus. In rats, aspartame treatment for 56 days increased expression of HRas and decreased levels of Cdkn1b. In a lifetime study in rats, hyperplasia was observed in the renal pelvis of females and in the olfactory epithelium in both females and males. However, there was no evidence of increased cell proliferation or hyperplasia in the urinary bladders of male rats exposed via drinking-water and food for 4 weeks. In in vitro systems, aspartame increased cell proliferation in a growth-factor dependent mouse mast cell line, but increased gene expression associated with apoptosis induction in rat adrenal pheochromocytoma cells.

With respect to angiogenesis, the evidence was suggestive. There were no studies in exposed humans. In human primary umbilical vein endothelial cells, aspartame increased markers of angiogenesis, such as tubule formation and vascular endothelial growth factor (VEGF), after exposure for 14 days in one study, but not after a shorter exposure in two other studies. Rats treated with aspartame for 3 days showed an increase in VEGF expression in the placenta. In experimental systems in vitro, a qualitative increase in angiogenesis was reported in a rat skin model, and a dose-dependent increase was observed in the vascular area in the chick chorioallantoic membrane. Aspartame inhibited ATPase activity in membranes isolated from human lymphocytes.

For the key characteristic "is genotoxic", the database was extensive; however, the evidence was considered inconsistent, and many studies presented limitations in design and quality. No data were available in exposed humans. Three studies in human primary cells reported induction of chromosomal aberrations and micronucleus formation; however, two had limitations. One chromosomal aberration study employed only a single concentration and no information on aberrant types and mitotic index was given. One study using the micronucleus assay showed a small statistically significant increase in micronuclei frequency by the t-test, which was not corrected for multiple comparisons. In studies in human cell lines, both positive and negative results were observed for DNA strand breaks; however, some of the studies that reported positive results had limitations. Aspartame induced chromosomal aberrations or micronucleus formation in several studies that showed limitations; however, the findings were negative for both end-points in two well-designed in vivo studies. Aspartame did not induce dominant lethal mutations or reverse mutations in host-mediated assays. In rodents, mixed results were obtained for DNA strand breaks and DNA fragmentation; some of the studies were associated with apoptosis. In studies of limited quality in non-human mammalian cells in vitro, aspartame induced DNA strand breaks in Madin–Darby canine kidney (MDCK) cells but did not induce unscheduled DNA synthesis in rat primary hepatocytes. Aspartame did not induce SMART (somatic mutation and recombination by wing spot test) in *Drosophila melanogaster*. In two studies of limited quality in *D. melanogaster* or *Danio rerio*, aspartame induced DNA fragmentation. Aspartame did not induce reverse mutation in standard sets of *Salmonella typhimurium* and *Escherichia coli* strains, with or without metabolic activation.

Regarding the key characteristic "modulates receptor-mediated effects", the few available studies gave negative or inconsistent results. Aspartame had no effect on the transcriptional activity of the aryl hydrocarbon receptor and glucocorticoid receptor-dependent expression of CYP1A1 in human primary hepatocytes or in hepatic and intestinal cell lines. In rodents, there were no consistent effects on estrogen, androgen, progesterone, and glucocorticoid receptor function after acute exposure to aspartame. Two studies examining thyroid hormone levels found that aspartame decreased circulating triiodothyronine (T3) with conflicting changes in thyroxine (T4). Aspartame exposure had inconsistent effects on glutamate receptors in rodent brain tissue.

For the other key characteristics, i.e. "is electrophilic or metabolized to an electrophile", "is immunosuppressive", and "causes immortalization", there was either a paucity of data or the results were negative.

Aspartame was essentially without effects in the assay battery of the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the government of the USA.

With regard to other relevant evidence, several studies investigated the effects of aspartame exposure on the release of insulin and other hormones such as leptin, C-peptide, glucagon, and glucagon-like peptide-1 (GLP-1), or on glucose metabolism in healthy or diabetic subjects. Several intervention trials of up to 12 weeks in duration gave largely negative results. A small number of cross-sectional studies were uninformative regarding the effects of aspartame on insulin, because of methodological issues.

On the other hand, there was consistent evidence that exposure to aspartame induces an increase in insulin in rodents. One study also reported that aspartame, through its metabolite phenylalanine, induces post-translational inactivation of insulin receptor β (IRβ).

Aspartame was reported to alter the gut (and oral) microbiome in some studies, but not all, in exposed humans. Alteration of the gut microbiome was also identified in studies in experimental systems in vivo and in vitro.

Recent evidence reported that aspartame binds to the heterodimer G-protein-coupled taste receptor type 1 member 2 (T1R2) and taste receptor type 1 member 3 (T1R3) sweet taste receptors, which modulate glucose signalling and metabolism in both humans and rodents. In human colorectal adenocarcinoma cells, aspartame was shown to increase epithelial barrier permeability and induce ROS production, effects that were attenuated by knockdown of the T1R3 receptor. Aspartame was also shown to increase markers of stemness in human pancreatic adenocarcinoma cells, an effect modulated by the T1R1 receptor.

6.   Evaluation and Rationale

6.1. Cancer in humans

There is *limited evidence* in humans for the carcinogenicity of aspartame. Positive associations have been observed between aspartame and cancer of the liver (hepatocellular carcinoma).

6.2 Cancer in experimental animals

There is *limited evidence* in experimental animals for the carcinogenicity of aspartame.

6.3. Mechanistic evidence

There is *limited mechanistic evidence*.

6.4 Overall evaluation

Aspartame is *possibly carcinogenic to humans (Group 2B)*.

6.5   Rationale

The Group 2B evaluation for aspartame is based on *limited evidence* for cancer in humans. There is also *limited evidence* for cancer in experimental animals and *limited mechanistic evidence*. There is *limited evidence* that aspartame causes liver cancer (hepatocellular carcinoma) in humans. Among the available studies of cancer in humans, only three studies (including four cohorts) on the consumption of artificially sweetened beverages were available for assessing the association between aspartame and liver cancer. Consumption of artificially sweetened beverages was considered to be a proxy for aspartame exposure, supported by evidence on the country and time period of aspartame use in beverages. A positive association was observed between consumption of artificially sweetened beverages and risk of liver cancer in all three studies, but bias or confounding could not be ruled out as an explanation for the findings. For all other cancer sites, the evidence is *inadequate*, because the studies did not show consistent positive associations, were few in number, or were of low informativeness for aspartame specifically.

The evidence for cancer in experimental animals was *limited* because, although increases in tumour incidence were observed in both species (mice and rats) in three studies, concerns existed regarding the statistical analyses and pathological diagnoses. Thus, the Working Group considered that there are unresolved questions about the adequacy of the design, conduct, or interpretation of the available studies. A minority of the Working Group did not have concerns about the observations of solid and myeloid tumours reported in these studies, and therefore considered that the evidence for cancer in experimental animals was *sufficient* on the basis of an increased incidence of malignant neoplasms or a combination of benign and malignant neoplasms in two species (mouse and rat) of both sexes in three well-conducted studies.

There was also *limited mechanistic evidence*, which was based on consistent and coherent evidence that aspartame induces oxidative stress in experimental systems and suggestive evidence that aspartame induces chronic inflammation and alters cell proliferation, cell death, or nutrient supply in experimental systems.

A minority of the Working Group supported a Group 2A classification for aspartame, based on a combination of *limited evidence* for cancer in humans and *sufficient evidence* for cancer in experimental animals, supported by the *limited mechanistic evidence*, but these conclusions were not supported by the Working Group overall, thus leading to a Group 2B evaluation.

References

Abd Elfatah AA, Ghaly IS, Hanafy SM (2012). Cytotoxic effect of aspartame (diet sweet) on the histological and genetic structures of female albino rats and their offspring. *Pak J Biol Sci*. 15(19):904–18. doi:10.3923/pjbs.2012.904.918 PMID:24159687

Abdel-Salam OM, Salem NA, El-Shamarka ME, Hussein JS, Ahmed NA, El-Nagar ME (2012a). Studies on the effects of aspartame on memory and oxidative stress in brain of mice. *Eur Rev Med Pharmacol Sci*. 16(15):2092–101. PMID:23280025

Abdel-Salam OM, Salem NA, Hussein JS (2012b). Effect of aspartame on oxidative stress and monoamine neurotransmitter levels in lipopolysaccharide-treated mice. *Neurotox Res*. 21(3):245–55. doi:10.1007/s12640-011-9264-9 PMID:21822758

Abegaz EG, Mayhew DA, Butchko HH, Stargel WW, Comer CP, Andress SE (2012). Aspartame. In: O'Brien Nabors L, editor. Alternative sweeteners. 4th ed. Boca Raton (FL), USA: CRC Press; pp. 74–93. doi:10.1201/b11242-8

Abhilash M, Paul MV, Varghese MV, Nair RH (2011). Effect of long term intake of aspartame on antioxidant defense status in liver. *Food Chem Toxicol*. 49(6):1203–7. doi:10.1016/j.fct.2011.02.019 PMID:21376768

Abu-Taweel GM, A ZM, Ajarem JS, Ahmad M (2014). Cognitive and biochemical effects of monosodium glutamate and aspartame, administered individually and in combination in male albino mice. *Neurotoxicol Teratol*. 42:60–7. doi:10.1016/j.ntt.2014.02.001 PMID:24556450

Adaramoye OA, Akanni OO (2016). Effects of long-term administration of aspartame on biochemical indices, lipid profile and redox status of cellular system of male rats. *J Basic Clin Physiol Pharmacol*. 27(1):29–37. doi:10.1515/jbcpp-2014-0130 PMID:26247507

Ahmad SY, Friel J, Mackay D (2020). The effects of non-nutritive artificial sweeteners, aspartame and sucralose, on the gut microbiome in healthy adults: secondary outcomes of a randomized double-blinded crossover clinical trial. *Nutrients*. 12(11):3408. doi:10.3390/nu12113408 PMID:33171964

Ahmad SY, Friel JK, MacKay DS (2020a). The effect of the artificial sweeteners on glucose metabolism in healthy adults: a randomized, double-blinded, crossover clinical trial. *Appl Physiol Nutr Metab*. 45(6):606–12. doi:10.1139/apnm-2019-0359 PMID:31697573

Ahmad SY, Friel J, Mackay D (2020b). The effects of non-nutritive artificial sweeteners, aspartame and sucralose, on the gut microbiome in healthy adults: secondary outcomes of a randomized double-blinded crossover clinical trial. *Nutrients*. 12(11):3408. doi:10.3390/nu12113408 PMID:33171964

Al-Eisa RA, Al-Salmi FA, Hamza RZ, El-Shenawy NS (2018). Role of L-carnitine in protection against the cardiac oxidative stress induced by aspartame in Wistar albino rats. *PLoS One*. 13(11):e0204913. doi:10.1371/journal.pone.0204913 PMID:30403670

Al Humaid J (2018). Sweetener content and cariogenic potential of pediatric oral medications: a literature. *Int J Health Sci (Qassim)*. 12(3):75–82. PMID:29896075

Alkafafy M-S, Ibrahim ZS, Ahmed MM, El-Shazly SA (2015). Impact of aspartame and saccharin on the rat liver: biochemical, molecular, and histological approach. *Int J Immunopathol Pharmacol*. 28(2):247–55. doi:10.1177/0394632015586134 PMID:26015492

Alkhedaide A, Soliman MM, Salah-Eldin AE, Ismail TA, Alshehiri ZS, Attia HF (2016). Chronic effects of soft drink consumption on the health state of Wistar rats: a biochemical, genetic and histopathological study. *Mol Med Rep*. 13(6):5109–17. doi:10.3892/mmr.2016.5199 PMID:27121771

Alleva R, Borghi B, Santarelli L, Strafella E, Carbonari D, Bracci M, et al. (2011). In vitro effect of aspartame in angiogenesis induction. *Toxicol In Vitro*. 25(1):286–93. doi:10.1016/j.tiv.2010.09.002 PMID:20837131

Alsuhaibani ES (2010). In vivo cytogenetic studies on aspartame. *Comp Funct Genomics*. 2010:2010. doi:10.1155/2010/605921 PMID:20689731

Alves PDCC, Rodrigues-Silva C, Ribeiro AR, Rath S (2021). Removal of low-calorie sweeteners at five Brazilian wastewater treatment plants and their occurrence in surface water. *J Environ Manage*. 289:112561. doi:10.1016/j.jenvman.2021.112561 PMID:33865021

Anbara H, Kian M, Darya GH, Sheibani MT (2022). Long-term intake of aspartame-induced cardiovascular toxicity is reflected in altered histochemical

parameters, evokes oxidative stress, and trigger p53-dependent apoptosis in a mouse model. *Int J Exp Pathol.* 103(6):252–62. doi:10.1111/iep.12458 PMID:36251541

Anbara H, Sheibani MT, Razi M (2020). Long-term effect of aspartame on male reproductive system: evidence for testicular histomorphometrics, Hsp70-2 protein expression and biochemical status. *Int J Fertil Steril.* 14(2):91–101. doi:10.22074/ijfs.2020.6065 PMID:32681620

Anbara H, Sheibani MT, Razi M, Kian M (2021). Insight into the mechanism of aspartame-induced toxicity in male reproductive system following long-term consumption in mice model. *Environ Toxicol.* 36(2):223–37. doi:10.1002/tox.23028 PMID:32951320

Andreatta MM, Muñoz SE, Lantieri MJ, Eynard AR, Navarro A (2008). Artificial sweetener consumption and urinary tract tumors in Cordoba, Argentina. *Prev Med.* 47(1):136–9. doi:10.1016/j.ypmed.2008.03.015 PMID:18495230

Anton SD, Martin CK, Han H, Coulon S, Cefalu WT, Geiselman P, et al. (2010). Effects of stevia, aspartame, and sucrose on food intake, satiety, and postprandial glucose and insulin levels. *Appetite.* 55(1):37–43. doi:10.1016/j.appet.2010.03.009 PMID:20303371

Arcego DM, Olivo LB, Moraes RO, Garcia EDS, Silveira AC, Krolow R, et al. (2020). Neurometabolic effects of sweetened solution intake during adolescence related to depressive-like phenotype in rats. *Nutrition.* 75-76:110770. doi:10.1016/j.nut.2020.110770 PMID:32276242

Arcella D, Le Donne C, Piccinelli R, Leclercq C (2004). Dietary estimated intake of intense sweeteners by Italian teenagers. Present levels and projections derived from the INRAN-RM-2001 food survey. *Food Chem Toxicol.* 42(4):677–85. doi:10.1016/j.fct.2003.12.004 PMID:15019193

Ashok I, Poornima PS, Wankhar D, Ravindran R, Sheeladevi R (2017). Oxidative stress evoked damages on rat sperm and attenuated antioxidant status on consumption of aspartame. *Int J Impot Res.* 29(4):164–70. doi:10.1038/ijir.2017.17 PMID:28446800

Ashok I, Sheeladevi R (2014). Biochemical responses and mitochondrial mediated activation of apoptosis on long-term effect of aspartame in rat brain. *Redox Biol.* 2:820–31. doi:10.1016/j.redox.2014.04.011 PMID:25009784

Ashok I, Sheeladevi R (2015). Oxidant stress evoked damage in rat hepatocyte leading to triggered nitric oxide synthase (NOS) levels on long term consumption of aspartame. *Yao Wu Shi Pin Fen Xi.* 23(4):679–91. doi:10.1016/j.jfda.2014.07.011 PMID:28911484

Ashok I, Sheeladevi R, Wankhar D (2015). Acute effect of aspartame-induced oxidative stress in Wistar albino rat brain. *J Biomed Res.* 29(5):390–6. doi:10.7555/JBR.28.20120118 PMID:26445572

Aspinall RL, Saunders RN, Pautsch WF, Nutting EF (1980). The biological properties of aspartame. V. Effects on a variety of physiological parameters related to inflammation and metabolism. *J Environ Pathol Toxicol.* 3(5–6):387–95. PMID:6108346

Auerbach O, Garfinkel L (1989). Histologic changes in the urinary bladder in relation to cigarette smoking and use of artificial sweeteners. *Cancer.* 64(5):983–7. doi:10.1002/1097-0142(19890901)64:5<983::AID-CNCR2820640502>3.0.CO;2-9 PMID:2758391

Azad MB, Archibald A, Tomczyk MM, Head A, Cheung KG, de Souza RJ, et al. (2020). Nonnutritive sweetener consumption during pregnancy, adiposity, and adipocyte differentiation in offspring: evidence from humans, mice, and cells. *Int J Obes.* 44(10):2137–48. doi:10.1038/s41366-020-0575-x PMID:32366959

Baena-Nogueras RM, Traverso-Soto JM, Biel-Maeso M, Villar-Navarro E, Lara-Martín PA (2018). Sources and trends of artificial sweeteners in coastal waters in the bay of Cadiz (NE Atlantic). *Mar Pollut Bull.* 135:607–16. doi:10.1016/j.marpolbul.2018.07.069 PMID:30301079

Bajahzer MF, Bruun JM, Rosqvist F, Marklund M, Richelsen B, Risérus U (2022). Effects of sugar-sweetened soda on plasma saturated and monounsaturated fatty acids in individuals with obesity: a randomized study. *Front Nutr.* 9:936828. doi:10.3389/fnut.2022.936828 PMID:36118751

Bandyopadhyay A, Ghoshal S, Mukherjee A (2008). Genotoxicity testing of low-calorie sweeteners: aspartame, acesulfame-K, and saccharin. *Drug Chem Toxicol.* 31(4):447–57. doi:10.1080/01480540802390270 PMID:18850355

Bao Y, Stolzenberg-Solomon R, Jiao L, Silverman DT, Subar AF, Park Y, et al. (2008). Added sugar and sugar-sweetened foods and beverages and the risk of pancreatic cancer in the National Institutes of Health-AARP Diet and Health Study. *Am J Clin Nutr.* 88(2):431–40. doi:10.1093/ajcn/88.2.431 PMID:18689380

Bär A, Biermann C (1992). Intake of intense sweeteners in Germany. *Z Ernährungswiss.* 31(1):25–39. doi:10.1007/BF01612550 PMID:1374988

Baraniuk JN, Follett JV, Schiffman SS, Massey EW, Sampson HA, Warick ZS, et al. (1988). 74 Immunophysiologic correlates of cutaneous histamine reactivity in patients with alleged aspartame sensitivity. *J Allergy Clin Immunol.* 81(1):187. doi:10.1016/0091-6749(88)90311-9

Barceloux DG, Bond GR, Krenzelok EP, Cooper H, Vale JA; American Academy of Clinical Toxicology Ad Hoc Committee on the Treatment Guidelines for Methanol Poisoning (2002). American Academy of Clinical Toxicology practice guidelines on the treatment of methanol poisoning. *J Toxicol Clin Toxicol.* 40(4):415–46. doi:10.1081/CLT-120006745 PMID:12216995

Barraj L, Scrafford C, Bi X, Tran N (2021). Intake of low and no-calorie sweeteners (LNCS) by the Brazilian population. *Food Addit Contam Part A Chem Anal*

Control Expo Risk Assess. 38(2):181–94. doi:10.1080/19440049.2020.1846083 PMID:33337974

Basílio M, Silva LJG, Pereira AMPT, Pena A, Lino CM (2020). Artificial sweeteners in non-alcoholic beverages: occurrence and exposure estimation of the Portuguese population. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 37(12):2040–50. doi:10.1080/19440049.2020.1812734 PMID:32910867

Bassett JK, Milne RL, English DR, Giles GG, Hodge AM (2020). Consumption of sugar-sweetened and artificially sweetened soft drinks and risk of cancers not related to obesity. *Int J Cancer.* 146(12):3329–34. doi:10.1002/ijc.32772 PMID:31693185

Baudrimont I, Ahouandjivo R, Creppy EE (1997). Prevention of lipid peroxidation induced by ochratoxin A in Vero cells in culture by several agents. *Chem Biol Interact.* 104(1):29–40. doi:10.1016/S0009-2797(97)03764-2 PMID:9158693

Beck B, Burlet A, Max JP, Stricker-Krongrad A (2002). Effects of long-term ingestion of aspartame on hypothalamic neuropeptide Y, plasma leptin and body weight gain and composition. *Physiol Behav.* 75(1–2):41–7. doi:10.1016/S0031-9384(01)00654-0 PMID:11890951

Belpoggi F, Soffritti M, Padovani M, Degli Esposti D, Lauriola M, Minardi F (2006). Results of long-term carcinogenicity bioassay on Sprague-Dawley rats exposed to aspartame administered in feed. *Ann N Y Acad Sci.* 1076(1):559–77. doi:10.1196/annals.1371.080 PMID:17119233

Best D, Nelson L (1993). Low-calorie foods and sweeteners. *Prepared Foods.* (June):47–57.

