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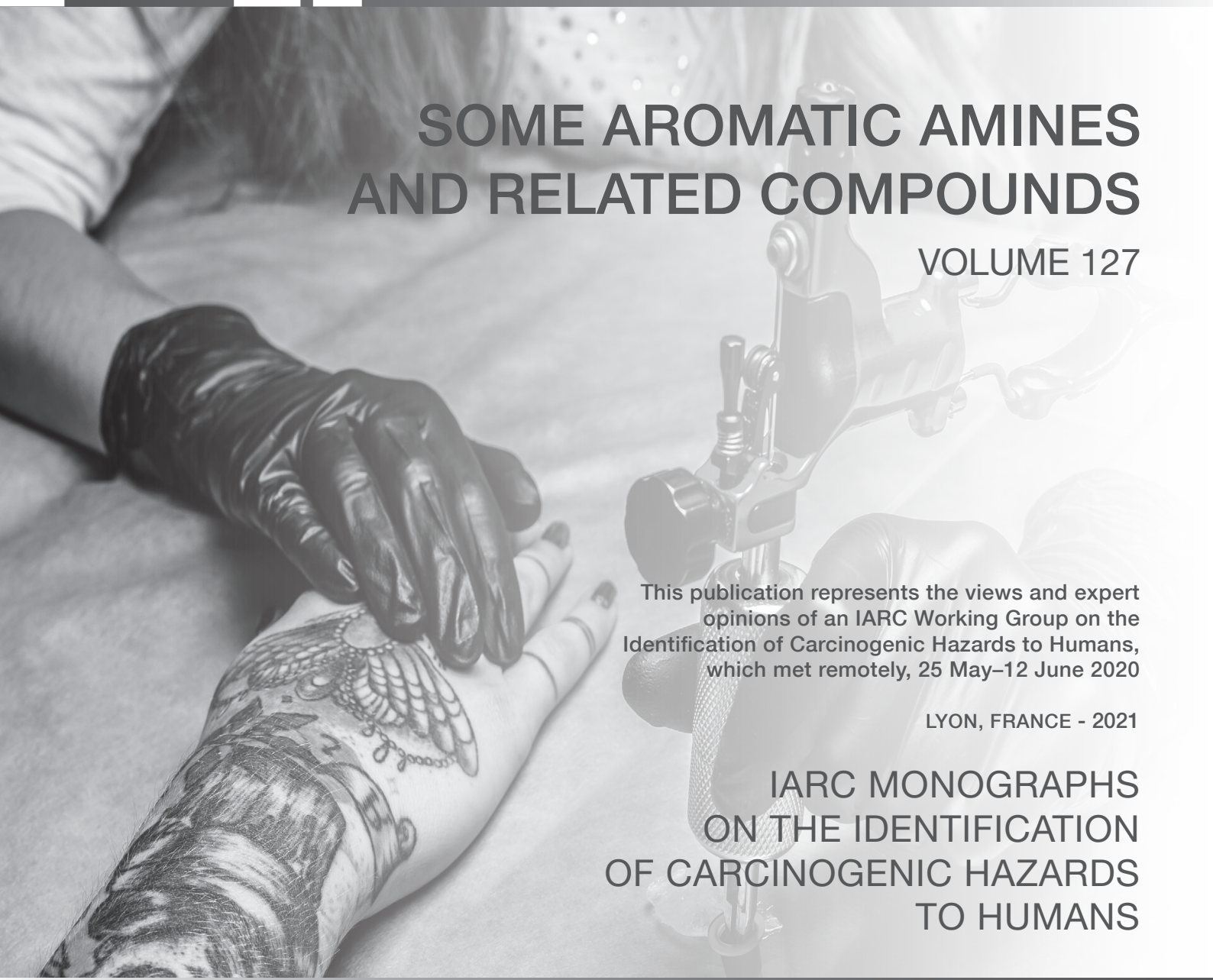

SOME AROMATIC AMINES AND RELATED COMPOUNDS

VOLUME 127

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IARC MONOGRAPHS ON THE IDENTIFICATION OF CARCINOGENIC HAZARDS TO HUMANS

International Agency for Research on Cancer

World Health Organization

ANILINE AND ANILINE HYDROCHLORIDE

1. Exposure Characterization

1.1 Identification of the agent

1.1.1 Nomenclature

(a) Aniline

Chem. Abstr. Serv. Reg. No.: 62-53-3

EC No.: 200-539-3

IUPAC systematic name: aniline

Synonyms and abbreviations: benzenamine; phenylamine; aminobenzene; aminophen; aniline oil.

(b) Aniline hydrochloride

Chem. Abstr. Serv. Reg. No.: 142-04-1

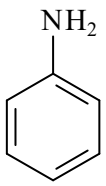
EC No.: 205-519-8

IUPAC systematic name: aniline hydrochloride

Synonyms: aniline chloride; anilinium chloride; benzenamine hydrochloride; aniline. HCl; phenylamine hydrochloride; phenylammonium chloride.

1.1.2 Structural and molecular formulae, and relative molecular mass

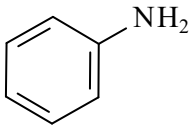
(a) Aniline



Molecular formula: C_6H_7N

Relative molecular mass: 93.13 (NCBI, 2020a).

(b) Aniline hydrochloride



• HCl

Molecular formula: C_6H_8ClN

Relative molecular mass: 129.59 (NCBI, 2020b).

1.1.3 Chemical and physical properties of the pure substance

Aniline is a basic compound and will undergo acid–base reactions. Aniline and its hydrochloride salt will achieve a pH-dependent acid–base equilibrium in the body.