Bianchi RG, Muir ET, Cook DL, Nutting EF (1980). The biological properties of aspartame. II. Actions involving the gastrointestinal system. *J Environ Pathol Toxicol.* 3(5-6):355–62. PMID:6777445

Bishop NC, Gleeson M, Nicholas CW, Ali A (2002). Influence of carbohydrate supplementation on plasma cytokine and neutrophil degranulation responses to high intensity intermittent exercise. *Int J Sport Nutr Exerc Metab.* 12(2):145–56. doi:10.1123/ijsnem.12.2.145 PMID:12187615

Bizarre S, Janshekar H, Kishi A (2006). High-intensity sweeteners. CEH Marketing Research Report. Report No. 543.6500. November 2003.

Bleich SN, Wolfson JA, Vine S, Wang YC (2014). Diet-beverage consumption and caloric intake among US adults, overall and by body weight. *Am J Public Health.* 104(3):e72–8. doi:10.2105/AJPH.2013.301556 PMID:24432876

Boehm MF, Bada JL (1984). Racemization of aspartic acid and phenylalanine in the sweetener aspartame at 100 degrees C. *Proc Natl Acad Sci USA.* 81(16):5263–6. doi:10.1073/pnas.81.16.5263 PMID:6591191

Bosetti C, Gallus S, Talamini R, Montella M, Franceschi S, Negri E, et al. (2009). Artificial sweeteners and the risk of gastric, pancreatic, and endometrial cancers in Italy. *Cancer Epidemiol Biomarkers Prev.* 18(8):2235–8. doi:10.1158/1055-9965.EPI-09-0365 PMID:19661082

Bruce DG, Storlien LH, Furler SM, Chisholm DJ (1987). Cephalic phase metabolic responses in normal weight adults. *Metabolism.* 36(8):721–5. doi:10.1016/0026-0495(87)90106-5 PMID:3298939

Bryan GT (1984). Artificial sweeteners and bladder cancer: assessment of potential urinary bladder carcinogenicity of aspartame and its diketopiperazine derivative in mice. In: Stegink F, Filer LJ Jr, editors. Aspartame: physiology and biochemistry. 1st ed. Boca Raton (FL), USA: CRC Press; pp. 321–348.

Buffini M, Goscinny S, Van Loco J, Nugent AP, Walton J, Flynn A, et al. (2018). Dietary intakes of six intense sweeteners by Irish adults. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 35(3):425–38. doi:10.1080/19440049.2017.1411619 PMID:29210609

Burdock GA (2010). Fenaroli's handbook of flavor ingredients. 6th ed. Boca Raton (FL), USA: CRC Press; pp. 122–3. doi:10.1201/9781439847503

Burgert SL, Andersen DW, Stegink LD, Takeuchi H, Schedl HP (1991). Metabolism of aspartame and its L-phenylalanine methyl ester decomposition product by the porcine gut. *Metabolism.* 40(6):612–8. doi:10.1016/0026-0495(91)90052-X PMID:1865825

Burns TS, Stargel WW, Tschanz C, Kotsonis FN, Hurwitz A (1991). Aspartame and sucrose produce a similar increase in the plasma phenylalanine to large neutral amino acid ratio in healthy subjects. *Pharmacology.* 43(4):210–9. doi:10.1159/000138847 PMID:1771173

Burton EG, Schoenhard GL, Hill JA, Schmidt RE, Hribar JD, Kotsonis FN, et al. (1989). Identification of *N*-β-L-aspartyl-L-phenylalanine as a normal constituent of human plasma and urine. *J Nutr.* 119(5):713–21. doi:10.1093/jn/119.5.713 PMID:2723819

Butchko HH, Stargel WW, Comer CP, Mayhew DA, Benninger C, Blackburn GL, et al. (2002). Aspartame: review of safety. *Regul Toxicol Pharmacol.* 35(2 Pt 2):S1–93. doi:10.1006/rtph.2002.1542 PMID:12180494

Caballero B, Mahon BE, Rohr FJ, Levy HL, Wurtman RJ (1986). Plasma amino acid levels after single-dose aspartame consumption in phenylketonuria, mild hyperphenylalaninemia, and heterozygous state for phenylketonuria. *J Pediatr.* 109(4):668–71. doi:10.1016/S0022-3476(86)80239-6 PMID:3761084

Cabaniols C, Giorgi R, Chinot O, Ferahta N, Spinelli V, Alla P, et al. (2011). Links between private habits, psychological stress and brain cancer: a case-control pilot study in France. *J Neurooncol.* 103(2):307–16. doi:10.1007/s11060-010-0388-1 PMID:20835749

Cacoub P, Saadoun D (2021). Extrahepatic manifestations of chronic HCV infection. *N Engl J Med.* 384(11):1038–52. doi:10.1056/NEJMra2033539 PMID:33730456

Çadirci K, Özdemir Tozlu Ö, Türkez H, Mardinoğlu A (2020). The in vitro cytotoxic, genotoxic, and oxidative damage potentials of the oral artificial

sweetener aspartame on cultured human blood cells. *Turk J Med Sci.* 50(2):448–54. doi:10.3906/sag-2001-113 PMID:32222132

Carlson HE, Shah JH (1989). Aspartame and its constituent amino acids: effects on prolactin, cortisol, growth hormone, insulin, and glucose in normal humans. *Am J Clin Nutr.* 49(3):427–32. doi:10.1093/ajcn/49.3.427 PMID:2923074

Carvalho C, Correia D, Severo M, Magalhães V, Casal S, Ramos E, et al. (2022). Dietary exposure to artificial sweeteners and associated factors in the Portuguese population. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 39(7):1206–21. doi:10.1080/19440049.2022.2075039 PMID:35604339

Castaño-Vinyals G, Aragonés N, Pérez-Gómez B, Martín V, Llorca J, Moreno V, et al.; MCC-Spain Study Group (2015). Population-based multicase–control study in common tumors in Spain (MCC-Spain): rationale and study design. *Gac Sanit.* 29(4):308–15. doi:10.1016/j.gaceta.2014.12.003 PMID:25613680

CEN (1996). Foodstuffs – determination of aspartame in table top sweetener preparations – method by high performance liquid chromatography. Technical specification. CEN/TS 1378:1996. Brussels, Belgium: Comité Européen de Normalisation [European Committee for Standardization].

CEN (1999). Foodstuffs – determination of acesulfame-K, aspartame and saccharin sodium in foods. high performance liquid chromatography method. (Adoption of the identical standard DIN EN 12856, July 1999 edition, as a replacement for the previous official method L 00.00–28). EN 12856:1999. Brussels, Belgium: Comité Européen de Normalisation [European Committee for Standardization].

CEN (2009). Foodstuffs – determination of acesulfame-K, aspartame, neohesperidine-dihydrochalcone and saccharin. – high performance liquid chromatographic method. CEN/TS 15606:2009. Brussels, Belgium: Comité Européen de Normalisation [European Committee for Standardization].

CEN (2010). Foodstuffs – simultaneous determination of nine sweeteners by high performance liquid chromatography and evaporative light scattering detection. BS EN 15911:2010. Available from: https://knowledge.bsigroup.com/products/foodstuffs-simultaneous-determination-of-nine-sweeteners-by-high-performance-liquid-chromatography-and-evaporative-light-scattering-detection/standard, accessed June 2023.

Chan JM, Wang F, Holly EA (2009). Sweets, sweetened beverages, and risk of pancreatic cancer in a large population-based case–control study. *Cancer Causes Control.* 20(6):835–46. doi:10.1007/s10552-009-9323-1 PMID:19277880

Chang CS, Yeh TS (2014). Detection of 10 sweeteners in various foods by liquid chromatography/tandem mass spectrometry. *J Food Drug Anal.* 22(3):318–28. doi:10.1016/j.jfda.2014.01.024 PMID:28911421

Chazelas E, Druesne-Pecollo N, Esseddik Y, de Edelenyi FS, Agaesse C, De Sa A, et al. (2021). Exposure to food additive mixtures in 106,000 French adults from the NutriNet-Santé cohort. *Sci Rep.* 11(1):19680. doi:10.1038/s41598-021-98496-6 PMID:34608173

Chazelas E, Srour B, Desmetz E, Kesse-Guyot E, Julia C, Deschamps V, et al. (2019). Sugary drink consumption and risk of cancer: results from NutriNet-Santé prospective cohort. *BMJ.* 366:l2408. doi:10.1136/bmj.l2408 PMID:31292122

Chemical Abstracts Service (2022a). Aspartame. CAS registry number 22839-47-0. CAS Common Chemistry [online database]. Columbus (OH), USA: Chemical Abstracts Service, American Chemical Society. Available from: https://commonchemistry.cas.org/detail?cas_rn=22839-47-0, accessed 22 November 2022.

Chemical Abstracts Service (2022b). Suppliers for CAS No. 22839-47-0. SciFinder [online database]. Columbus (OH), USA: Chemical Abstracts Service, American Chemical Society. Available from: https://www.cas.org/solutions/cas-scifinder-discovery-platform/cas-scifinder/substances.

Chen YC, Yeh YC, Lin YF, Au HK, Hsia SM, Chen YH, et al. (2022). Aspartame consumption, mitochondrial disorder-induced impaired ovarian function, and infertility risk. *Int J Mol Sci.* 23(21):12740. doi:10.3390/ijms232112740 PMID:36361530

Chiozzotto D; Soffritti M; Falcioni L, et al. (2011). Results of life span carcinogenicity bioassay on Sprague-Dawley rats exposed to aspartame since foetal life. *Eur J Oncol.* 16(2):81–97.

Cho ES, Coon JD, Steglink LD (1987). Plasma and urine diketopiperazine concentrations in normal adults ingesting large quantities of aspartame. *Food Chem Toxicol.* 25(7):499–504. doi:10.1016/0278-6915(87)90200-6 PMID:3623338

Choi HM, Doss HM, Kim KS (2020). Multifaceted physiological roles of adiponectin in inflammation and diseases. *Int J Mol Sci.* 21(4):1219. doi:10.3390/ijms21041219 PMID:32059381

Choi JY, Park MN, Kim CS, Lee YK, Choi EY, Chun WY, et al. (2017). Long-term consumption of sugar-sweetened beverage during the growth period promotes social aggression in adult mice with proinflammatory responses in the brain. *Sci Rep.* 7(1):45693. doi:10.1038/srep45693 PMID:28393871

Choudhary AK, Sheela Devi R (2015). Longer period of oral administration of aspartame on cytokine response in Wistar albino rats. *Endocrinol Nutr.* 62(3):114–22. doi:10.1016/j.endonu.2014.11.004 PMID:25681123

Choudhary AK, Sheela Devi RS (2016). Effects of aspartame on hsp70, bcl-2 and bax expression in immune organs of Wistar albino rats. *J Biomed Res*. 30(5):427–35. doi:10.7555/JBR.30.20140097 PMID:27845306

Chu YY, Chen YH, Hsieh RH, Hsia SM, Wu HT, Chen YC (2022). Development and validation of the Chinese version non-nutritive sweetener food frequency questionnaire with urinary biomarker in children and adolescents. *Public Health Nutr*. 25(8):1–23. PMID:35414373

Chung MS, Suh HJ, Yoo W, Choi SH, Cho YJ, Cho YH, et al. (2005). Daily intake assessment of saccharin, stevioside, D-sorbitol and aspartame from various processed foods in Korea. *Food Addit Contam*. 22(11):1087–97. doi:10.1080/02652030500202092 PMID:16332631

Colagiuri S, Miller JJ, Edwards RA (1989). Metabolic effects of adding sucrose and aspartame to the diet of subjects with noninsulin-dependent diabetes mellitus. *Am J Clin Nutr*. 50(3):474–8. doi:10.1093/ajcn/50.3.474 PMID:2672774

Collison KS, Inglis A, Shibin S, Andres B, Ubungen R, Thiam J, et al. (2016). Differential effects of early-life NMDA receptor antagonism on aspartame-impaired insulin tolerance and behavior. *Physiol Behav*. 167:209–21. doi:10.1016/j.physbeh.2016.09.011 PMID:27640132

Collison KS, Inglis A, Shibin S, Saleh S, Andres B, Ubungen R, et al. (2018). Effect of developmental NMDAR antagonism with CGP 39551 on aspartame-induced hypothalamic and adrenal gene expression. *PLoS One*. 13(3):e0194416. doi:10.1371/journal.pone.0194416 PMID:29561882

Collison KS, Makhoul NJ, Zaidi MZ, Al-Rabiah R, Inglis A, Andres BL, et al. (2012a). Interactive effects of neonatal exposure to monosodium glutamate and aspartame on glucose homeostasis. *Nutr Metab (Lond)*. 9(1):58. doi:10.1186/1743-7075-9-58 PMID:22697049

Collison KS, Makhoul NJ, Zaidi MZ, Inglis A, Andres BL, Ubungen R, et al. (2013). Prediabetic changes in gene expression induced by aspartame and monosodium glutamate in *trans* fat-fed C57Bl/6 J mice. *Nutr Metab (Lond)*. 10(1):44. doi:10.1186/1743-7075-10-44 PMID:23783067

Collison KS, Makhoul NJ, Zaidi MZ, Saleh SM, Andres B, Inglis A, et al. (2012b). Gender dimorphism in aspartame-induced impairment of spatial cognition and insulin sensitivity. *PLoS One*. 7(4):e31570. doi:10.1371/journal.pone.0031570 PMID:22509243

Council of the European Union (2001). Council Directive 2001/112/EC of 20 December 2001 relating to fruit juices and certain similar products intended for human consumption. Document 32001L0112. *Off J Eur Commun*. L10:58–66. Available from: https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=celex%3A32001L0112.

Creppy EE, Baudrimont I, Anne-Marie (1998). How aspartame prevents the toxicity of ochratoxin A. *J Toxicol Sci*. 23(Suppl 2):165–72. doi:10.2131/jts.23.SupplementII_165 PMID:9760456

Crosby GA, Furia TE (1980). New sweeteners. In: Furia TE, editor. CRC handbook of food additives. Boca Raton (FL), USA: CRC Press; pp. 203–216.

Curtius HC, Endres W, Blau N (1994). Effect of high-protein meal plus aspartame ingestion on plasma phenylalanine concentrations in obligate heterozygotes for phenylketonuria. *Metabolism*. 43(4):413–6. doi:10.1016/0026-0495(94)90068-X PMID:8159095

da Silva LC, Carvalho TS, da Silva FB, Pires RF, Giugliani R, Pereira ML (2000). Aspartame loading test in PKU heterozygous individuals bearing severe and moderate mutations. *Clin Genet*. 58(1):86–8. doi:10.1034/j.1399-0004.2000.580118.x PMID:10945671

Daher M, Fahd C, Nour AA, Sacre Y (2022). Trends and amounts of consumption of low-calorie sweeteners: a cross-sectional study. *Clin Nutr ESPEN*. 48:427–33. doi:10.1016/j.clnesp.2022.01.006 PMID:35331524

Davis EW, McCann SE, Joseph JM, Yeary KHK, Fountzilas C, Moysich KB (2023). Sugar sweetened and artificially sweetened beverage consumption and pancreatic cancer: a retrospective study. *Nutrients*. 15(2):275. doi:10.3390/nu15020275 PMID:36678146

Davoli E, Cappellini L, Airoldi L, Fanelli R (1986). Serum methanol concentrations in rats and in men after a single dose of aspartame. *Food Chem Toxicol*. 24(3):187–9. doi:10.1016/0278-6915(86)90227-9 PMID:3957170

Debras C, Chazelas E, Sellem L, Porcher R, Druesne-Pecollo N, Esseddik Y, et al. (2022a). Artificial sweeteners and risk of cardiovascular diseases: results from the prospective NutriNet-Santé cohort. *BMJ*. 378:e071204. doi:10.1136/bmj-2022-071204 PMID:36638072

Debras C, Chazelas E, Srour B, Druesne-Pecollo N, Esseddik Y, Szabo de Edelenyi F, et al. (2022b). Artificial sweeteners and cancer risk: results from the NutriNet-Santé population-based cohort study. *PLoS Med*. 19(3):e1003950. doi:10.1371/journal.pmed.1003950 PMID:35324894

Debras C, Deschasaux-Tanguy M, Chazelas E, Sellem L, Druesne-Pecollo N, Esseddik Y, et al. (2023). Artificial sweeteners and risk of type 2 diabetes in the prospective NutriNet-Santé cohort. *Diabetes Care*. 46(9):1681–90. doi:10.2337/dc23-0206 PMID:37490630

DeLima Associates (2022). Aspartame. Consumer Product Information Database (CPID) [online database]. McLean (VA): USA: DeLima Associates. Available from: https://www.whatsinproducts.com/chemicals/view/1/3857/022839-47-0/Aspartame, accessed 14 November 2022.

Dewinter L, Casteels K, Corthouts K, Van de Kerckhove K, Van der Vaerent K, Vanmeerbeeck K, et al. (2016). Dietary intake of non-nutritive sweeteners in type 1 diabetes mellitus children. *Food Addit Contam Part*

A Chem Anal Control Expo Risk Assess. 33(1):19–26. PMID:26523968

Dietrich AM, Pang Z, Zheng H, Ma X (2021). Mini review: will artificial sweeteners discharged to the aqueous environment unintentionally "sweeten" the taste of tap water? *Chem Eng J Adv.* 6:100100. Available from: https://www.sciencedirect.com/science/article/pii/S2666821121000168.

Dooley J, Lagou V, Dresselaers T, van Dongen KA, Himmelreich U, Liston A (2017). No effect of dietary aspartame or stevia on pancreatic acinar carcinoma development, growth, or induced mortality in a murine model. *Front Oncol.* 7:18. doi:10.3389/fonc.2017.00018 PMID:28232906

Duarte LM, Ferreira SMR, Almeida CCB, Duran ACDFL, Grilo MF, Macedo MS, et al. (2022). Dietary exposure to low-calorie sweeteners in a sample of Brazilian pregnant women. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 39(10):1650–62. doi:10.1080/19440049.2022.2112763 PMID:35993876

Dunford EK, Coyle DH, Louie JCY, Rooney K, Blaxland A, Pettigrew S, et al. (2022). Changes in the presence of nonnutritive sweeteners, sugar alcohols, and free sugars in Australian foods. *J Acad Nutr Diet.* 122(5):991–999. e7. doi:10.1016/j.jand.2021.11.018 PMID:34864247

Dunford EK, Miles DR, Ng SW, Popkin B (2020). Types and amounts of nonnutritive sweeteners purchased by US households: a comparison of 2002 and 2018 Nielsen Homescan purchases. *J Acad Nutr Diet.* 120 10:1662–1671.e10. doi:10.1016/j.jand.2020.04.022 PMID:32739278

Duodecim Drug Database (2023). Kustannus oy Duodecim. Available from: https://www.terveysportti.fi/apps/laake/, accessed July 2023. [Finnish]

Durán Agüero S, Rodríguez M, Noel R, Arrivillaga K, Ariza J, Cornwall J, et al. (2017). Comparison of nonnutritive artificial sweetener consumption among university students in Latin American: multicentric study. *Arch Latinoam Nutr.* 67(3).

Durnev AD, Oreshchenko AV, Kulakova AV, Beresten' NF, Seredenin SB (1995). [Clastogenic activity of dietary sugar substitutes.] *Vopr Med Khim.* 41(4):31–3. [Russian] PMID:8571583

ECHA (2022). Aspartame. Substance infocard. Helsinki, Finland: European Chemicals Agency. Available from: https://echa.europa.eu/de/substance-information/-/substanceinfo/100.041.132, accessed 22 November 2022.

ECHEMI (2020). The development of new sweetener. 4 February 2020. ECHEMI Group. Available from: https://www.echemi.com/cms/97880.html.

EFSA (2013). Scientific opinion on the re-evaluation of aspartame (E 951) as a food additive. EFSA Panel on Food Additives and Nutrient Sources added to Food. *EFSA J.* 11(12):3496. Available from https://www.efsa.europa.eu/en/efsajournal/pub/3496, accessed May 2023.

EFSA (2021). Review and synthesis of data on the potential environmental impact of artificial sweeteners. EFSA supporting publication 2021:EN-6918. Helsinki, Finland: European Chemicals Agency. Available from: https://efsa.onlinelibrary.wiley.com/doi/abs/10.2903/sp.efsa.2021.EN-6918, accessed 14 November 2022.

EFSA_E1 (2011). SC-18862: a sweetening agent. 1 June 1972. Prepared by the Department of Biological Services, Searle Laboratories, USA. Submitted by Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E12 (2011). Mutagenic study in rats. SC-18862. Final report. 31 July 1970. Prepared by Hazleton Laboratories, Inc., for GD Searle & Co., USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E15 (2011a). VI. Pharmacokinetics and metabolism of SC-18862 in man. In: The metabolism of aspartame. Vol. 1, Parts I–XIV. 10 July 1972. Prepared by the Department of Biochemical Research, Searle Laboratories, USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E15 (2011b). IX. Pharmacokinetics and metabolism of SC-19192 in man. In: The metabolism of aspartame. Vol. 1, Parts I–XIV. 10 July 1972. Prepared by the Department of Biochemical Research, Searle Laboratories, USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E17 (2011). The metabolism of aspartame. Vol. 2, Parts XV–XIX. 9 October 1972. Prepared by the Department of Biological Services, Searle Laboratories, USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E18 (2011). The metabolism of aspartame. Vol. 3, Parts XX–XXIII. 1 September 1972. Prepared by the Department of Biochemical Research, Searle Laboratories, USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E19 (2011). SC-18862. A sweetening agent. Endocrine studies. Prepared by the Department of Biological Research, Searle Laboratories, USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available

from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E20 (2011). 2-Month oral administration – rats. SC-18862. Final report. 20 January 1969. Prepared by Hazelton Laboratories, Inc., for GD Searle & Company, USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E21 (2011). 2-Month oral toxicity – dogs. SC-18862. Final Report. 8 February 1969. Prepared by Hazelton Laboratories, Inc., for GD Searle & Company, USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E25 (2011). Short term tolerance of aspartame by adult PKU heterozygotes. 1972. Prepared by Koch R, Peterson RM, Scriver CR, for GD Searle & Co., USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E26 (2011). Tolerance of loading doses of aspartame by phenylketonuric (PKU) homozygous children. 1972. Prepared by Koch R, for GD Searle & Co., USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E27 (2011). SC-18862: 46 week oral toxicity study in the hamster. Pathology-Toxicology Project No. 852S72. 8 December 1972. Prepared by the Department of Biological Research (Pathology-Toxicology), Searle Laboratories, USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E28 (2011). SC-18862: 106 week oral toxicity study in the dog. Pathology-Toxicology Project No. 855S70. 1 August 1972. Prepared by the Department of Biological Services (Pathology-Toxicology), Searle Laboratories, USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E30 (2011). SC-19192: an evaluation of mutagenic potential employing the in vivo cytogenetics method in the rat. Final Report. P-T No. 1027H72. 29 August 1972. Prepared by Hazelton Laboratories, Inc., for Searle Laboratories, USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E31 (2011). SC-19192: an evaluation of mutagenic potential employing the host-mediated assay in the rat. Final Report. P-T No. 1029H72. 28 August 1972. Prepared by Hazelton Laboratories, Inc., for Searle Laboratories, USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E33 (2011). Appendix. SC-18862: two-year toxicity study in the rat; P-T No. 838H71. 12 January 1973. 12 January 1973. Prepared by Hazleton Laboratories, Inc., for Searle & Co., USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E34 (2011). SC-18862: two-year toxicity study in the rat. P-T No. 838H71. Final Report. 12 January 1973. Report prepared by Hazleton Laboratories, Inc., for Searle Laboratories, USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E35 (2011). Supplement No. I, Part I. SC-18862: 46-week oral toxicity study in the hamster. Pathology-Toxicology Project No. 852S72. 8 December 1972. Prepared by the Department of Biological Research (Pathology-Toxicology), Searle Laboratories, USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E36 (2011). Supplement No. I, Part II. SC-18862: 46-week oral toxicity study in the hamster. Pathology-Toxicology Project No. 852S72. 8 December 1972. Prepared by the Department of Biological Research, Searle Laboratories, USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E40 (2011). SC-18862: an evaluation of the mutagenic potential in the rat employing the dominant lethal assay. Pathology-Toxicology Project No. 868H70. 16 January 1973. Prepared by the Department of Biological Research, Searle Laboratories, USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E41 (2011). SC-18862: an evaluation of the mutagenic potential in the rat employing the dominant lethal assay. Pathology-Toxicology Project No. 1007S72. 17 January 1973. Report prepared by the Department of Biological Research, Searle Laboratories, USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available

from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E42 (2011). SC-19192: an evaluation of the mutagenic potential in the rat employing the dominant lethal assay. Pathology-Toxicology Project No. 1008S72. 16 January 1973. Prepared by the Department of Biological Research, Searle Laboratories, USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E43 (2011). SC-18862: an evaluation of mutagenic potential employing the in vivo cytogenetics method in the rat. P-T No. 1026H72. Final Report. 29 August 1972. Prepared by Hazleton Laboratories, Inc., for Searle Laboratories, USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E44 (2011). SC-18862: an evaluation of mutagenic potential employing the host-mediated assay in the rat. P-T No. 1028H72. Final Report. 25 August 1972. Prepared by Hazleton Laboratories, Inc., for Searle Laboratories, USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E58 (2011). Final report. SC-18862: a 26 week urinary bladder tumorigenicity study in the mouse by the intravesical pellet implant technique. P-T No. 1031of72. 23 January 1973. Prepared by Bryan GT, for Searle Laboratories, Searle & Co., USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531.

EFSA_E60 (2011). Long term tolerance of aspartame by normal adults. 1972. Prepared by Hill Top Research, Inc. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E65 (2011). Tolerance of aspartame by diabetic subjects. 1973. Prepared by Bleicher SJ, Stern SB, for Searle Laboratories, USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E67 (2011). Long term tolerance of aspartame by adult PKU heterozygotes. 1972. Prepared by Koch R, Peterson RM, for GD Searle & Co., USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E70 (2011). Final report. SC-18862: lifetime toxicity study in the rat. P-T No. 892H72. 11 January 1974. Prepared by Hazleton Laboratories, Inc., for Searle Laboratories, Searle & Co., USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E71 (2011). Study of possible nitrosamide formation from APM And DKP under simulated physiological conditions. Prepared by Searle Laboratories, GD Searle & Co., USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E72 (2011). SC-18862: a 56-week urinary bladder tumorigenicity study in the mouse by the intravesical pellet implant technique. Pathology-Toxicology Project Nos 1033of73, 1035of72, 1037of72. 28 January 1974. Prepared by Bryan GT, for Searle Laboratories, Searle & Co., USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E75 (2011). Final report. SC-18862: 104-week toxicity study in the mouse. P-T No. 984H73. 6 September 1974. Prepared by Hazelton Laboratories, Inc., for Searle Laboratories, USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E80 (2011). The metabolism of aspartame. Vol. 4, Parts XXIV–XXXI. October 1974. Prepared by the Department of Drug Metabolism & Radiochemistry, Searle Laboratories, USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E81 (2011). SC-18862: an evaluation of mutagenic potential employing the host-mediated assay in the mouse. Pathology-Toxicology Project No. 1087S73. October 1974. Prepared by the Department of Pathology-Toxicology, Searle Laboratories, USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E82 (2011). SC-19192: an evaluation of mutagenic potential employing the host-mediated assay in the mouse. Pathology-Toxicology Project No. 1095S73. 1974. Prepared by the Department of Pathology-Toxicology, Searle Laboratories, USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.

efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E86 (2011). SC-18862: a supplemental study of dog brains from a 106-week oral toxicity study. (P-T No. 855S70). Pathology-Toxicology Project No. 1226. 1973. Prepared by the Department of Pathology-Toxicology, Searle Laboratories, USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E87 (2011). SC-18862 (aspartame): a supplemental study of rat brains from two tumorigenicity studies (P-T Nos 838H71 and 892H72). Pathology-Toxicology Project No. 1227. 1973. Prepared by the Department of Biological Services, Searle Laboratories, USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E91 (2011). Table of contents. Studies on aspartame. Section I. A. Introduction B. Pivotal animal safety studies. C. Overview of data reassurance activities. Section II. Review of metabolism studies. Section III. Progress report on aspartame studies (University of Iowa). 1976. Prepared by the Department of Biological Services, Searle Laboratories, USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E97 (2011). SC-18862: an evaluation of mutagenic potential employing the Ames Salmonella/microsome assay. Safety Assessment Project No. 1377. 30 January 1978. Prepared by Molinary SV, for the Department of Product Safety Assessment, Searle Laboratories, USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E98 (2011). SC-19192: an evaluation of mutagenic potential employing the Ames Salmonella/microsome assay. Report release. Safety Assessment Project No. 1378. 30 January 1978. Prepared by Molinary SV, for the Department of Product Safety Assessment, Searle Laboratories, USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E101 (2011). An evaluation of the mutagenic potential of SC-18862 employing the Ames Salmonella/microsome assay. Report release. 27 April 1978. Prepared by the Department of Biological Services, Searle Laboratories, USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E103 (2011). Effects of aspartame (SC-18862) on gonadotropin secretion in rats. Biology document review and approval sign-off. 1 May 1978. Prepared by the Department of Biological Services, Searle Laboratories, USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E106 (2011). An evaluation of the mutagenic potential of SC-19192 employing the Ames Salmonella/microsome assay. Report release. 1978. Prepared by the Department of Biological Services, Searle Laboratories, USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E108 (2011). Effect of aspartame on plasma and red cell amino acids of apparently healthy female adults and on presumed phenylketonuric heterozygotes. MED-76-06-055. 1978. Prepared by Koch R, Blaskovics M. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_E109 (2011). Effect of aspartame loading at 100 mg per kg body weight upon plasma and erythrocyte levels of free amino acids in normal subjects and subjects presumed to be heterozygous for phenylketonuria. Final report. 30 July 1978. Prepared by Stegink LD, Filer LJ Jr, Baker GL, McDonnel JE, for the GD Searle & Co., USA. Submitted by Ajinomoto and Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_UA04 (2011). Final report. Histopathological examination in a carcinogenicity study of SC-18862 in Slc:Wistar rats. Experiment No. 9748. 16 August 2006. Submitted by Ajinomoto. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_UN01 (2011). Effects of aspartame ingestion without food on serum glucose, insulin, and glucagon. Final clinical research report. Document No. N04-84-02-088. 18 January 1988. Prepared by Clinical Research Department, The Nutrasweet Company. Submitted by Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_UN07 (2011). Multi-center clinical study to evaluate allergic reactions allegedly due to aspartame consumption. Protocol No. N22-86-02-002. 21 October 1992. Submitted by Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_UN08 (2011). Safety of long-term aspartame administration in normal subjects. Final clinical research report. Protocol No. N07-84-02-093. 4 August 1988. Submitted by Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

EFSA_UN09 (2011). A study of the clinical and biochemical effects of aspartame in subjects with chronic stable alcoholic liver disease. Protocol No. N23-87-02-001. 15 October 1991. Prepared by the Nutrasweet Company. Submitted by Nutrasweet. Parma, Italy: European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/consultations/call/110531, accessed May 2023.

Elballat SE, Abas A-SM (2020). Ameliorative role of silymarin against aspartame–induced biochemical changes, oxidative stress, inflammatory effect and genotoxicity in male albino rats. *Int J Curr Pharm Res*. 12(1):2020. doi:10.22159/ijcpr.2020v12i1.36831

EMA (2017). Information for the package leaflet regarding aspartame and phenylalanine used as excipients in medicinal products for human use. London, UK: European Medicines Agency. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/information-package-leaflet-regarding-aspartame-phenylalanine-used-excipients-medicinal-products_en.pdf, accessed 14 November 2022.

Enuwosa E, Gautam L, King L, Chichger H (2021). Saccharin and sucralose protect the glomerular microvasculature in vitro against VEGF-induced permeability. *Nutrients*. 13(8):2746. doi:10.3390/nu13082746 PMID:34444906

Erbaş O, Erdoğan MA, Khalilnezhad A, Solmaz V, Gürkan FT, Yiğittürk G, et al. (2018). Evaluation of long-term effects of artificial sweeteners on rat brain: a biochemical, behavioral, and histological study. *J Biochem Mol Toxicol*. 32(6):e22053. doi:10.1002/jbt.22053 PMID:29660801

European Commission (1999). Commission Directive 1999/21/EC of 25 March 1999 on dietary foods for special medical purposes. Document 31999L0021. *Off J Eur Commun*. L91:29–36. Available from: https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=celex%3A31999L0021, accessed February 2024.

European Commission (2001). Report from the Commission on dietary food additive intake in the European Union. COM(2001) 542 final. Brussels, Belgium: Commission of the European Communities. Available from: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=COM:2001:0542:FIN:EN:PDF, accessed February 2024.

European Commission (2008). Commission Regulation (EC) No. 889/2008 of 5 September 2008 laying down detailed rules for the implementation of Council Regulation (EC) No. 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control. *Off J Eur Commun*. 250:1–84. Available from https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32008R0889&from=DE, accessed May 2023.

European Commission (2011). Commission Regulation (EU) No. 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No. 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives. *Off J Eur Commun*. L295:1–177. Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32011R1129&qid=1708593659465, accessed February 2024.

European Commission (2012). Commission Regulation (EU) No. 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No. 1333/2008 of the European Parliament and of the Council. *Off J Eur Commun*. L83:1–295. Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32012R0231&from=EN, accessed May 2023.

European Commission (2016). Commission Implementing Regulation (EU) 2016/262 of 25 February 2016 imposing a provisional anti-dumping duty on imports of aspartame originating in the People's Republic of China. *Off J Eur Commun*. L50:4–24. Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32016R0262&from=EN, accessed 28 April 2023.

European Commission (2022). Commission Implementing Regulation (EU) 2022/2001 of 21 October 2022 imposing a definitive anti-dumping duty on imports of aspartame originating in the People's Republic of China, following an expiry review pursuant to Article 11(2) of Regulation (EU) 2016/1036 of the European Parliament and of the Council. *Off J Eur Commun*. L274:24–62. Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32022R2001&from=EN, accessed June 2023.

European Commission (2022a). Aspartame. CosIng – Cosmetics Ingredients [online database]. Brussels, Belgium: European Commission. Available from: https://ec.europa.eu/growth/tools-databases/cosing/, accessed June 2023.

European Commission (2022b). Commission Implementing Regulation (EU) 2022/2001 of 21 October 2022 imposing a definitive anti-dumping duty on imports of aspartame originating in the People's Republic of China, following an expiry review pursuant to Article 11(2) of Regulation (EU) 2016/1036 of the European Parliament and of the Council. *Off J Eur Commun*. L274:24–63. Available from: https://

eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32022R2001&from=EN, accessed 28 April 2023.

European Medicines Agency (2019). Annex to the European Commission guideline on 'Excipients in the labelling and package leaflet of medicinal products for human use'. SANTE-2017-11668. Revision 2, EMA/CHMP/302620/2017, March 2018. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/annex-european-commission-guideline-excipients-labelling-package-leaflet-medicinal-products-human_en-1.pdf, accessed May 2023.

European Parliament and Council (1994). European Parliament and Council Directive 94/35/EC of 30 June 1994 on sweeteners for use in food-stuffs. *Off J Eur Commun*. L273:3–12. Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex:31994L0035, accessed May 2023.

European Parliament and Council (2000). Directive 2000/36/EC of the European Parliament and of the Council of 23 June 2000 relating to cocoa and chocolate products intended for human consumption. Document 32000L0036. *Off J Eur Commun*. L197:19–25. Available from: https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32000L0036, accessed February 2024.

European Parliament and Council (2002). Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements. Document 32002L0046. *Off J Eur Commun*. L183:51–57. Available from: https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=celex%3A32002L0046, accessed February 2024.

European Parliament and Council (2008). Regulation (EC) No. 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. *Off J Eur Commun*. L354:16–33. Available from: https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX:32008R1333, accessed May 2023.

European Parliament and Council (2011). Regulation (EU) No. 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No. 1924/2006 and (EC) No. 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No. 608/2004. *Off J Eur Commun*. L304:18–63. Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32011R1169, accessed June 2023.

European Pharmacopoeia (2020). Aspartame. In: European pharmacopoeia, 10th ed. Strasbourg, France: Council of Europe; pp. 2821–3. [German]

Ewertz M, Gill C (1990). Dietary factors and breast-cancer risk in Denmark. *Int J Cancer*. 46(5):779–84. doi:10.1002/ijc.2910460505 PMID:2228305

FAO/WHO (1995). General standards for food additives. CODEX STAN 192-1995. Rome, Italy: Codex Alimentarius, Food and Agriculture Organization of the United Nations and the World Health Organization. Available from: https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B192-1995%252FCXS_192e.pdf, accessed May 2023.

Fareed SA, Mostafa HE (2021). Could aspartame exacerbate caffeine effects on renal maturation in rat's offspring? A biochemical and histological study. *Birth Defects Res*. 113(1):90–107. doi:10.1002/bdr2.1836 PMID:33128303

Faruque S, Tong J, Lacmanovic V, Agbonghae C, Minaya DM, Czaja K (2019). The dose makes the poison: sugar and obesity in the United States - a review. *Pol J Food Nutr Sci*. 69(3):219–33. doi:10.31883/pjfns/110735 PMID:31938015

Fatoki TH, Ibraheem O, Awofisayo OA, Oyedele AS, Akinlolu OS (2020). In silico investigation of first-pass effect on selected small molecule excipients and structural dynamics of P-glycoprotein. *Bioinform Biol Insights*. 14:1177932220943183. doi:10.1177/1177932220943183 PMID:32782427

Ferland A, Brassard P, Poirier P (2007). Is aspartame really safer in reducing the risk of hypoglycemia during exercise in patients with type 2 diabetes? *Diabetes Care*. 30(7):e59. doi:10.2337/dc06-1888 PMID:17596482

Feskanich D, Rimm EB, Giovannucci EL, Colditz GA, Stampfer MJ, Litin LB, et al. (1993). Reproducibility and validity of food intake measurements from a semi-quantitative food frequency questionnaire. *J Am Diet Assoc*. 93(7):790–6. doi:10.1016/0002-8223(93)91754-E PMID:8320406

Filer LJ Jr, Baker GL, Stegink LD (1983). Effect of aspartame loading on plasma and erythrocyte free amino acid concentrations in one-year-old infants. *J Nutr*. 113(8):1591–9. doi:10.1093/jn/113.8.1591 PMID:6135765

Filer LJ Jr, Stegink LD (1989). Aspartame metabolism in normal adults, phenylketonuric heterozygotes, and diabetic subjects. *Diabetes Care*. 12(1):67–74. doi:10.2337/diacare.12.1.67 PMID:2653751

Finamor I, Pérez S, Bressan CA, Brenner CE, Rius-Pérez S, Brittes PC, et al. (2017). Chronic aspartame intake causes changes in the trans-sulphuration pathway, glutathione depletion and liver damage in mice. *Redox Biol*. 11:701–7. doi:10.1016/j.redox.2017.01.019 PMID:28187322

Finamor IA, Bressan CA, Torres-Cuevas I, Rius-Pérez S, da Veiga M, Rocha MI, et al. (2021). Long-term aspartame administration leads to fibrosis, inflammasome activation, and gluconeogenesis impairment in the liver of mice. *Biology (Basel)*. 10(2):82. doi:10.3390/biology10020082 PMID:33499218

Finamor IA, Ourique GM, Pês TS, Saccol EM, Bressan CA, Scheid T, et al. (2014). The protective effect of *N*-acetylcysteine on oxidative stress in the brain caused by the long-term intake of aspartame by rats. *Neurochem Res*. 39(9):1681–90. doi:10.1007/s11064-014-1360-9 PMID:24970110

Food Advisory Committee (1991). Changes in the UK market for sweeteners and their implications for the intakes of sweeteners. Agenda item. 14 March 1991. FdAC/Sweeteners/3. Food Advisory Committee, UK Parliament. Available from: http://data.parliament.uk/DepositedPapers/Files/DEP2013-0273/1FdACSweeteners31.pdf.

Fowke JH, Schlundt D, Gong Y, Jin F, Shu XO, Wen W, et al. (2004). Impact of season of food frequency questionnaire administration on dietary reporting. *Ann Epidemiol*. 14(10):778–85. doi:10.1016/j.annepidem.2004.02.002 PMID:15519900

Frankenfeld CL, Sikaroodi M, Lamb E, Shoemaker S, Gillevet PM (2015). High-intensity sweetener consumption and gut microbiome content and predicted gene function in a cross-sectional study of adults in the United States. *Ann Epidemiol*. 25(10):736–42.e4. doi:10.1016/j.annepidem.2015.06.083 PMID:26272781

Frezza M, di Padova C, Pozzato G, Terpin M, Baraona E, Lieber CS (1990). High blood alcohol levels in women. The role of decreased gastric alcohol dehydrogenase activity and first-pass metabolism. *N Engl J Med*. 322(2):95–9. doi:10.1056/NEJM199001113220205 PMID:2248624

Fry J (1999). The world market for intense sweeteners. *World Rev Nutr Diet*. 85:201–11. doi:10.1159/000059691 PMID:10647352

Fuentealba Arévalo F, Espinoza Espinoza J, Salazar Ibacahe C, Durán Agüero S (2019). Consumption of non-caloric sweeteners among pregnant Chileans: a cross-sectional study. *Nutr Hosp*. 36(4):890–7. PMID:31192687

Fulgoni VL 3rd, Drewnowski A (2022). No association between low-calorie sweetener (LCS) use and overall cancer risk in the nationally representative database in the US: analyses of NHANES 1988–2018 data and 2019 public-use linked mortality files. *Nutrients*. 14(23):4957. doi:10.3390/nu14234957 PMID:36500986

Gallus S, Scotti L, Negri E, Talamini R, Franceschi S, Montella M, et al. (2007). Artificial sweeteners and cancer risk in a network of case–control studies. *Ann Oncol*. 18(1):40–4. doi:10.1093/annonc/mdl346 PMID:17043096

Gammon MD, Schoenberg JB, Ahsan H, Risch HA, Vaughan TL, Chow WH, et al. (1997). Tobacco, alcohol, and socioeconomic status and adenocarcinomas of the esophagus and gastric cardia. *J Natl Cancer Inst*. 89(17):1277–84. doi:10.1093/jnci/89.17.1277 PMID:9293918

Gan Z, Sun H, Feng B, Wang R, Zhang Y (2013). Occurrence of seven artificial sweeteners in the aquatic environment and precipitation of Tianjin, China. *Water Res*. 47(14):4928–37. doi:10.1016/j.watres.2013.05.038 PMID:23866151

Gan Z, Sun H, Yao Y, Zhao Y, Li Y, Zhang Y, et al. (2014). Distribution of artificial sweeteners in dust and soil in China and their seasonal variations in the environment of Tianjin. *Sci Total Environ*. 488–489:168–75. doi:10.1016/j.scitotenv.2014.04.084 PMID:24830929

Garavaglia MB, Rodríguez García V, Zapata ME, Rovirosa A, González V, Flax Marcó F, et al. (2018). Non-nutritive sweeteners: children and adolescent consumption and food sources. *Arch Argent Pediatr*. 116(3):186–91. PMID:29756702

Garnier-Sagne I, Leblanc JC, Verger P (2001). Calculation of the intake of three intense sweeteners in young insulin-dependent diabetics. *Food Chem Toxicol*. 39(7):745–9. doi:10.1016/S0278-6915(01)00003-5 PMID:11397521

Garriga MM, Berkebile C, Metcalfe DD (1991). A combined single-blind, double-blind, placebo-controlled study to determine the reproducibility of hypersensitivity reactions to aspartame. *J Allergy Clin Immunol*. 87(4):821–7. doi:10.1016/0091-6749(91)90128-B PMID:2013676

Gezginci-Oktayoglu S, Ercin M, Sancar S, Celik E, Koyuturk M, Bolkent S, et al. (2021). Aspartame induces cancer stem cell enrichment through p21, NICD and GLI1 in human PANC-1 pancreas adenocarcinoma cells. *Food Chem Toxicol*. 153:112264. doi:10.1016/j.fct.2021.112264 PMID:33992720

Giovannucci E, Harlan DM, Archer MC, Bergenstal RM, Gapstur SM, Habel LA, et al. (2010). Diabetes and cancer: a consensus report. *Diabetes Care*. 33(7):1674–85. doi:10.2337/dc10-0666 PMID:20587728

Gnudi F, Panzacchi S, Tibaldi E, Iuliani M, Sgargi D, Bua L, et al. (2023). Hemolymphoreticular neoplasias from the Ramazzini Institute long-term mice and rat studies on aspartame. *Ann Glob Health*. 89(1):43. doi:10.5334/aogh.4163 PMID:37362827

Gold EB, Gordis L, Diener MD, Seltser R, Boitnott JK, Bynum TE, et al. (1985). Diet and other risk factors for cancer of the pancreas. *Cancer*. 55(2):460–7. doi:10.1002/1097-0142(19850115)55:2<460::AID-CNCR2820550229>3.0.CO;2-V PMID:3965101

Gombos K, Varjas T, Orsós Z, Polyák E, Peredi J, Varga Z, et al. (2007). The effect of aspartame administration on oncogene and suppressor gene expressions. *In Vivo*. 21(1):89–92. PMID:17354619

Griebsch LV, Theiss EL, Janitschke D, Erhardt VKJ, Erhardt T, Haas EC, et al. (2023). Aspartame and its metabolites cause oxidative stress and mitochondrial and lipid alterations in SH-SY5Y cells. *Nutrients*. 15(6):1467. doi:10.3390/nu15061467 PMID:36986196

Gul SS, Hamilton AR, Munoz AR, Phupitakphol T, Liu W, Hyoju SK, et al. (2017). Inhibition of the gut enzyme intestinal alkaline phosphatase may explain how aspartame promotes glucose intolerance and obesity in mice. *Appl Physiol Nutr Metab*. 42(1):77–83. doi:10.1139/apnm-2016-0346 PMID:27997218

Guo W, Li J, Liu Q, Shi J, Gao Y (2021). Tracking the fate of artificial sweeteners within the coastal waters of Shenzhen city, China: from wastewater treatment plants to sea. *J Hazard Mater*. 414:125498. doi:10.1016/j.jhazmat.2021.125498 PMID:33667807

Gupta V, Cochran C, Parker TF, Long DL, Ashby J, Gorman MA, et al. (1989). Effect of aspartame on plasma amino acid profiles of diabetic patients with chronic renal failure. *Am J Clin Nutr*. 49(6):1302–6. doi:10.1093/ajcn/49.6.1302 PMID:2729170

Gurney JG, Pogoda JM, Holly EA, Hecht SS, Preston-Martin S (1997). Aspartame consumption in relation to childhood brain tumor risk: results from a case-control study. *J Natl Cancer Inst*. 89(14):1072–4. doi:10.1093/jnci/89.14.1072 PMID:9230890

Hafner E, Pravst I (2021). The sharp rise in the use of low- and no-calorie sweeteners in non-alcoholic beverages in Slovenia: an update based on 2020 data. *Front Nutr*. 8:778178. doi:10.3389/fnut.2021.778178 PMID:34869543

Hagiwara A, Fukushima S, Kitaori M, Shibata M, Ito N (1984). Effects of three sweeteners on rat urinary bladder carcinogenesis initiated by *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine. *Gan*. 75(9):763–8. PMID:6500232

Hall LN, Sanchez LR, Hubbard J, Lee H, Looby SE, Srinivasa S, et al. (2017). Aspartame intake relates to coronary plaque burden and inflammatory indices in human immunodeficiency virus. *Open Forum Infect Dis*. 4(2):ofx083. doi:10.1093/ofid/ofx083 PMID:28695142

Hall WL, Millward DJ, Rogers PJ, Morgan LM (2003). Physiological mechanisms mediating aspartame-induced satiety. *Physiol Behav*. 78(4–5):557–62. doi:10.1016/S0031-9384(03)00034-9 PMID:12782208

Hamza RZ, Al-Eisa RA, Mehana AE, El-Shenawy NS (2019). Effect of ʟ-carnitine on aspartame-induced oxidative stress, histopathological changes, and genotoxicity in liver of male rats. *J Basic Clin Physiol Pharmacol*. 30(2):219–32. doi:10.1515/jbcpp-2018-0064 PMID:30645201

Hardcastle JE, Bruch RJ (1997). Effect of L-aspartyl-L-phenylalanine methyl ester on leukotriene biosynthesis in macrophage cells. *Prostaglandins Leukot Essent Fatty Acids*. 57(3):331–3. doi:10.1016/S0952-3278(97)90552-4 PMID:9384524

Harder NHO, Hieronimus B, Stanhope KL, Shibata NM, Lee V, Nunez MV, et al. (2020). Effects of dietary glucose and fructose on copper, iron, and zinc metabolism parameters in humans. *Nutrients*. 12(9):2581. doi:10.3390/nu12092581 PMID:32854403

Hautanen V, Toimela T, Paparella M, Heinonen T (2023). A human cell-based assay to assess the induction of vasculature formation for non-genotoxic carcinogenicity testing purposes: A pilot study. *Altern Lab Anim*. 51(3):188–203. doi:10.1177/02611929231171165 PMID:37125451

Health Canada (2022). Packaging and labelling guide for cannabis products. Available from: https://www.canada.ca/en/health-canada/services/cannabis-regulations-licensed-producers/packaging-labelling-guide-cannabis-products/guide.html#shr-pg0, accessed 14 November 2022.

Heath AK, Clasen JL, Jayanth NP, Jenab M, Tjønneland A, Petersen KEN, et al. (2021). Soft drink and juice consumption and renal cell carcinoma incidence and mortality in the European prospective investigation into cancer and nutrition. *Cancer Epidemiol Biomarkers Prev*. 30(6):1270–4. doi:10.1158/1055-9965.EPI-20-1726 PMID:33849969

Hedrick VE, Passaro EM, Davy BM, You W, Zoellner JM (2017). Characterization of non-nutritive sweetener intake in rural Southwest Virginian adults living in a health-disparate region. *Nutrients*. 9(7):757. doi:10.3390/nu9070757 PMID:28708096

Hercberg S, Castetbon K, Czernichow S, Malon A, Mejean C, Kesse E, et al. (2010). The Nutrinet-Santé Study: a web-based prospective study on the relationship between nutrition and health and determinants of dietary patterns and nutritional status. *BMC Public Health*. 10(1):242. doi:10.1186/1471-2458-10-242 PMID:20459807

Hertelendy ZI, Mendenhall CL, Rouster SD, Marshall L, Weesner R (1993). Biochemical and clinical effects of aspartame in patients with chronic, stable alcoholic liver disease. *Am J Gastroenterol*. 88(5):737–43. PMID:8480740

Hess EL, Myers EA, Swithers SE, Hedrick VE (2018). Associations between nonnutritive sweetener intake and metabolic syndrome in adults. *J Am Coll Nutr*. 37(6):487–93. doi:10.1080/07315724.2018.1440658 PMID:29601264

Hieronimus B, Medici V, Bremer AA, Lee V, Nunez MV, Sigala DM, et al. (2020). Synergistic effects of fructose and glucose on lipoprotein risk factors for cardiovascular disease in young adults. *Metabolism*. 112:154356. doi:10.1016/j.metabol.2020.154356 PMID:32916151

Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, et al., editors (2023). Cochrane handbook for systematic reviews of interventions. Version 6.4 (updated August 2023). Available from: www.

training.cochrane.org/handbook, accessed 3 November 2023.

Higgins KA, Considine RV, Mattes RD (2018). Aspartame consumption for 12 weeks does not affect glycemia, appetite, or body weight of healthy, lean adults in a randomized controlled trial. *J Nutr*. 148(4):650–7. doi:10.1093/jn/nxy021 PMID:29659969

Higgins KA, Mattes RD (2019). A randomized controlled trial contrasting the effects of 4 low-calorie sweeteners and sucrose on body weight in adults with overweight or obesity. *Am J Clin Nutr*. 109(5):1288–301. doi:10.1093/ajcn/nqy381 PMID:30997499

Hjelle JJ, Dudley RE, Marietta MP, Sanders PG, Dickie BC, Brisson J, et al. (1992). Plasma concentrations and pharmacokinetics of phenylalanine in rats and mice administered aspartame. *Pharmacology*. 44(1):48–60. doi:10.1159/000138873 PMID:1553388

Hodge AM, Bassett JK, Milne RL, English DR, Giles GG (2018). Consumption of sugar-sweetened and artificially sweetened soft drinks and risk of obesity-related cancers. *Public Health Nutr*. 21(9):1618–26. doi:10.1017/S1368980017002555 PMID:29463332

Hollie PG (1983). Coke to buy new Searle sweetener. *The New York Times*. 3 August 1983. Available from: https://www.nytimes.com/1983/08/03/business/coke-to-buy-new-searle-sweetener.html, accessed 28 April 2023.

Homler BE (1984). Aspartame: implications for the food scientist. In: Stegink LD, Filer LJ Jr, editors. Aspartame: physiology and biochemistry. Boca Raton (FL), USA: CRC Press; pp. 247–62. doi:10.1201/9781003065289-14

Hooper NM, Hesp RJ, Tieku S (1994). Metabolism of aspartame by human and pig intestinal microvillar peptidases. *Biochem J*. 298(Pt 3):635–9. doi:10.1042/bj2980635 PMID:8141778

Horio Y, Sun Y, Liu C, Saito T, Kurasaki M (2014). Aspartame-induced apoptosis in PC12 cells. *Environ Toxicol Pharmacol*. 37(1):158–65. doi:10.1016/j.etap.2013.11.021 PMID:24355796

Horwitz DL, McLane M, Kobe P (1988). Response to single dose of aspartame or saccharin by NIDDM patients. *Diabetes Care*. 11(3):230–4. doi:10.2337/diacare.11.3.230 PMID:3046854

Huang S-Y, Sun R, Chen Y-C, Kang L, Wang C-T, Chiu C-F, et al. (2023a). Aspartame consumption during pregnancy impairs placenta growth in mice through sweet taste receptor-reactive oxygen species-dependent pathway. *J Nutr Biochem*. 113:109228. doi:10.1016/j.jnutbio.2022.109228 PMID:36435291

Huang Y, Chen Z, Chen B, Li J, Yuan X, Li J, et al. (2023b). Dietary sugar consumption and health: umbrella review. *BMJ*. 381:e071609. doi:10.1136/bmj-2022-071609 PMID:37019448

Hur J, Otegbeye E, Joh HK, Nimptsch K, Ng K, Ogino S, et al. (2021). Sugar-sweetened beverage intake in adulthood and adolescence and risk of early-onset colorectal cancer among women. *Gut*. 70(12):2330–6. doi:10.1136/gutjnl-2020-323450 PMID:33958435

Huttala O, Vuorenpää H, Toimela T, Uotila J, Kuokkanen H, Ylikomi T, et al. (2015). Human vascular model with defined stimulation medium - a characterization study. *Altern Anim Exp*. 32(2):125–36. doi:10.14573/altex.1411271 PMID:25742497

Huvaere K, Vandevijvere S, Hasni M, Vinkx C, Van Loco J (2012). Dietary intake of artificial sweeteners by the Belgian population. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess*. 29(1):54–65. doi:10.1080/19440049.2011.627572 PMID:22088137

Hwang LD, Lin C, Gharahkhani P, Cuellar-Partida G, Ong JS, An J, et al. (2019). New insight into human sweet taste: a genome-wide association study of the perception and intake of sweet substances. *Am J Clin Nutr*. 109(6):1724–37. doi:10.1093/ajcn/nqz043 PMID:31005972

IARC (1993). International classification of rodent tumours. Part I – the rat. 4. Haematopoietic system. *IARC Sci Publ*. 122(Pt 1–4):1–27. PMID:8270318

IARC (2012). Personal habits and indoor combustions. *IARC Monogr Eval Carcinog Risks Hum*. 100E:1–575. Available from: https://publications.iarc.who.int/122 PMID:23193840

IARC (2018a). Drinking coffee, mate, and very hot beverages. *IARC Monogr Eval Carcinog Risks Hum*. 116:1–499. Available from: https://publications.iarc.who.int/566 PMID:31310458

IARC (2018b). Red meat and processed meat. *IARC Monogr Eval Carcinog Risks Hum*. 114:1–506. Available from: https://publications.iarc.who.int/564 PMID:29949327

IARC (2018c). Absence of excess body fatness. *IARC Handb Cancer Prev*. 16:1–645. Available from: https://publications.iarc.who.int/570.

IARC (2019). Preamble to the *IARC Monographs* (amended January 2019). Lyon, France: International Agency for Research on Cancer. Available from: https://monographs.iarc.who.int/iarc-monographs-preamble-preamble-to-the-iarc-monographs/, accessed 9 October 2023.

IARC (2020). Night shift work. *IARC Monogr Identif Carcinog Hazard Hum*. 124:1–371. Available from: https://publications.iarc.who.int/593 PMID:33656825

IASR (2004). World market of sugar and sweeteners. Leinfelden-Echterdingen, Germany: International Association for Stevia Research. Available from: https://www.uni-hohenheim.de/fileadmin/einrichtungen/stevia/downloads/World_Market_Sugar.pdf.

Ibrahim RYM, Hammad HBI, Gaafar AA, Saber AA (2022). The possible role of the seaweed *Sargassum vulgare* as a promising functional food ingredient minimizing aspartame-associated toxicity in rats. *Int J Environ Health Res*. 32(4):752–71. doi:10.1080/09603123.2020.1797642 PMID:32705899

INCI Beauty (2022). Aspartame. No. CAS: 22839-47-0. Ingrédients. INCI Beauty. Available from: https://incibeauty.com/ingredients/6782-aspartame, accessed 14 November 2022. [French]

Inoue-Choi M, Robien K, Mariani A, Cerhan JR, Anderson KE (2013). Sugar-sweetened beverage intake and the risk of type I and type II endometrial cancer among postmenopausal women. *Cancer Epidemiol Biomarkers Prev.* 22(12):2384–94. doi:10.1158/1055-9965.EPI-13-0636 PMID:24273064

INRAE, ANSES (2023). OQALI. L'observatoire de l'alimentation [online database]. France: Institut national de recherche pour l'agriculture, l'alimentation et l'environnement and Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail. Available from: https://www.oqali.fr/.

Ishii H (1981). Incidence of brain tumors in rats fed aspartame. *Toxicol Lett.* 7(6):433–7. doi:10.1016/0378-4274(81)90089-8 PMID:7245229

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(2):91–4. doi:10.1016/0300-483X(81)90119-0 PMID:7281205

Ito N, Fukushima S, Shirai T, Hagiwara A, Imaida K (1984). Drugs, food additives and natural products as promoters in rat urinary bladder carcinogenesis. *IARC Sci Publ.* (56):399–407. PMID:6536604

Iyaswamy A, Kammella AK, Thavasimuthu C, Wankupar W, Dapkupar W, Shanmugam S, et al. (2018). Oxidative stress evoked damages leading to attenuated memory and inhibition of NMDAR-CaMKII-ERK/CREB signalling on consumption of aspartame in rat model. *J Food Drug Anal.* 26(2):903–16. doi:10.1016/j.jfda.2017.11.001 PMID:29567262

Iyyaswamy A, Rathinasamy S (2012). Effect of chronic exposure to aspartame on oxidative stress in the brain of albino rats. *J Biosci.* 37(4):679–88. doi:10.1007/s12038-012-9236-0 PMID:22922192

Jang HJ, Kokrashvili Z, Theodorakis MJ, Carlson OD, Kim BJ, Zhou J, et al. (2007). Gut-expressed gustducin and taste receptors regulate secretion of glucagon-like peptide-1. *Proc Natl Acad Sci USA.* 104(38):15069–74. doi:10.1073/pnas.0706890104 PMID:17724330

Janssens S, Ciapaite J, Wolters JC, van Riel NA, Nicolay K, Prompers JJ (2017). An in vivo magnetic resonance spectroscopy study of the effects of caloric and non-caloric sweeteners on liver lipid metabolism in rats. *Nutrients.* 9(5):476. doi:10.3390/nu9050476 PMID:28489050

Janvier S, Goscinny S, Le Donne C, Van Loco J (2015). Low-calorie sweeteners in food and food supplements on the Italian market. *Food Addit Contam Part B Surveill.* 8(4):298–308. doi:10.1080/19393210.2015.1094829 PMID:26406785

Jatho A, Cambia JM, Myung SK (2021). Consumption of artificially sweetened soft drinks and risk of gastro-intestinal cancer: a meta-analysis of observational studies. *Public Health Nutr.* 24(18):6122–36. doi:10.1017/S136898002100104X PMID:33691824

JECFA (1975). Evaluation of certain food additives. Some food colours, thickening agents, smoke condensates, and certain other substances. Nineteenth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series No. 576. Geneva, Switzerland: Joint FAO/WHO Expert Committee on Food Additives, World Health Organization. Available from: https://apps.who.int/iris/handle/10665/41166, accessed May 2023.

JECFA (1976). Evaluation of certain food additives. Twentieth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series No. 599. Geneva, Switzerland: Joint FAO/WHO Expert Committee on Food Additives, World Health Organization. Available from: https://apps.who.int/iris/handle/10665/41217, accessed May 2023.

JECFA (1980). Aspartame. In: Evaluation of certain food additives. Twenty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series No. 653. Geneva, Switzerland: Joint FAO/WHO Expert Committee on Food Additives, World Health Organization. Available from: https://iris.who.int/bitstream/handle/10665/41410/WHO_TRS_653.pdf?sequence=1&isAllowed=y, accessed February 2024.

JECFA (1981). Aspartame. In: Toxicological evaluation of certain food additives. WHO Food Additives Series No 16. Geneva, Switzerland: Joint FAO/WHO Expert Committee on Food Additives, World Health Organization. Available from: https://inchem.org/documents/jecfa/jecmono/v16je03.htm, accessed May 2023.

Jeffrey AM, Williams GM (2000). Lack of DNA-damaging activity of five non-nutritive sweeteners in the rat hepatocyte/DNA repair assay. *Food Chem Toxicol.* 38(4):335–8. doi:10.1016/S0278-6915(99)00163-5 PMID:10722887

Jensen U, Christiansen M (2016). [Project 2011-20-793-00320 Colourants and sweeteners in non-alcoholic flavoured beverages]. Copenhagen, Denmark. The Danish Food Agency. [Danish]

Jensen U, Christiansen M (2014). [Project 2011-20-64-00320 Colourants and sweeteners in non-alcoholic beverages, 2014]. Copenhagen, Denmark. The Danish Food Agency. [Danish]

Joh HK, Lee DH, Hur J, Nimptsch K, Chang Y, Joung H, et al. (2021). Simple sugar and sugar-sweetened beverage intake during adolescence and risk of colorectal cancer precursors. *Gastroenterology.* 161(1):128–142.e20. doi:10.1053/j.gastro.2021.03.028 PMID:33753105

Jones GS, Graubard BI, Ramirez Y, Liao LM, Huang WY, Alvarez CS, et al. (2022). Sweetened beverage consumption and risk of liver cancer by diabetes status: a pooled analysis. *Cancer Epidemiol*. 79:102201. doi:10.1016/j.canep.2022.102201 PMID:35728406

Judson RS, Magpantay FM, Chickarmane V, Haskell C, Tania N, Taylor J, et al. (2015). Integrated model of chemical perturbations of a biological pathway using 18 in vitro high-throughput screening assays for the estrogen receptor. *Toxicol Sci*. 148(1):137–54. doi:10.1093/toxsci/kfv168 PMID:26272952

Kamath S, Vijaynarayana K, Prashanth Shetty D, Shetty P (2010). Evaluation of genotoxic potential of aspartame. *Pharmacologyonline*. 1:753–69.

Kamenickova A, Pecova M, Bachleda P, Dvorak Z (2013). Effects of artificial sweeteners on the AhR- and GR-dependent CYP1A1 expression in primary human hepatocytes and human cancer cells. *Toxicol In Vitro*. 27(8):2283–8. doi:10.1016/j.tiv.2013.10.001 PMID:24120730

Karikas GA, Schulpis KH, Reclos G, Kokotos G (1998). Measurement of molecular interaction of aspartame and its metabolites with DNA. *Clin Biochem*. 31(5):405–7. doi:10.1016/S0009-9120(98)00016-2 PMID:9721441

Kashima H, Taniyama K, Sugimura K, Endo MY, Kobayashi T, Fukuba Y (2019). Suppression of sweet sensing with glucose, but not aspartame, delays gastric emptying and glycemic response. *Nutr Res*. 68:62–9. doi:10.1016/j.nutres.2019.06.005 PMID:31421394

Kawasaki H, Takagi S, Onishi Y, Urashima Y, Sekine Y, Sato M, et al. (2011). Estimation of daily intakes of food additives using the market basket method in Japan. *Jpn J Food Chem Safety*. 18(3):150–62.

Kheirdoosh F, Kashanian S, Khodaei MM, Sariaslani M, Falsafi M, Moghadam NH, et al. (2021). Spectroscopic studies on the interaction of aspartame with human serum albumin. *Nucleosides Nucleotides Nucleic Acids*. 40(3):300–16. doi:10.1080/15257770.2021.1872792 PMID:33455539

Khidr BM, El-Sokkary GH, Saleh SMM (2017). Study on morphological changes induced by aspartame on liver of normal and diabetic male albino rats. *J Histol Histopathol*. 4(1):1. doi:10.7243/2055-091X-4-1

Kim GD, Park YS, Ahn HJ, Cho JJ, Park CS (2015a). Aspartame attenuates 2, 4-dinitrofluorobenzene-induced atopic dermatitis-like clinical symptoms in NC/Nga mice. *J Invest Dermatol*. 135(11):2705–13. doi:10.1038/jid.2015.234 PMID:26099025

Kim M, Lee G, Lim HS, Yun SS, Hwang M, Hong JH, et al. (2017). Safety assessment of 16 sweeteners for the Korean population using dietary intake monitoring and poundage method. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess*. 34(9):1500–9. doi:10.1080/19440049.2017.1349344 PMID:28699833

Kim Y, Keogh JB, Clifton PM (2020). Consumption of a beverage containing aspartame and acesulfame K for two weeks does not adversely influence glucose metabolism in adult males and females: a randomized crossover study. *Int J Environ Res Public Health*. 17(23):9049. doi:10.3390/ijerph17239049 PMID:33291649

Kimura T, Kanasaki A, Hayashi N, Yamada T, Iida T, Nagata Y, et al. (2017). d-Allulose enhances postprandial fat oxidation in healthy humans. *Nutrition*. 43–44:16–20. doi:10.1016/j.nut.2017.06.007 PMID:28935140

Knopp RH, Brandt K, Arky RA (1976). Effects of aspartame in young persons during weight reduction. *J Toxicol Environ Health*. 2(2):417–28. doi:10.1080/15287397609529443 PMID:796476

Kobeissi LH, Yassine IA, Jabbour ME, Moussa MA, Dhaini HR (2013). Urinary bladder cancer risk factors: a Lebanese case–control study. *Asian Pac J Cancer Prev*. 14(5):3205–11. doi:10.7314/APJCP.2013.14.5.3205 PMID:23803105

Koch R, Schaeffler G, Shaw NF (1976). Results of loading doses of aspartame by two phenylketonuric (PKU) children compared with two normal children. *J Toxicol Environ Health*. 2(2):459–69. doi:10.1080/15287397609529447 PMID:1011297

Kokotou MG, Thomaidis NS (2013). Determination of eight artificial sweeteners in wastewater by hydrophilic interaction liquid chromatography-tandem mass spectrometry. *Anal Methods*. 5(16):3825–33. doi:10.1039/c3ay40599k

Kubica P, Namieśnik J, Wasik A (2015). Determination of eight artificial sweeteners and common *Stevia rebaudiana* glycosides in non-alcoholic and alcoholic beverages by reversed-phase liquid chromatography coupled with tandem mass spectrometry. *Anal Bioanal Chem*. 407(5):1505–12. doi:10.1007/s00216-014-8355-x PMID:25471292

Kubica P, Namieśnik J, Wasik A (2016). Comparison of hydrophilic interaction and reversed phase liquid chromatography coupled with tandem mass spectrometry for the determination of eight artificial sweeteners and common steviol glycosides in popular beverages. *J Pharm Biomed Anal*. 127:184–92. doi:10.1016/j.jpba.2016.01.006 PMID:26782293

Kuk JL, Brown RE (2016). Aspartame intake is associated with greater glucose intolerance in individuals with obesity. *Appl Physiol Nutr Metab*. 41(7):795–8. doi:10.1139/apnm-2015-0675 PMID:27216413

LaBuda CJ, Fuchs PN (2001). A comparison of chronic aspartame exposure to aspirin on inflammation, hyperalgesia and open field activity following carrageenan-induced monoarthritis. *Life Sci*. 69(4):443–54. doi:10.1016/S0024-3205(01)01136-5 PMID:11459435

Lachenmeier DW, Ganss S, Rychlak B, Rehm J, Sulkowska U, Skiba M, et al. (2009). Association between quality of cheap and unrecorded alcohol products and public health consequences in Poland. *Alcohol Clin Exp Res*.

33(10):1757–69. doi:10.1111/j.1530-0277.2009.01013.x PMID:19572980

Laguna JC, Alegret M, Cofán M, Sánchez-Tainta A, Díaz-López A, Martínez-González MA, et al. (2021). Simple sugar intake and cancer incidence, cancer mortality and all-cause mortality: A cohort study from the PREDIMED trial. *Clin Nutr.* 40(10):5269–77. doi:10.1016/j.clnu.2021.07.031 PMID:34536637

Lambert N, Rostock C, Hetland S (2010). Environmental risks associated to plasticizers and sweeteners in the Nordic countries. Project carried out for the Nordic Screening Group. Oslo, December 2010. Oslo, Norway: Bergfald Miljørådgiver. Available from: https://www.miljodirektoratet.no/globalassets/publikasjoner/klif/publikasjoner/2801/ta2801.pdf, accessed May 2023.

Larsson SC, Giovannucci EL, Wolk A (2016). Sweetened beverage consumption and risk of biliary tract and gallbladder cancer in a prospective study. *J Natl Cancer Inst.* 108(10):djw125. doi:10.1093/jnci/djw125 PMID:27281756

Le Donne C, Mistura L, Goscinny S, Janvier S, Cuypers K, D'Addezio L, et al. (2017). Assessment of dietary intake of 10 intense sweeteners by the Italian population. *Food Chem Toxicol.* 102:186–97. doi:10.1016/j.fct.2017.02.014 PMID:28216168

Lebda MA, Sadek KM, El-Sayed YS (2017a). Aspartame and soft drink-mediated neurotoxicity in rats: implication of oxidative stress, apoptotic signalling pathways, electrolytes and hormonal levels. *Metab Brain Dis.* 32(5):1639–47. doi:10.1007/s11011-017-0052-y PMID:28660358

Lebda MA, Tohamy HG, El-Sayed YS (2017b). Long-term soft drink and aspartame intake induces hepatic damage via dysregulation of adipocytokines and alteration of the lipid profile and antioxidant status. *Nutr Res.* 41:47–55. doi:10.1016/j.nutres.2017.04.002 PMID:28465000

Leclercq C, Berardi D, Sorbillo MR, Lambe J (1999). Intake of saccharin, aspartame, acesulfame K and cyclamate in Italian teenagers: present levels and projections. *Food Addit Contam.* 16(3):99–109. doi:10.1080/026520399284145 PMID:10492702

Lee SL, Shih HT, Chi YC, Li YP, Yin SJ (2011). Oxidation of methanol, ethylene glycol, and isopropanol with human alcohol dehydrogenases and the inhibition by ethanol and 4-methylpyrazole. *Chem Biol Interact.* 191(1–3):26–31. doi:10.1016/j.cbi.2010.12.005 PMID:21167143

Lee TD (2000). Sweeteners. First published 26 January 2001. Kirk–Othmer encyclopedia of chemical technology. New York (NY), USA: John Wiley & Sons. doi:10.1002/0471238961.19230505120505.a01

Lee TD (2006). Sweeteners. First published 24 March 2006. Kirk–Othmer encyclopedia of chemical technology. New York (NY), USA: John Wiley & Sons. doi:10.1002/0471238961.19230505120505.a01.pub2

Lee Y, Do B, Lee G, Lim HS, Yun SS, Kwon H (2017). Simultaneous determination of sodium saccharin, aspartame, acesulfame-K and sucralose in food consumed in Korea using high-performance liquid chromatography and evaporative light-scattering detection. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 34(5):666–77. doi:10.1080/19440049.2017.1284348 PMID:28277180

Leon AS, Hunninghake DB, Bell C, Rassin DK, Tephly TR (1989). Safety of long-term large doses of aspartame. *Arch Intern Med.* 149(10):2318–24. doi:10.1001/archinte.1989.00390100120026 PMID:2802896

Leth T, Jensen U, Fagt S, Andersen R (2008). Estimated intake of intense sweeteners from non-alcoholic beverages in Denmark, 2005. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 25(6):662–8. doi:10.1080/02652030701765749 PMID:18484294

Lewis KA, Tzilivakis J (2021). Review and synthesis of data on the potential environmental impact of artificial sweeteners. *EFSA Supporting Publications.* 18(10):1. doi:10.2903/sp.efsa.2021.EN-6918

Li D, O'Brien JW, Tscharke BJ, Choi PM, Zheng Q, Ahmed F, et al. (2020). National wastewater reconnaissance of artificial sweetener consumption and emission in Australia. *Environ Int.* 143:105963. doi:10.1016/j.envint.2020.105963 PMID:32688159

Li D, O'Brien JW, Tscharke BJ, Okoffo ED, Mueller JF, Sun H, et al. (2021b). Artificial sweeteners in end-use biosolids in Australia. *Water Res.* 200:117237. doi:10.1016/j.watres.2021.117237 PMID:34051459

Li D, Zheng Q, Thomas KV, Dang AK, Binh VN, Anh NTK, et al. (2023). Use of artificial sweeteners and caffeine in a population of Hanoi: an assessment by wastewater-based epidemiology. *Sci Total Environ.* 868:161515. doi:10.1016/j.scitotenv.2023.161515 PMID:36634775

Li X, Li S, Li H, Wang J, Luo Q, Yin X (2021a). Quantification of artificial sweeteners in alcoholic drinks using direct analysis in real-time QTRAP mass spectrometry. *Food Chem.* 342:128331. doi:10.1016/j.foodchem.2020.128331 PMID:33097326

Lim U, Subar AF, Mouw T, Hartge P, Morton LM, Stolzenberg-Solomon R, et al. (2006). Consumption of aspartame-containing beverages and incidence of hematopoietic and brain malignancies. *Cancer Epidemiol Biomarkers Prev.* 15(9):1654–9. doi:10.1158/1055-9965.EPI-06-0203 PMID:16985027

Lin SY, Cheng YD (2000). Simultaneous formation and detection of the reaction product of solid-state aspartame sweetener by FT-IR/DSC microscopic system. *Food Addit Contam.* 17(10):821–7. doi:10.1080/026520300420385 PMID:11103265

Lino CM, Costa IM, Pena A, Ferreira R, Cardoso SM (2008). Estimated intake of the sweeteners, acesulfame-K and aspartame, from soft drinks, soft drinks based on mineral waters and nectars for a group of Portuguese teenage students. *Food Addit Contam Part*

A Chem Anal Control Expo Risk Assess. 25(11):1291–6. doi:10.1080/02652030802195309 PMID:19680835

Lipton WE, Li YN, Younoszai MK, Stegink LD (1991). Intestinal absorption of aspartame decomposition products in adult rats. *Metabolism.* 40(12):1337–45. doi:10.1016/0026-0495(91)90040-4 PMID:1961131

Liu D, Li ZH, Shen D, Zhang PD, Song WQ, Zhang WT, et al. (2022b). Association of sugar-sweetened, artificially sweetened, and unsweetened coffee consumption with all-cause and cause-specific mortality: a large prospective cohort study. *Ann Intern Med.* 175(7):909–17. doi:10.7326/M21-2977 PMID:35635846

Liu Y, Li X, Wu Y, Su Q, Qin L, Ma J (2022a). The associations between maternal serum aspartame and sucralose and metabolic health during pregnancy. *Nutrients.* 14(23):5001. doi:10.3390/nu14235001 PMID:36501030

Llaha F, Gil-Lespinard M, Unal P, de Villasante I, Castañeda J, Zamora-Ros R (2021). Consumption of sweet beverages and cancer risk. a systematic review and meta-analysis of observational studies. *Nutrients.* 13(2):516. doi:10.3390/nu13020516 PMID:33557387

Lo J, Abbara S, Shturman L, Soni A, Wei J, Rocha-Filho JA, et al. (2010). Increased prevalence of subclinical coronary atherosclerosis detected by coronary computed tomography angiography in HIV-infected men. *AIDS.* 24(2):243–53. doi:10.1097/QAD.0b013e328333ea9e PMID:19996940

Logue C, Dowey LRC, Strain JJ, Verhagen H, McClean S, Gallagher AM (2017). Application of liquid chromatography-tandem mass spectrometry to determine urinary concentrations of five commonly used low-calorie sweeteners: a novel biomarker approach for assessing recent intakes? *J Agric Food Chem.* 65(22):4516–25. doi:10.1021/acs.jafc.7b00404 PMID:28506059

Ma XY, Hu H, Lu SJ, Li QS, Xue LF, Li XC, et al. (2020). [Distribution of four artificial sweeteners in water treatment and water supply system in City H of Zhejiang Province]. *Huan Jing Ke Xue.* 41(4):1629–35. doi:10.13227/j.hjkx.201910084 PMID:32608668 [Chinese]

Mackenzie T, Brooks B, O'Connor G (2006). Beverage intake, diabetes, and glucose control of adults in America. *Ann Epidemiol.* 16(9):688–91. doi:10.1016/j.annepidem.2005.11.009 PMID:16458538

Maersk M, Belza A, Stødkilde-Jørgensen H, Ringgaard S, Chabanova E, Thomsen H, et al. (2012). Sucrose-sweetened beverages increase fat storage in the liver, muscle, and visceral fat depot: a 6-mo randomized intervention study. *Am J Clin Nutr.* 95(2):283–9. doi:10.3945/ajcn.111.022533 PMID:22205311

Maes P, Monakhova YB, Kuballa T, Reusch H, Lachenmeier DW (2012). Qualitative and quantitative control of carbonated cola beverages using ¹H NMR spectroscopy. *J Agric Food Chem.* 60(11):2778–84. doi:10.1021/jf204777m PMID:22356160

Maghiari AL, Coricovac D, Pinzaru IA, Macaşoi IG, Marcovici I, Simu S, et al. (2020). High concentrations of aspartame induce pro-angiogenic effects in ovo and cytotoxic effects in HT-29 human colorectal carcinoma cells. *Nutrients.* 12(12):3600. doi:10.3390/nu12123600 PMID:33255204

Magnuson BA, Burdock GA, Doull J, Kroes RM, Marsh GM, Pariza MW, et al. (2007). Aspartame: a safety evaluation based on current use levels, regulations, and toxicological and epidemiological studies. *Crit Rev Toxicol.* 37(8):629–727. doi:10.1080/10408440701516184 PMID:17828671

Mahfouz EM, Sadek RR, Abdel-Latief WM, Mosallem FA, Hassan EE (2014). The role of dietary and lifestyle factors in the development of colorectal cancer: case–control study in Minia, Egypt. *Cent Eur J Public Health.* 22(4):215–22. doi:10.21101/cejph.a3919 PMID:25622477

Malaisse WJ, Vanonderbergen A, Louchami K, Jijakli H, Malaisse-Lagae F (1998). Effects of artificial sweeteners on insulin release and cationic fluxes in rat pancreatic islets. *Cell Signal.* 10(10):727–33. doi:10.1016/S0898-6568(98)00017-5 PMID:9884024

Malarkey DE, Bucher JR (2011). Summary report of the National Toxicology Program and Environmental Protection Agency-sponsored review of pathology materials from selected Ramazzini Institute rodent cancer bioassays. 29 November 2011. Research Triangle Park (NC): USA: National Toxicology Program.

Malik VS, Li Y, Pan A, De Koning L, Schernhammer E, Willett WC, et al. (2019). Long-term consumption of sugar-sweetened and artificially sweetened beverages and risk of mortality in US adults. *Circulation.* 139(18):2113–25. doi:10.1161/CIRCULATIONAHA.118.037401 PMID:30882235

Mancini FR, Paul D, Gauvreau J, Volatier JL, Vin K, Hulin M (2015). Dietary exposure to benzoates (E210–E213), parabens (E214–E219), nitrites (E249–E250), nitrates (E251–E252), BHA (E320), BHT (E321) and aspartame (E951) in children less than 3 years old in France. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 32(3):293–306. doi:10.1080/19440049.2015.1007535 PMID:25686474

Markus V, Share O, Shagan M, Halpern B, Bar T, Kramarsky-Winter E, et al. (2021). Inhibitory effects of artificial sweeteners on bacterial quorum sensing. *Int J Mol Sci.* 22(18):9863. doi:10.3390/ijms22189863 PMID:34576027

Martínez X, Zapata Y, Pinto V, Cornejo C, Elbers M, Graaf MV, et al. (2020). Intake of non-nutritive sweeteners in Chilean children after enforcement of a new food labeling law that regulates added sugar content in processed foods. *Nutrients.* 12(6):12. doi:10.3390/nu12061594 PMID:32485840

Martyn DM, Nugent AP, McNulty BA, O'Reilly E, Tlustos C, Walton J, et al. (2016). Dietary intake of four artificial sweeteners by Irish pre-school children. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess*. 33(4):592–602. doi:10.1080/19440049.2016.1152880 PMID:26939625

Mateo-Fernández M, González-Jiménez MJ, Celestino MDR, Font R, Alonso-Moraga Á, Merinas-Amo T (2022). Toxicological and nutraceutical screening assays of some artificial sweeteners. *Processes (Basel)*. 10(2):410. doi:10.3390/pr10020410

Matsuzawa Y, O'Hara Y (1984). Tissue distribution of orally administered isotopically labeled aspartame in the rat. Chapter 8. In: Stegink F, Filer LJ Jr, editors. Aspartame: physiology and biochemistry. 1st ed. Boca Raton (FL), USA: CRC Press; pp. 161–200.

Mayne ST, Risch HA, Dubrow R, Chow WH, Gammon MD, Vaughan TL, et al. (2006). Carbonated soft drink consumption and risk of esophageal adenocarcinoma. *J Natl Cancer Inst*. 98(1):72–5. doi:10.1093/jnci/djj007 PMID:16391374

Mazur RH, Ripper A (1979). Peptide-based sweeteners. In: Hough CAM, Parker KJ, Vlitos AJ, editors. Developments in sweeteners – 1. London, UK: Elsevier Applied Science; pp. 125–34.

Mazur RH, Schlatter JM, Goldkamp AH (1969). Structure–taste relationships of some dipeptides. *J Am Chem Soc*. 91(10):2684–91. doi:10.1021/ja01038a046 PMID:5784944

Mbambo NP, Dlamini SN, Chukwuma CI, Islam MS, Xu Y, Li X, et al. (2020). Comparative effects of commonly used commercially available non-nutritive sweeteners on diabetes-related parameters in non-diabetic rats. *J Food Biochem*. 44(11):e13453. doi:10.1111/jfbc.13453 PMID:32869881

McCullough ML, Hodge RA, Campbell PT, Guinter MA, Patel AV (2022). Sugar- and artificially-sweetened beverages and cancer mortality in a large US prospective cohort. *Cancer Epidemiol Biomarkers Prev*. 31(10):1907–18. doi:10.1158/1055-9965.EPI-22-0392 PMID:36107009

McCullough ML, Teras LR, Shah R, Diver WR, Gaudet MM, Gapstur SM (2014). Artificially and sugar-sweetened carbonated beverage consumption is not associated with risk of lymphoid neoplasms in older men and women. *J Nutr*. 144(12):2041–9. doi:10.3945/jn.114.197475 PMID:25342696

Medrano LC, Flores-Aguilar JF, Islas G, Rodríguez JA, Ibarra IS (2019). Solid-phase extraction and large-volume sample stacking-capillary electrophoresis for determination of artificial sweeteners in water samples. *Food Anal Methods*. 12(2):526–33. doi:10.1007/s12161-018-1383-y

Meier I, Shephard SE, Lutz WK (1990). Nitrosation of aspartic acid, aspartame, and glycine ethylester. Alkylation of 4-(*p*-nitrobenzyl)pyridine (NBP) in vitro and binding to DNA in the rat. *Mutat Res*. 238(3):193–201. doi:10.1016/0165-1110(90)90011-Y PMID:2342512

Mendoza-Pérez S, Guzmán-Gómez MB, García-Gómez RS, Ordaz-Nava G, Gracia-Mora MI, Macías-Rosales L, et al. (2021). Effects on weaned male Wistar rats after 104, 197, and 288 days of chronic consumption of nutritive and non-nutritive additives in water. *J Food Sci Technol*. 58(6):2349–59. doi:10.1007/s13197-020-04746-2 PMID:33967331

Miao S, Beach ES, Sommer TJ, Zimmerman JB, Jordt SE (2016). High-intensity sweeteners in alternative tobacco products. *Nicotine Tob Res*. 18(11):2169–73. doi:10.1093/ntr/ntw141 PMID:27217475

Mintel Group Ltd (2023). GNPD. Mintel Global New Products Database [online database]. London, UK: Mintel Group Ltd. Available from: https://www.mintel.com/products/gnpd/.

Møller SE (1991). Effect of aspartame and protein, administered in phenylalanine-equivalent doses, on plasma neutral amino acids, aspartate, insulin and glucose in man. *Pharmacol Toxicol*. 68(5):408–12. doi:10.1111/j.1600-0773.1991.tb01262.x PMID:1946186

Momas I, Daures JP, Festy B, Bontoux J, Gremy F (1994). Bladder cancer and black tobacco cigarette smoking. Some results from a French case–control study. *Eur J Epidemiol*. 10(5):599–604. doi:10.1007/BF01719579 PMID:7859861

Monastyrskaia K, Lundstrom K, Plahl D, Acuna G, Schweitzer C, Malherbe P, et al. (1999). Effect of the umami peptides on the ligand binding and function of rat mGlu4a receptor might implicate this receptor in the monosodium glutamate taste transduction. *Br J Pharmacol*. 128(5):1027–34. doi:10.1038/sj.bjp.0702885 PMID:10556940

Morales-Ríos EI, García-Machorro J, Briones-Aranda A, Gómez-Pliego R, Espinosa-Raya J (2022). Effect of long-term intake of nutritive and non-nutritive sweeteners on metabolic health and cognition in adult male rats. *J Med Food*. 25(11):1059–65. doi:10.1089/jmf.2022.0016 PMID:35951019

Morini G, Bassoli A, Temussi PA (2005). From small sweeteners to sweet proteins: anatomy of the binding sites of the human T1R2_T1R3 receptor. *J Med Chem*. 48(17):5520–9. doi:10.1021/jm0503345 PMID:16107151

Mullee A, Romaguera D, Pearson-Stuttard J, Viallon V, Stepien M, Freisling H, et al. (2019). Association between soft drink consumption and mortality in 10 European countries. *JAMA Intern Med*. 179(11):1479–90. doi:10.1001/jamainternmed.2019.2478 PMID:31479109

Myers EA, Passaro EM, Hedrick VE (2018). The comparative reproducibility and validity of a non-nutritive sweetener food frequency questionnaire. *Nutrients*. 10(3):10. doi:10.3390/nu10030334 PMID:29534454

Najam K, Altaf I, Ashraf M, Rasheed MA, Saleem F, Munir N, et al. (2017). In vitro evaluation of mutagenicity and genotoxicity of sitagliptin alone and in

combination with artificial sweeteners. *Trop J Pharm Res*. 16(8):1841–7. doi:10.4314/tjpr.v16i8.13

Nakagawa Y, Nagasawa M, Yamada S, Hara A, Mogami H, Nikolaev VO, et al. (2009). Sweet taste receptor expressed in pancreatic beta-cells activates the calcium and cyclic AMP signaling systems and stimulates insulin secretion. *PLoS One*. 4(4):e5106. doi:10.1371/journal.pone.0005106 PMID:19352508

Nakano Y, Maeda A, Uchida S, Namiki N (2013). Preparation and evaluation of unpleasant taste-masked pioglitazone orally disintegrating tablets. *Int J Pharm*. 446(1–2):160–5. doi:10.1016/j.ijpharm.2013.02.019 PMID:23419665

Navarrete-Muñoz EM, Wark PA, Romaguera D, Bhoo-Pathy N, Michaud D, Molina-Montes E, et al. (2016). Sweet-beverage consumption and risk of pancreatic cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Am J Clin Nutr*. 104(3):760–8. doi:10.3945/ajcn.116.130963 PMID:27510540

NCBI (2022). Aspartame. Compound summary for CID 134601. PubChem [online database]. Bethesda (MD), USA: National Center for Biotechnology Information, United States National Library of Medicine. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/aspartame, accessed May 2023.

NCBI (2023). PubMed [online database]. Bethesda (MD), USA: National Library of Medicine. Available from: https://pubmed.ncbi.nlm.nih.gov/

Nehrling JK, Kobe P, McLane MP, Olson RE, Kamath S, Horwitz DL (1985). Aspartame use by persons with diabetes. *Diabetes Care*. 8(5):415–7. doi:10.2337/diacare.8.5.415 PMID:3902420

Neshat S, Rezaei A, Farid A, Sarallah R, Javanshir S, Ahmadian S, et al. (2022). The tangled web of dyslipidemia and cancer: Is there any association? *J Res Med Sci*. 27(1):93. doi:10.4103/jrms.jrms_267_22 PMID:36685020

Nettleton JE, Cho NA, Klancic T, Nicolucci AC, Shearer J, Borgland SL, et al. (2020). Maternal low-dose aspartame and stevia consumption with an obesogenic diet alters metabolism, gut microbiota and mesolimbic reward system in rat dams and their offspring. *Gut*. 69(10):1807–17. doi:10.1136/gutjnl-2018-317505 PMID:31996393

Nguyen LH, Ma W, Wang DD, Cao Y, Mallick H, Gerbaba TK, et al. (2020). Association between sulfur-metabolizing bacterial communities in stool and risk of distal colorectal cancer in men. *Gastroenterology*. 158(5):1313–25. doi:10.1053/j.gastro.2019.12.029 PMID:31972239

Nguyen UN, Dumoulin G, Henriet MT, Regnard J (1998). Aspartame ingestion increases urinary calcium, but not oxalate excretion, in healthy subjects. *J Clin Endocrinol Metab*. 83(1):165–8. doi:10.1210/jcem.83.1.4511 PMID:9435435

Nielsen S, Zederkopff Ballin N (2009). [Monitoring and control of additives: intense sweeteners, colours and preservatives in non-alcoholic beverages (Part III).] Project No. 2007-20-64-00728. Food region east. Copenhagen, Denmark: The Danish Food Agency. [Danish]

NIOSH (1992). Health hazard evaluation report. HETA-86-035-2224. Nabisco Brands, Inc., Seville, Ohio. Cincinnati (OH), USA: United States Department of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health; pp. 1–57. Available from: https://www.cdc.gov/niosh/hhe/reports/pdfs/1986-0035-2224.pdf, accessed June 2023.

NIOSH (1994). Aspartame. Method 5031. NIOSH manual of analytical methods, 4th ed. 15 August 1994. Atlanta (GA), USA: National Institute for Occupational Safety and Health. Available from: https://www.cdc.gov/niosh/docs/2003-154/pdfs/5031.pdf, accessed February 2024.

Nomura AM, Kolonel LN, Hankin JH, Yoshizawa CN (1991). Dietary factors in cancer of the lower urinary tract. *Int J Cancer*. 48(2):199–205. doi:10.1002/ijc.2910480208 PMID:2019466

Norell SE, Ahlbom A, Erwald R, Jacobson G, Lindberg-Navier I, Olin R, et al. (1986). Diet and pancreatic cancer: a case–control study. *Am J Epidemiol*. 124(6):894–902. doi:10.1093/oxfordjournals.aje.a114479 PMID:3776972

Nosti-Palacios R, Gómez-Garduño J, Molina-Ortiz D, Calzada-León R, Dorado-González VM, Vences-Mejía A (2014). Aspartame administration and insulin treatment altered brain levels of CYP2E1 and CYP3A2 in streptozotocin-induced diabetic rats. *Int J Toxicol*. 33(4):325–31. doi:10.1177/1091581814540480 PMID:25038063

NTP (2005). NTP report on the toxicology studies of aspartame (CAS No. 22839-47-0) in genetically modified (FVB Tg.AC hemizygous) and B6.129-*Cdkn2a*tm1Rdp (N2) deficient mice and carcinogenicity studies of aspartame in genetically modified [B6.129-*Trp53*tm1Brd (N5) haploinsufficient] mice (feed studies). *Natl Toxicol Program Genet Modif Model Rep*. 2005(1):1–222. PMID:18685711

Nunn R, Young L, Ni Mhurchu C (2021). Prevalence and types of non-nutritive sweeteners in the New Zealand food supply, 2013 and 2019. *Nutrients*. 13(9):3228. doi:10.3390/nu13093228 PMID:34579101

Nutrition Coordinating Center (2023). Nutrition Data System for Research software. USA: Nutrition Coordinating Center, University of Minnesota. Available from: http://www.ncc.umn.edu/.

OECD (2018). Test No. 451. Carcinogenicity studies. OECD guidelines for the testing of chemicals. Paris, France: Organisation for Economic Co-operation and Development. Available from: https://www.

oecd.org/env/test-no-451-carcinogenicity-studies-9789264071186-en.htm

Office of the Federal Register (1984). Food additives permitted for direct addition to food for human consumption; aspartame. 21 CR Part 172. Docket Nos. 75F-0355 and 82F-0305. Rules and Regulations. United States Food and Drug Administration. Federal Register Vol. 49, No. 36. Available from: https://www.govinfo.gov/content/pkg/FR-1984-02-22/pdf/FR-1984-02-22.pdf.

Office of the Federal Register (1981). Aspartame; commissioner's final decision. Docket No. 75F-0355. Part IV. Public Health Service, Food and Drug Administration, Department of Health and Human Services. Federal Register Vol. 46, No. 142, 24 July 1981. Available from: https://www.fda.gov/media/89219/download.

Office of the Federal Register (1996). Food additives permitted for direct addition to food for human consumption; aspartame. 21 CFR Part 172. Docket No. 94F-0405. Part IV. Department of Health and Human Services, Food and Drug Administration. Federal Register Vol. 61, No. 126, p. 33652, 28 June 1996. Available from: http://www.gpo.gov/fdsys/pkg/FR-1996-06-28/pdf/96-16522.pdf, accessed May 2023.

Oktavirina V, Prabawati NB, Fathimah RN, Palma M, Kurnia KA, Darmawan N, et al. (2021). Analytical methods for determination of non-nutritive sweeteners in foodstuffs. *Molecules*. 26(11):3135. doi:10.3390/molecules26113135 PMID:34073913

Okuno G, Kawakami F, Tako H, Kashihara T, Shibamoto S, Yamazaki T, et al. (1986). Glucose tolerance, blood lipid, insulin and glucagon concentration after single or continuous administration of aspartame in diabetics. *Diabetes Res Clin Pract*. 2(1):23–7. doi:10.1016/S0168-8227(86)80025-0 PMID:3522147

Onaolapo AY, Abdusalam SZ, Onaolapo OJ (2017a). Silymarin attenuates aspartame-induced variation in mouse behaviour, cerebrocortical morphology and oxidative stress markers. *Pathophysiology*. 24(2):51–62. doi:10.1016/j.pathophys.2017.01.002 PMID:28254270

Onaolapo AY, Onaolapo OJ, Nwoha PU (2016). Alterations in behaviour, cerebral cortical morphology and cerebral oxidative stress markers following aspartame ingestion. *J Chem Neuroanat*. 78:42–56. doi:10.1016/j.jchemneu.2016.08.006 PMID:27565676

Onaolapo AY, Onaolapo OJ, Nwoha PU (2017b). Methyl aspartylphenylalanine, the pons and cerebellum in mice: an evaluation of motor, morphological, biochemical, immunohistochemical and apoptotic effects. *J Chem Neuroanat*. 86:67–77. doi:10.1016/j.jchemneu.2017.09.001 PMID:28890110

Onaolapo AY, Onaolapo OJ, Nwoha PU (2017c). Aspartame and the hippocampus: revealing a bi-directional, dose/time-dependent behavioural and morphological shift in mice. *Neurobiol Learn Mem*. 139:76–88. doi:10.1016/j.nlm.2016.12.021 PMID:28049023

Open Food Facts (2023). Open Food Facts [online database]. Saint-Maur-des-Fossés, France: Open Food Facts. Available from: https://world.openfoodfacts.org/.

Oppermann JA (1984). Metabolism in animals. Chapter 7. In: Stegink F, Filer LJ Jr, editors. Aspartame: physiology and biochemistry. 1st ed. Boca Raton (FL), USA: CRC Press; pp. 141–160.

Oppermann JA, Muldoon E, Ranney RE (1973a). Metabolism of aspartame in monkeys. *J Nutr*. 103(10): 1454–9. doi:10.1093/jn/103.10.1454 PMID:4200872

Oppermann JA, Muldoon E, Ranney RE (1973b). Effect of aspartame on phenylalanine metabolism in the monkey. *J Nutr*. 103(10):1460–6. doi:10.1093/jn/103.10.1460 PMID:4200873

Otabe A, Ohta F, Takumi A, Lynch B (2019). Mutagenicity and genotoxicity studies of aspartame. *Regul Toxicol Pharmacol*. 103:345–51. doi:10.1016/j.yrtph.2018.01.023 PMID:29408486

Palmnäs MS, Cowan TE, Bomhof MR, Su J, Reimer RA, Vogel HJ, et al. (2014). Low-dose aspartame consumption differentially affects gut microbiota-host metabolic interactions in the diet-induced obese rat. *PLoS One*. 9(10):e109841. doi:10.1371/journal.pone.0109841 PMID:25313461

Palomar-Cros A, Straif K, Romaguera D, Aragones N, Castano-Vinyals G, Martin V, et al. (2023). Consumption of aspartame and other artificial sweeteners and risk of cancer in the Spanish Multicase–Control (MCC-Spain) study. *Int J Cancer*. 153(5):979–993. doi:10.1002/ijc.34577

Pan B, Ge L, Lai H, Wang Q, Wang Q, Zhang Q, et al. (2022). Association of soft drink and 100% fruit juice consumption with all-cause mortality, cardiovascular diseases mortality, and cancer mortality: a systematic review and dose-response meta-analysis of prospective cohort studies. *Crit Rev Food Sci Nutr*. 62(32):8908–19. doi:10.1080/10408398.2021.1937040 PMID:34121531

Pan B, Lai H, Ma N, Li D, Deng X, Wang X, et al. (2023). Association of soft drinks and 100% fruit juice consumption with risk of cancer: a systematic review and dose-response meta-analysis of prospective cohort studies. *Int J Behav Nutr Phys Act*. 20(1):58. doi:10.1186/s12966-023-01459-5 PMID:37189146

Pan-Hou H, Suda Y, Ohe Y, Sumi M, Yoshioka M (1990). Effect of aspartame on *N*-methyl-D-aspartate-sensitive L-[³H]glutamate binding sites in rat brain synaptic membranes. *Brain Res*. 520(1–2):351–3. doi:10.1016/0006-8993(90)91729-Z PMID:1976410

Pandurangan M, Enkhtaivan G, Kim DH (2016a). Cytotoxic effects of aspartame on human cervical carcinoma cells. *Toxicol Res (Camb)*. 5(1):45–52. doi:10.1039/C5TX00269A PMID:30090325

Pandurangan M, Enkhtaivan G, Mistry B, Chandrasekaran M, Noorzai R, Kim DH (2016b). Investigation of role of aspartame on apoptosis process in HeLa cells. *Saudi*

J Biol Sci. 23(4):503–6. doi:10.1016/j.sjbs.2015.06.001 PMID:27298583

Pandurangan M, Enkhtaivan G, Veerappan M, Mistry B, Patel R, Moon SH, et al. (2017). Renal-protective and ameliorating impacts of omega-3 fatty acids against aspartame damaged MDCK cells. *Biofactors*. 43(6):847–57. doi:10.1002/biof.1387 PMID:28881099

Pandurangan M, Park J, Kim E (2014). Aspartame down-regulates 3T3–L1 differentiation. *In Vitro Cell Dev Biol Anim*. 50(9):851–7. doi:10.1007/s11626-014-9789-3 PMID:24961835

Pin JP, Galvez T, Prézeau L (2003). Evolution, structure, and activation mechanism of family 3/C G-protein-coupled receptors. *Pharmacol Ther*. 98(3):325–54. doi:10.1016/S0163-7258(03)00038-X PMID:12782243

Prankerd RJ (2002). Aspartame. In: Brittain HG, editor. Analytical profiles of drug substances and excipients. 29:7–55. doi:10.1016/S1075-6280(02)29003-5

Prejean JD, Peckham JC, Casey AE, Griswold DP, Weisburger EK, Weisburger JH (1973). Spontaneous tumors in Sprague-Dawley rats and Swiss mice. *Cancer Res*. 33(11):2768–73. PMID:4748432

Prokić MD, Paunovic MG, Matic MM, Djordjevic NZ, Ognjanovic BI, Stajn AS, et al. (2014). Prooxidative effects of aspartame on antioxidant defense status in erythrocytes of rats. *J Biosci*. 39(5):859–66. doi:10.1007/s12038-014-9487-z PMID:25431414

Prokić MD, Paunović MG, Matić MM, Djordjević NZ, Ognjanović BI, Štajn AŠ, et al. (2015). Effect of aspartame on biochemical and oxidative stress parameters in rat blood. *Arch Biol Sci*. 67(2):535–45. doi:10.2298/ABS141009016P

Puthrasingam S, Heybroek WM, Johnston A, Maskrey V, Swift CG, Turner P, et al. (1996). Aspartame pharmacokinetics - the effect of ageing. *Age Ageing*. 25(3):217–20. doi:10.1093/ageing/25.3.217 PMID:8670556

Qu D, Jiang M, Huang D, Zhang H, Feng L, Chen Y, et al. (2019). Synergistic effects of the enhancements to mitochondrial ROS, p53 activation and apoptosis generated by aspartame and potassium sorbate in HepG2 cells. *Molecules*. 24(3):457. doi:10.3390/molecules24030457 PMID:30696035

Ragi ME, El-Haber R, El-Masri F, Obeid OA (2021). The effect of aspartame and sucralose intake on body weight measures and blood metabolites: role of their form (solid and/or liquid) of ingestion. *Br J Nutr*. 128(2):352–360. doi:10.1017/s0007114521003238 PMID:34420538

Rahiman F, Pool EJ (2014). The in vitro effects of artificial and natural sweeteners on the immune system using whole blood culture assays. *J Immunoassay Immunochem*. 35(1):26–36. doi:10.1080/15321819.2013.784197 PMID:24063614

Ramne S, Brunkwall L, Ericson U, Gray N, Kuhnle GGC, Nilsson PM, et al. (2021). Gut microbiota composition in relation to intake of added sugar, sugar-sweetened beverages and artificially sweetened beverages in the Malmö Offspring Study. *Eur J Nutr*. 60(4):2087–97. doi:10.1007/s00394-020-02392-0 PMID:33030577

Ranney RE, Oppermann JA, Muldoon E, McMahon FG (1976). Comparative metabolism of aspartame in experimental animals and humans. *J Toxicol Environ Health*. 2(2):441–51. doi:10.1080/15287397609529445 PMID:827618

Rebolledo N, Reyes M, Popkin BM, Adair L, Avery CL, Corvalán C, et al. (2022). Changes in nonnutritive sweetener intake in a cohort of preschoolers after the implementation of Chile's Law of Food Labelling and Advertising. *Pediatr Obes*. 17(7):e12895. doi:10.1111/ijpo.12895 PMID:35088571

Reilly MA, Lajtha A (1995). Aspartame does not affect aminergic and glutamatergic receptor kinetics in rat brain. *Ann N Y Acad Sci*. 757(1):311–3. doi:10.1111/j.1749-6632.1995.tb17488.x PMID:7611687

Reisch MS (2014). Artificial sweetener producers hurt by Chinese competition. *Chem Eng News*. 92(40):6. Available from: https://cen.acs.org/articles/92/i40/Artificial-Sweetener-Producers-Hurt-Chinese.html, accessed 28 April 2023. doi:10.1021/cen-v068n040.p006

Reisfeld B, de Conti A, El Ghissassi F, Benbrahim-Tallaa L, Gwinn W, Grosse Y, et al. (2022). kc-hits: a tool to aid in the evaluation and classification of chemical carcinogens. *Bioinformatics*. 38(10):2961–2. doi:10.1093/bioinformatics/btac189 PMID:35561175

Rencüzoğulları E, Tüylü BA, Topaktaş M, Ila HB, Kayraldiz A, Arslan M, et al. (2004). Genotoxicity of aspartame. *Drug Chem Toxicol*. 27(3):257–68. doi:10.1081/DCT-120037506 PMID:15478947

Reshman F, Sumitha R, Parvathi D, et al. (2015). In-vivo toxicity assessment of aspartame in *Drosophila melanogaster* and *Danio rerio*. *Indian J Sci Technol*. 8(34):1–7. doi:10.17485/ijst/2015/v8i34/70677

Reynolds WA, Stegink LD, Filer LJ Jr, Renn E (1980). Aspartame administration to the infant monkey: hypothalamic morphology and plasma amino acid levels. *Anat Rec*. 198(1):73–85. doi:10.1002/ar.1091980106 PMID:7457932

Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, et al. (2002). European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. *Public Health Nutr*. 5(6B):1113–24. doi:10.1079/PHN2002394 PMID:12639222

Rimm EB, Giovannucci EL, Stampfer MJ, Colditz GA, Litin LB, Willett WC (1992). Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals. *Am J Epidemiol*. 135(10):1114–26, discussion 1127–36. doi:10.1093/oxfordjournals.aje.a116211 PMID:1632423

Ringel NE, Hovey KM, Andrews CA, Mossavar-Rahmani Y, Shadyab AH, Snetselaar LG, et al. (2023). Association of artificially sweetened beverage consumption and

urinary tract cancers in the Women's Health Initiative Observational Study. *Eur Urol Open Sci*. 47:80–6. doi:10.1016/j.euros.2022.11.016 PMID:36601047

Rodin J (1990). Comparative effects of fructose, aspartame, glucose, and water preloads on calorie and macronutrient intake. *Am J Clin Nutr*. 51(3):428–35. doi:10.1093/ajcn/51.3.428 PMID:2178391

Rodrigues H, Silva C, Martel F (2022). The effects of aspartame on the HTR8/SVneo extravillous trophoblast cell line. *Reprod Biol*. 22(3):100678. doi:10.1016/j.repbio.2022.100678 PMID:35963112

Rojas A, Schneider I, Lindner C, Gonzalez I, Morales MA (2023). Association between diabetes and cancer. Current mechanistic insights into the association and future challenges. *Mol Cell Biochem*. 478(8):1743–58. doi:10.1007/s11010-022-04630-x PMID:36565361

Romaguera D, Gracia-Lavedan E, Molinuevo A, de Batlle J, Mendez M, Moreno V, et al. (2017). Adherence to nutrition-based cancer prevention guidelines and breast, prostate and colorectal cancer risk in the MCC-Spain case–control study. *Int J Cancer*. 141(1):83–93. doi:10.1002/ijc.30722 PMID:28380695

Romano M, Casacci F, De Marchi F, Pacei T, Esteve A, Lomuscio G, et al. (1989). Effects of aspartame and carbohydrate administration on human and rat plasma large neutral amino acid levels and rat brain amino acid and monoamine levels. *J Nutr*. 119(1):75–81. doi:10.1093/jn/119.1.75 PMID:2913236

Romano M, Diomede L, Guiso G, Caccia S, Perego C, Salmona M (1990). Plasma and brain kinetics of large neutral amino acids and of striatum monoamines in rats given aspartame. *Food Chem Toxicol*. 28(5):317–21. doi:10.1016/0278-6915(90)90105-V PMID:2379890

Romanos-Nanclares A, Collins LC, Hu FB, Willett WC, Rosner BA, Toledo E, et al. (2021). Sugar-sweetened beverages, artificially sweetened beverages, and breast cancer risk: results from 2 prospective US cohorts. *J Nutr*. 151(9):2768–79. doi:10.1093/jn/nxab172 PMID:34114021

Ryuk JA, Kang S, Daily JW, Ko BS, Park S (2019). Moderate intake of aspartame and sucralose with meals, but not fructose, does not exacerbate energy and glucose metabolism in estrogen-deficient rats. *J Clin Biochem Nutr*. 65(3):223–31. doi:10.3164/jcbn.19-15 PMID:31777424

Samaniego-Vaesken ML, Ruiz E, Partearroyo T, Aranceta-Bartrina J, Gil Á, González-Gross M, et al. (2018). Added sugars and low- and no-calorie sweeteners in a representative sample of food products consumed by the Spanish ANIBES study population. *Nutrients*. 10(9):10. doi:10.3390/nu10091265 PMID:30205522

Sasaki YF, Kawaguchi S, Kamaya A, Ohshita M, Kabasawa K, Iwama K, et al. (2002). The comet assay with 8 mouse organs: results with 39 currently used food additives. *Mutat Res*. 519(1–2):103–19. doi:10.1016/S1383-5718(02)00128-6 PMID:12160896

Saunders FJ, Pautsch WF, Nutting EF (1980). The biological properties of aspartame. III. Examination for endocrine-like activities. *J Environ Pathol Toxicol*. 3(5-6):363–73. PMID:7441091

Schernhammer ES, Bertrand KA, Birmann BM, Sampson L, Willett WC, Feskanich D (2012). Consumption of artificial sweetener- and sugar-containing soda and risk of lymphoma and leukemia in men and women. *Am J Clin Nutr*. 96(6):1419–28. doi:10.3945/ajcn.111.030833 PMID:23097267

Schernhammer ES, Hu FB, Giovannucci E, Michaud DS, Colditz GA, Stampfer MJ, et al. (2005). Sugar-sweetened soft drink consumption and risk of pancreatic cancer in two prospective cohorts. *Cancer Epidemiol Biomarkers Prev*. 14(9):2098–105. doi:10.1158/1055-9965.EPI-05-0059 PMID:16172216

Scheurer M, Brauch HJ, Lange FT (2009). Analysis and occurrence of seven artificial sweeteners in German waste water and surface water and in soil aquifer treatment (SAT). *Anal Bioanal Chem*. 394(6):1585–94. doi:10.1007/s00216-009-2881-y PMID:19533103

Schiano C, Grimaldi V, Franzese M, Fiorito C, De Nigris F, Donatelli F, et al. (2020). Non-nutritional sweeteners effects on endothelial vascular function. *Toxicol In Vitro*. 62:104694. doi:10.1016/j.tiv.2019.104694 PMID:31655124

Schorb S, Gleiss K, Wedekind R, Suonio E, Kull A-K, Kuntz M, et al. (2023). Assessment of aspartame (E951) occurrence in selected foods and beverages on the German market 2000–2022. *Foods*. 12(11):2156. doi:10.3390/foods12112156 PMID:37297402

Schulpis KH, Papassotiriou I, Parthimos T, Tsakiris T, Tsakiris S (2006). The effect of L-cysteine and glutathione on inhibition of Na+, K+-ATPase activity by aspartame metabolites in human erythrocyte membrane. *Eur J Clin Nutr*. 60(5):593–7. doi:10.1038/sj.ejcn.1602355 PMID:16391576

Shah S, de Jager LS (2017). Recent analytical methods for the analysis of sweeteners in food: a regulatory perspective. Food and Drug Administration Papers. Vol. 5; pp. 13–32.

Shalaby AM, Ibrahim MAAH, Aboregela AM (2019). Effect of aspartame on the placenta of adult albino rat. A histological and immunohistochemical study. *Ann Anat*. 224:133–41. doi:10.1016/j.aanat.2019.04.007 PMID:31108189

Sharma BM, Bečanová J, Scheringer M, Sharma A, Bharat GK, Whitehead PG, et al. (2019). Health and ecological risk assessment of emerging contaminants (pharmaceuticals, personal care products, and artificial sweeteners) in surface and groundwater (drinking water) in the Ganges River Basin, India. *Sci Total Environ*. 646:1459–67. doi:10.1016/j.scitotenv.2018.07.235 PMID:30235631

Shephard SE, Wakabayashi K, Nagao M (1993). Mutagenic activity of peptides and the artificial sweetener aspartame after nitrosation. *Food Chem Toxicol*. 31(5):323–9. doi:10.1016/0278-6915(93)90186-3 PMID:8505016

Shibui Y, Fujitani S, Iwata H, Lynch B, Roberts A (2019). Histological analyses of the Ishii (1981) rat carcinogenicity study of aspartame and comparison with the Ramazzini Institute studies. *Regul Toxicol Pharmacol*. 102:23–9. doi:10.1016/j.yrtph.2018.12.010 PMID:30572082

Shigeta H, Yoshida T, Nakai M, Mori H, Kano Y, Nishioka H, et al. (1985). Effects of aspartame on diabetic rats and diabetic patients. *J Nutr Sci Vitaminol (Tokyo)*. 31(5):533–40. doi:10.3177/jnsv.31.533 PMID:3908628

Shil A, Chichger H (2021). Artificial sweeteners negatively regulate pathogenic characteristics of two model gut bacteria, *E. coli* and *E. faecalis*. *Int J Mol Sci*. 22(10):5228. doi:10.3390/ijms22105228 PMID:34063332

Shil A, Olusanya O, Ghufoor Z, Forson B, Marks J, Chichger H (2020). Artificial sweeteners disrupt tight junctions and barrier function in the intestinal epithelium through activation of the sweet taste receptor, T1R3. *Nutrients*. 12(6):1862. doi:10.3390/nu12061862 PMID:32580504

Shinggu DY, Bekab J (2018). Determination and comparison of aspartame levels in some selected soft drinks consumed in Mubi, Adamawa State, Nigeria. *Int Res J Pure Appl Chem*. 16(3):1–7. doi:10.9734/IRJPAC/2018/41441

Siegler J, Howell K, Vince R, Bray J, Towlson C, Peart D, et al. (2012). Aspartame in conjunction with carbohydrate reduces insulin levels during endurance exercise. *J Int Soc Sports Nutr*. 9(1):36. doi:10.1186/1550-2783-9-36 PMID:22853297

Sigala DM, Hieronimus B, Medici V, Lee V, Nunez MV, Bremer AA, et al. (2021). Consuming sucrose- or HFCS-sweetened beverages increases hepatic lipid and decreases insulin sensitivity in adults. *J Clin Endocrinol Metab*. 106(11):3248–64. doi:10.1210/clinem/dgab508 PMID:34265055

Sigala DM, Hieronimus B, Medici V, Lee V, Nunez MV, Bremer AA, et al. (2022). The dose–response effects of consuming high fructose corn syrup-sweetened beverages on hepatic lipid content and insulin sensitivity in young adults. *Nutrients*. 14(8):1648. doi:10.3390/nu14081648 PMID:35458210

Sigala DM, Widaman AM, Hieronimus B, Nunez MV, Lee V, Benyam Y, et al. (2020). Effects of consuming sugar-sweetened beverages for 2 weeks on 24-h circulating leptin profiles, ad libitum food intake and body weight in young adults. *Nutrients*. 12(12):3893. doi:10.3390/nu12123893 PMID:33352724

Singh N, Singh Lubana S, Arora S, Sachmechi I (2020). A study of artificial sweeteners and thyroid cancer risk. *J Clin Med Res*. 12(8):492–8. doi:10.14740/jocmr4258 PMID:32849937

Singleton MJ, Heiser C, Jamesen K, Mattes RD (1999). Sweetener augmentation of serum triacylglycerol during a fat challenge test in humans. *J Am Coll Nutr*. 18(2):179–85. doi:10.1080/07315724.1999.10718847 PMID:10204835

Smith MT, Guyton KZ, Gibbons CF, Fritz JM, Portier CJ, Rusyn I, et al. (2016). Key characteristics of carcinogens as a basis for organizing data on mechanisms of carcinogenesis. *Environ Health Perspect*. 124(6):713–21. doi:10.1289/ehp.1509912 PMID:26600562

Soffritti M, Belpoggi F, Degli Esposti D, Lambertini L (2005). Aspartame induces lymphomas and leukaemias in rats. *Eur J Oncol*. 10:107–16.

Soffritti M, Belpoggi F, Degli Esposti D, Lambertini L, Tibaldi E, Rigano A (2006). First experimental demonstration of the multipotential carcinogenic effects of aspartame administered in the feed to Sprague-Dawley rats. *Environ Health Perspect*. 114(3):379–85. doi:10.1289/ehp.8711 PMID:16507461

Soffritti M, Belpoggi F, Lambertin L, Lauriola M, Padovani M, Maltoni C (2002). Results of long-term experimental studies on the carcinogenicity of formaldehyde and acetaldehyde in rats. *Ann N Y Acad Sci*. 982(1):87–105. doi:10.1111/j.1749-6632.2002.tb04926.x PMID:12562630

Soffritti M, Belpoggi F, Manservigi M, Tibaldi E, Lauriola M, Falcioni L, et al. (2010). Aspartame administered in feed, beginning prenatally through life span, induces cancers of the liver and lung in male Swiss mice. *Am J Ind Med*. 53(12):1197–206. doi:10.1002/ajim.20896 PMID:20886530

Soffritti M, Belpoggi F, Tibaldi E, Esposti DD, Lauriola M (2007). Life-span exposure to low doses of aspartame beginning during prenatal life increases cancer effects in rats. *Environ Health Perspect*. 115(9):1293–7. doi:10.1289/ehp.10271 PMID:17805418

Sørensen LB, Raben A, Stender S, Astrup A (2005). Effect of sucrose on inflammatory markers in overweight humans. *Am J Clin Nutr*. 82(2):421–7. doi:10.1093/ajcn/82.2.421 PMID:16087988

Sorrentino ZA, Smith G, Palm L, Motwani K, Butterfield J, Archer C, et al. (2020). An erythritol-sweetened beverage induces satiety and suppresses ghrelin compared to aspartame in healthy non-obese subjects: a pilot study. *Cureus*. 12(11):e11409. doi:10.7759/cureus.11409 PMID:33194505

Sorriento D, Iaccarino G (2019). Inflammation and cardiovascular diseases: the most recent findings. *Int J Mol Sci*. 20(16):3879. doi:10.3390/ijms20163879 PMID:31395800

Souto NS, Dassi M, Braga ACM, Rosa ÉVF, Fighera MR, Royes LFF, et al. (2021). Hepatic susceptibility to oxidative damage after repeated concomitant exposure to aspartame and aflatoxin B1 in rats. *Drug Chem Toxicol*. 2021:1–6. PMID:34709106

SPIN (2023). CAS No. 22839-47-0. Substances in Preparations in Nordic Countries [online database]. Available from: http://www.spin2000.net/spinmyphp/?pid=22839470, accessed 28 April 2023.

Stanley L (2013). Review of data on the food additive aspartame. External scientific report. Supporting Publications 2013;10(3):EN-399. Parma, Italy: European Food Safety Authority. https://efsa.onlinelibrary.wiley.com/doi/abs/10.2903/sp.efsa.2013.EN-399, accessed 20 October 2023.

Stegink LD (1984). Aspartame metabolism in humans: acute dosing studies. Chapter 26. In: Stegink LD, Filer LJ Jr, editors. Aspartame – physiology and biochemistry. 1st ed. Boca Raton (FL), USA: CRC Press.; pp. 509–53.

Stegink LD (1987). The aspartame story: a model for the clinical testing of a food additive. *Am J Clin Nutr*. 46(1 Suppl):204–15. doi:10.1093/ajcn/46.1.204 PMID:3300262

Stegink LD, Brummel MC, Filer LJ Jr, Baker GL (1983b). Blood methanol concentrations in one-year-old infants administered graded doses of aspartame. *J Nutr*. 113(8):1600–6. doi:10.1093/jn/113.8.1600 PMID:6875695

Stegink LD, Brummel MC, McMartin K, Martin-Amat G, Filer LJ Jr, Baker GL, et al. (1981b). Blood methanol concentrations in normal adult subjects administered abuse doses of aspartame. *J Toxicol Environ Health*. 7(2):281–90. doi:10.1080/15287398109529979 PMID:7230276

Stegink LD, Filer LJ Jr, Baker GL (1977). Effect of aspartame and aspartate loading upon plasma and erythrocyte free amino acid levels in normal adult volunteers. *J Nutr*. 107(10):1837–45. doi:10.1093/jn/107.10.1837 PMID:903828

Stegink LD, Filer LJ Jr, Baker GL (1979a). Plasma, erythrocyte and human milk levels of free amino acids in lactating women administered aspartame or lactose. *J Nutr*. 109(12):2173–81. doi:10.1093/jn/109.12.2173 PMID:512705

Stegink LD, Filer LJ Jr, Baker GL (1981a). Plasma and erythrocyte concentrations of free amino acids in adult humans administered abuse doses of aspartame. *J Toxicol Environ Health*. 7(2):291–305. doi:10.1080/15287398109529980 PMID:7230277

Stegink LD, Filer LJ Jr, Baker GL (1982). Effect of aspartame plus monosodium L-glutamate ingestion on plasma and erythrocyte amino acid levels in normal adult subjects fed a high protein meal. *Am J Clin Nutr*. 36(6):1145–52. doi:10.1093/ajcn/36.6.1145 PMID:7148735

Stegink LD, Filer LJ Jr, Baker GL (1983a). Plasma amino acid concentrations in normal adults fed meals with added monosodium L-glutamate and aspartame. *J Nutr*. 113(9):1851–60. doi:10.1093/jn/113.9.1851 PMID:6886827

Stegink LD, Filer LJ Jr, Baker GL (1987b). Plasma amino acid concentrations in normal adults ingesting aspartame and monosodium L-glutamate as part of a soup/beverage meal. *Metabolism*. 36(11):1073–9. doi:10.1016/0026-0495(87)90028-X PMID:3670074

Stegink LD, Filer LJ Jr, Baker GL, Bell EF, Ziegler EE, Brummel MC, et al. (1989). Repeated ingestion of aspartame-sweetened beverage: effect on plasma amino acid concentrations in individuals heterozygous for phenylketonuria. *Metabolism*. 38(1):78–84. doi:10.1016/0026-0495(89)90184-4 PMID:2909831

Stegink LD, Filer LJ Jr, Baker GL, Brummel MC (1979c). Plasma and erythrocyte amino acid levels of adult humans given 100 mg/kg body weight aspartame. *Toxicology*. 14(2):131–40. doi:10.1016/0300-483X(79)90059-3 PMID:538766

Stegink LD, Filer LJ Jr, Baker GL, McDonnell JE (1979b). Effect of aspartame loading upon plasma and erythrocyte amino acid levels in phenylketonuric heterozygotes and normal adult subjects. *J Nutr*. 109(4):708–17. doi:10.1093/jn/109.4.708 PMID:430270

Stegink LD, Filer LJ Jr, Baker GL, McDonnell JE (1980). Effect of an abuse dose of aspartame upon plasma and erythrocyte levels of amino acids in phenylketonuric heterozygous and normal adults. *J Nutr*. 110(11):2216–24. doi:10.1093/jn/110.11.2216 PMID:7431122

Stegink LD, Filer LJ Jr, Bell EF, Ziegler EE, Tephly TR, Krause WL (1990). Repeated ingestion of aspartame-sweetened beverages: further observations in individuals heterozygous for phenylketonuria. *Metabolism*. 39(10):1076–81. doi:10.1016/0026-0495(90)90169-D PMID:2215254

Stegink LD, Filer LJ Jr, Brummel MC, Baker GL, Krause WL, Bell EF, et al. (1991). Plasma amino acid concentrations and amino acid ratios in normal adults and adults heterozygous for phenylketonuria ingesting a hamburger and milk shake meal. *Am J Clin Nutr*. 53(3):670–5. doi:10.1093/ajcn/53.3.670 PMID:2000820

Stegink LD, Koch R, Blaskovics ME, Filer LJ Jr, Baker GL, McDonnell JE (1981c). Plasma phenylalanine levels in phenylketonuric heterozygous and normal adults administered aspartame at 34 mg/kg body weight. *Toxicology*. 20(1):81–90. doi:10.1016/0300-483X(81)90108-6 PMID:7268794

Stegink LD, Wolf-Novak LC, Filer LJ Jr, Bell EF, Ziegler EE, Krause WL, et al. (1987a). Aspartame-sweetened beverage: effect on plasma amino acid concentrations in normal adults and adults heterozygous for phenylketonuria. *J Nutr*. 117(11):1989–95. doi:10.1093/jn/117.11.1989 PMID:2890728

Stepien M, Duarte-Salles T, Fedirko V, Trichopoulou A, Lagiou P, Bamia C, et al. (2016). Consumption of soft drinks and juices and risk of liver and biliary tract cancers in a European cohort. *Eur J Nutr*. 55(1):7–20. doi:10.1007/s00394-014-0818-5 PMID:25528243

Stern SB, Bleicher SJ, Flores A, Gombos G, Recitas D, Shu J (1976). Administration of aspartame in non-insulin-dependent diabetics. *J Toxicol Environ Health*. 2(2):429–39. doi:10.1080/15287397609529444 PMID:1011296

Subedi B, Kannan K (2014). Fate of artificial sweeteners in wastewater treatment plants in New York State, USA. *Environ Sci Technol*. 48(23):13668–74. doi:10.1021/es504769c PMID:25365516

Subedi B, Lee S, Moon HB, Kannan K (2014). Emission of artificial sweeteners, select pharmaceuticals, and personal care products through sewage sludge from wastewater treatment plants in Korea. *Environ Int*. 68:33–40. doi:10.1016/j.envint.2014.03.006 PMID:24695211

Suez J, Cohen Y, Valdés-Mas R, Mor U, Dori-Bachash M, Federici S, et al. (2022). Personalized microbiome-driven effects of non-nutritive sweeteners on human glucose tolerance. *Cell*. 185(18):3307–3328.e19. doi:10.1016/j.cell.2022.07.016 PMID:35987213

Suez J, Korem T, Zeevi D, Zilberman-Schapira G, Thaiss CA, Maza O, et al. (2014). Artificial sweeteners induce glucose intolerance by altering the gut microbiota. *Nature*. 514(7521):181–6. doi:10.1038/nature13793 PMID:25231862

Sun D, Liu L, Mao S, Zhu W, Liu J (2019). Aspartame supplementation in starter accelerates small intestinal epithelial cell cycle and stimulates secretion of glucagon-like peptide-2 in pre-weaned lambs. *J Anim Physiol Anim Nutr (Berl)*. 103(5):1338–50. doi:10.1111/jpn.13159 PMID:31342562

Sylvetsky AC, Brown RJ, Blau JE, Walter M, Rother KI (2016). Hormonal responses to non-nutritive sweeteners in water and diet soda. *Nutr Metab (Lond)*. 13(1):71. doi:10.1186/s12986-016-0129-3 PMID:27777606

Sylvetsky AC, Gardner AL, Bauman V, Blau JE, Garraffo HM, Walter PJ, et al. (2015). Nonnutritive sweeteners in breast milk. *J Toxicol Environ Health A*. 78(16):1029–32. doi:10.1080/15287394.2015.1053646 PMID:26267522

Sylvetsky AC, Rother KI (2016). Trends in the consumption of low-calorie sweeteners. *Physiol Behav*. 164(Pt B):446–50. doi:10.1016/j.physbeh.2016.03.030 PMID:27039282

Szucs EF, Barrett KE, Metcalfe DD (1986). The effects of aspartame on mast cells and basophils. *Food Chem Toxicol*. 24(2):171–4. doi:10.1016/0278-6915(86)90353-4 PMID:2420690

Takehara CT, Nicoluci ÍG, Andrade TFS, Arisseto-Bragotto AP (2022). A comprehensive database of declared high-intensity sweeteners in Brazilian commercial products and updated exposure assessment. *Food Res Int*. 161:111899. doi:10.1016/j.foodres.2022.111899 PMID:36192918

Tamez M, Monge A, López-Ridaura R, Fagherazzi G, Rinaldi S, Ortiz-Panozo E, et al. (2018). Soda intake is directly associated with serum C-reactive protein concentration in Mexican women. *J Nutr*. 148(1):117–24. doi:10.1093/jn/nxx021 PMID:29378052

Teff KL, Devine J, Engelman K (1995). Sweet taste: effect on cephalic phase insulin release in men. *Physiol Behav*. 57(6):1089–95. doi:10.1016/0031-9384(94)00373-D PMID:7652029

Temizkan S, Deyneli O, Yasar M, Arpa M, Gunes M, Yazici D, et al. (2015). Sucralose enhances GLP-1 release and lowers blood glucose in the presence of carbohydrate in healthy subjects but not in patients with type 2 diabetes. *Eur J Clin Nutr*. 69(2):162–6. doi:10.1038/ejcn.2014.208 PMID:25271009

Tepler A, Hoffman G, Jindal S, Narula N, Shah SC (2021). Intake of artificial sweeteners among adults is associated with reduced odds of gastrointestinal luminal cancers: a meta-analysis of cohort and case–control studies. *Nutr Res*. 93:87–98. doi:10.1016/j.nutres.2021.07.007 PMID:34461350

Thomas RS, Paules RS, Simeonov A, Fitzpatrick SC, Crofton KM, Casey WM, et al. (2018). The US Federal Tox21 Program: a strategic and operational plan for continued leadership. *Altern Anim Exp*. 35(2):163–8. doi:10.14573/altex.1803011 PMID:29529324

Tibaldi E, Gnudi F, Panzacchi S, Mandrioli D, Vornoli A, Manservigi M, et al. (2020). Identification of aspartame-induced haematopoietic and lymphoid tumours in rats after lifetime treatment. *Acta Histochem*. 122(5):151548. doi:10.1016/j.acthis.2020.151548 PMID:32622430

Tice RR, Hayashi M, MacGregor JT, Anderson D, Blakey DH, Holden HE, et al. (1994). Report from the working group on the in vivo mammalian bone marrow chromosomal aberration test. *Mutat Res*. 312(3):305–12. doi:10.1016/0165-1161(94)00016-6 PMID:7514742

Tobey NA, Heizer WD (1986). Intestinal hydrolysis of aspartylphenylalanine – the metabolic product of aspartame. *Gastroenterology*. 91(4):931–7. doi:10.1016/0016-5085(86)90697-9 PMID:3743970

Toews I, Lohner S, Küllenberg de Gaudry D, Sommer H, Meerpohl JJ (2019). Association between intake of non-sugar sweeteners and health outcomes: systematic review and meta-analyses of randomised and non-randomised controlled trials and observational studies. *BMJ*. 364:k4718. doi:10.1136/bmj.k4718 PMID:30602577

Tolouei S, Burnet JB, Autixier L, Taghipour M, Bonsteel J, Duy SV, et al. (2019). Temporal variability of parasites, bacterial indicators, and wastewater micropollutants in a water resource recovery facility under various weather conditions. *Water Res*. 148:446–58. doi:10.1016/j.watres.2018.10.068 PMID:30408731

Tovar AP, Navalta JW, Kruskall LJ, Young JC (2017). The effect of moderate consumption of non-nutritive sweeteners on glucose tolerance and body composition in rats. *Appl Physiol Nutr Metab*. 42(11):1225–7. doi:10.1139/apnm-2017-0120 PMID:28692807

Tran NL, Barraj LM, Hearty AP, Jack MM (2021). Tiered intake assessment for low- and no-calorie sweeteners in beverages. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess*. 38(2):208–22. doi:10.1080/19440049.2020.1843717 PMID:33451265

Trichopoulos D, Bamia C, Lagiou P, Fedirko V, Trepo E, Jenab M, et al. (2011). Hepatocellular carcinoma risk factors and disease burden in a European cohort: a nested case–control study. *J Natl Cancer Inst*. 103(22):1686–95. doi:10.1093/jnci/djr395 PMID:22021666

Trocho C, Pardo R, Rafecas I, Virgili J, Remesar X, Fernández-López JA, et al. (1998). Formaldehyde derived from dietary aspartame binds to tissue components in vivo. *Life Sci*. 63(5):337–49. doi:10.1016/S0024-3205(98)00282-3 PMID:9714421

Tumminia A, Vinciguerra F, Parisi M, Graziano M, Sciacca L, Baratta R, et al. (2019). Adipose tissue, obesity and adiponectin: role in endocrine cancer risk. *Int J Mol Sci*. 20(12):2863. doi:10.3390/ijms20122863 PMID:31212761

Tutelyan VA, Kravchenko LV, Kuzmina EE (1990). The effect of aspartame on the activity of rat liver xenobiotic-metabolizing enzymes. *Drug Metab Dispos*. 18(2):223–5. PMID:1971577

United States Pharmacopeia (2023). Aspartame. USP-NF/PF Abstract. Rockville (MD), USA: United States Pharmacopeia. Available from: usp.org/USPNF/USPNF_M6195_04_01.html, accessed May 2023. doi:usp.org/USPNF/USPNF_M6195_04_01.html

US EPA (2023). CompTox Chemicals Dashboard. Last updated on 3 February 2023. United States Environmental Protection Agency. Available from: https://www.epa.gov/chemical-research/comptox-chemicals-dashboard.

US FDA (1974). Aspartame. Code of Federal Regulations. Title 21 – Food and Drugs. Chapter I – food and drug administration. Department of Health and Human Services. Subchapter B – Food for Human Consumption (continued). Part 172 – Food additives permitted for direct addition to food for human consumption. Silver Spring (MD), USA: United States Food and Drug Administration. Available from: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=172.804, accessed June 2023.

USDA (1986). Sugar and sweetener. Outlook and situation report. SSRV11N1. Washington (DC), USA: United States Department of Agriculture. Available from: https://downloads.usda.library.cornell.edu/usda-esmis/files/pv63g024f/5d86p188f/2b88qf52r/SSS-03-30-1986.pdf, accessed November 2023.

USDA (2012). Sugar and sweeteners outlook. SSS-M-286. Electronic Outlook Report from the Economic Research Service. 18 June 2012. Washington (DC), USA: United States Department of Agriculture. Available from: https://www.ers.usda.gov/webdocs/outlooks/39309/28794_sssm286.pdf?v=81.6, accessed May 2023.

van Eyk AD (2015). The effect of five artificial sweeteners on Caco-2, HT-29 and HEK-293 cells. *Drug Chem Toxicol*. 38(3):318–27. doi:10.3109/01480545.2014.966381 PMID:25317478

van Spronsen FJ, Blau N, Harding C, Burlina A, Longo N, Bosch AM (2021). Phenylketonuria. *Nat Rev Dis Primers*. 7(1):36. doi:10.1038/s41572-021-00267-0 PMID:34017006

van Vliet K, Melis ES, de Blaauw P, van Dam E, Maatman RGHJ, Abeln D, et al. (2020). Aspartame and Phe-containing degradation products in soft drinks across Europe. *Nutrients*. 12(6):1887. doi:10.3390/nu12061887 PMID:32599819

Vences-Mejía A, Labra-Ruíz N, Hernández-Martínez N, Dorado-González V, Gómez-Garduño J, Pérez-López I, et al. (2006). The effect of aspartame on rat brain xenobiotic-metabolizing enzymes. *Hum Exp Toxicol*. 25(8):453–9. doi:10.1191/0960327106het646oa PMID:16937917

Villadsen J, Jakobsen M (2012). [Subproject 6: Monitoring and control of additives: intense sweeteners, colourings and preservatives in beverages]. Project No. 2009-20-64-00183. Copenhagen, Denmark: The Danish Food Agency. [Danish]

Vin K, Connolly A, McCaffrey T, McKevitt A, O'Mahony C, Prieto M, et al. (2013). Estimation of the dietary intake of 13 priority additives in France, Italy, the UK and Ireland as part of the FACET project. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess*. 30(12):2050–80. doi:10.1080/19440049.2013.851417 PMID:24304380

VKM (2014). Risk assessments of aspartame, acesulfame K, sucralose and benzoic acid from soft drinks, "saft", nectar and flavoured water. Opinion of the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics of the Norwegian Committee for Food Safety. VKM Report 2014: 26. Oslo, Norway: Vitenskapskomiteen for mattrygghet [Norwegian Scientific Committee for Food Safety]. Available from: https://vkm.no/download/18.a665c1015c865cc85bb7ae2/1501776952080/8055794778.pdf, accessed May 2023.

von Poser Toigo E, Huffell AP, Mota CS, Bertolini D, Pettenuzzo LF, Dalmaz C (2015). Metabolic and feeding behavior alterations provoked by prenatal exposure to aspartame. *Appetite*. 87:168–74. doi:10.1016/j.appet.2014.12.213 PMID:25543075

von Rymon Lipinski GW (2015). Sweeteners. In: Ullmann's encyclopedia of industrial chemistry. Weinheim, Germany: Wiley-VCH Verlag. doi:10.1002/14356007.a26_023.pub2

Wang Y, Nguyen LH, Mehta RS, Song M, Huttenhower C, Chan AT (2021). Association between the sulfur microbial diet and risk of colorectal cancer.

JAMA Netw Open. 4(11):e2134308. doi:10.1001/jamanetworkopen.2021.34308 PMID:34767023

Wasik A, McCourt J, Buchgraber M (2007). Simultaneous determination of nine intense sweeteners in foodstuffs by high performance liquid chromatography and evaporative light scattering detection–development and single-laboratory validation. *J Chromatogr A.* 1157(1–2):187–96. doi:10.1016/j.chroma.2007.04.068 PMID:17540386

Watanabe Y, Bach LT, Van Dinh P, Prudente M, Aguja S, Phay N, et al. (2016). Ubiquitous detection of artificial sweeteners and iodinated X-ray contrast media in aquatic environmental and wastewater treatment plant samples from Vietnam, the Philippines, and Myanmar. *Arch Environ Contam Toxicol.* 70(4):671–81. doi:10.1007/s00244-015-0220-1 PMID:26304512

WCRF/AICR (2018). Continuous update project. Recommendations and public health and policy implications. London, England: World Cancer Research Fund/American Institute for Cancer Research.

Welss T (2015). Oral hygiene products. In: Ullmann's encyclopedia of industrial chemistry. Weinheim, Germany: Wiley-VCH Verlag.

WHO, Rios-Leyvraz M, Montez J (2022). Health effects of the use of non-sugar sweeteners: a systematic review and meta-analysis. Geneva, Switzerland: World Health Organization. Available from: https://apps.who.int/iris/handle/10665/353064.

Wolf-Novak LC, Stegink LD, Brummel MC, Persoon TJ, Filer LJ Jr, Bell EF, et al. (1990). Aspartame ingestion with and without carbohydrate in phenylketonuric and normal subjects: effect on plasma concentrations of amino acids, glucose, and insulin. *Metabolism.* 39(4):391–6. doi:10.1016/0026-0495(90)90254-A PMID:2182973

Wouassi D, Mercier J, Ahmaidi S, Brun JF, Mercier B, Orsetti A, et al. (1997). Metabolic and hormonal responses during repeated bouts of brief and intense exercise: effects of pre-exercise glucose ingestion. *Eur J Appl Physiol Occup Physiol.* 76(3):197–202. doi:10.1007/s004210050236 PMID:9286597

Yan S, Yan F, Liu L, Li B, Liu S, Cui W (2022). Can artificial sweeteners increase the risk of cancer incidence and mortality: evidence from prospective studies. *Nutrients.* 14(18):3742. doi:10.3390/nu14183742 PMID:36145117

Yesildal F, Aydin FN, Deveci S, Tekin S, Aydin I, Mammadov R, et al. (2015). Aspartame induces angiogenesis in vitro and in vivo models. *Hum Exp Toxicol.* 34(3):260–5. doi:10.1177/0960327114537535 PMID:24925367

Yin T, Li J, Wang Y, Liu K, Long T, Cheng L (2022). Artificially sweetened beverage consumption and cancer risk: a comprehensive dose-response meta-analysis of prospective studies. *Nutrients.* 14(21):4445. doi:10.3390/nu14214445 PMID:36364707

Yokozeki K, Abe I (2021). A novel route for aspartame production by combining enzymatic and chemical reactions for industrial use. *Biosci Biotechnol Biochem.* 85(2):464–6. doi:10.1093/bbb/zbaa045 PMID:33604621

You D, Xu H, Chen X, Zhou J, Wu Y, Tang Y, et al. (2022). Association between soft drink consumption types and risk of lung cancer and all-cancer: a prospective study of PLCO data. *J Biomed Res.* 36(6):390–400. doi:10.7555/JBR.36.20220135 PMID:36424907

Yu Z, Ley SH, Sun Q, Hu FB, Malik VS (2018). Cross-sectional association between sugar-sweetened beverage intake and cardiometabolic biomarkers in US women. *Br J Nutr.* 119(5):570–80. doi:10.1017/S0007114517003841 PMID:29508692

Yuan C, Joh HK, Wang QL, Zhang Y, Smith-Warner SA, Wang M, et al. (2022). Sugar-sweetened beverage and sugar consumption and colorectal cancer incidence and mortality according to anatomic subsite. *Am J Clin Nutr.* 115(6):1481–9. doi:10.1093/ajcn/nqac040 PMID:35470384

Zamora-Ros R, Cayssials V, Clèries R, Torrents M, Byrnes G, Weiderpass E, et al. (2022). Sweetened beverages are associated with a higher risk of differentiated thyroid cancer in the EPIC cohort: a dietary pattern approach. *Eur J Nutr.* doi:10.1007/s00394-022-02953-5 PMID:35907037

Zhang T, Gan Z, Gao C, Ma L, Li Y, Li X, et al. (2016). Occurrence of artificial sweeteners in human liver and paired blood and urine samples from adults in Tianjin, China and their implications for human exposure. *Environ Sci Process Impacts.* 18(9):1169–76. doi:10.1039/C6EM00130K PMID:27383923

Zhang YB, Chen JX, Jiang YW, Xia PF, Pan A (2021). Association of sugar-sweetened beverage and artificially sweetened beverage intakes with mortality: an analysis of US National Health and Nutrition Examination Survey. *Eur J Nutr.* 60(4):1945–55. doi:10.1007/s00394-020-02387-x PMID:32945955

Zhou Q, Sun WW, Chen JC, Zhang HL, Liu J, Lin Y, et al. (2022). Phenylalanine impairs insulin signalling and inhibits glucose uptake through modification of IRβ. *Nat Commun.* 13(1):4291. doi:10.1038/s41467-022-32000-0 PMID:35879296