(a) Aniline

Description: aniline appears as a yellowish to brownish oily liquid with a musty fishy odour (NCBI, 2020a), detectable at 1 ppm [3.81 mg/m³] (European Commission, 2016; NIOSH, 2019)

Boiling point: 184.1 °C (NCBI, 2020a)

Melting point: −6 °C (NCBI, 2020a)

Flash point: 70 °C, closed cup (NCBI, 2020a)

Density: 1.02 g/cm³ (NCBI, 2020a)

Vapour density: 3.2 (air = 1) (NCBI, 2020a)

Vapour pressure: 40 Pa at 20 °C; 0.67 mm Hg at 25 °C (NCBI, 2020a)

Solubility: 36 g/L at 25 °C in water; soluble in alcohol, ether, benzene, ethyl ether, and carbon tetrachloride (NCBI, 2020a)

Dissociation constant: pK_a, 4.6 (at 25 °C; aniline conjugate acid) (NCBI, 2020a)

Octanol/water partition coefficient (P): log K_{ow}, 0.9 (US EPA, 2020a)

Conversion factor: 1 ppm = 3.81 mg/m³ (NIOSH, 2019) [calculated from: mg/m³ = (relative molecular mass/24.45) × ppm, assuming temperature (25 °C) and pressure (101 kPa)].

(b) Aniline hydrochloride

Description: aniline hydrochloride appears as a white to greenish coloured crystalline solid (NCBI, 2020b)

Boiling point: 245 °C (NCBI, 2020b)

Melting point: 198 °C (NCBI, 2020b)

Flash point: 193 °C, open cup (NCBI, 2020b)

Density: 1.22 g/cm³ (NCBI, 2020b)

Vapour density: 4.46 (air = 1) (NCBI 2020b)

Solubility: soluble in water: 1070 g/L at 20 °C (NCBI, 2020b)

Dissociation constant: pK_a, 4.6 (at 25 °C; aniline conjugate acid)

Octanol/water partition coefficient (P): log $K_{ow,}$ 0.936, predicted median (US EPA, 2020b)

Conversion factor: 1 ppm = 5.3 mg/m³ [calculated from: mg/m³ = (relative molecular mass/24.45) × ppm, assuming temperature (25 °C) and pressure (101 kPa)].

1.2 Production and use

1.2.1 Production process

Historically, aniline was produced by Otto Unverdorben in 1826 by dry distillation from the leaves of the indigo plant, genus *Indigofera* (most probably *Indigofera tinctoria*). Aniline was isolated from coal tar in 1834. Aniline was first synthesized in 1842 by reducing nitrobenzene with sodium sulfates (Zinin reaction; Zinin, 1842) and first manufactured commercially in 1847 (Kouris & Northcott, 1963). Aniline was made by the Bechamp process of reduction of nitrobenzene with iron and hydrochloric acid and, until 1966, by amination of chlorobenzene with ammonia (IARC, 1982). Presently, aniline is produced by catalytic reduction of nitrobenzene. The process can be divided into three main parts: nitrobenzene hydrogenation, dehydration, and purification. Nitrobenzene is converted to aniline (nearly 100%) in a single pass by feeding it with hydrogen into a reactor containing the catalyst metal. The excess hydrogen is removed, and the liquid product is dehydrated. The crude aniline is purified by distillation. The final product obtained is aniline with a purity of > 99.95 wt% containing less than 0.1 ppm [0.1 mg/kg] of nitrobenzene by weight. The conversion of nitrobenzene to aniline remains the most common pathway for aniline production. An alternative production pathway for aniline uses phenol and ammonia as the starting raw materials (Intratec Solutions, 2016).

Aniline hydrochloride is prepared by reacting aniline vapour and hydrogen chloride gas at temperatures of > 250 °C (Holt & Daudt, 1935).

1.2.2 Production volume

Aniline is a High Production Volume chemical (OECD, 2009; US EPA, 2020c). It is used as a starting material in several industries, including for the manufacture of a variety of plastics, rubber additives, colourants, and drugs (Käfferlein et al., 2014).

Global capacity for aniline production was 4.98 million tonnes per year in 2006, with the major producers being in western Europe, the USA, and the Asia-Pacific region (ICIS, 2008; see Table 1.1). Total volume for manufacture and use in the European Union is 1 million to 10 million tonnes per year (ECHA, 2020a). In an updated assessment report on aniline under the Canadian Environmental Protection Act of 1999 (Health Canada, 2011), Health Canada reported that more than 28 tonnes of aniline and its salts were synthesized as a by-product of chemical manufacturing in Canada in 2007. Additionally, between 13 and 48 tonnes of aniline and aniline salts were imported into Canada in 2000–2007 (Health Canada, 2011). During 2014–2019, global consumption of aniline grew at an average annual rate of 4.8%, reaching more than 6.7 million tonnes in 2019 (IHS Markit, 2020).

North-eastern Asia was the largest producer of aniline during 2013–2018, accounting for more than half of the global production of aniline. Western Europe and the USA were the next-largest suppliers. Most increases in production capacity for aniline were in China during 2013–2018, along with expansions in the Middle East and western Europe. In 2018, China accounted for almost 50% of the world's aniline production capacity, followed by western Europe and the USA (IHS Markit, 2019).

The national aggregate production volume in the USA in 2011 was approximately 1956 million pounds [887 000 tonnes], whereas in 2012–2015, it was between 1000 million and 5000 million pounds [450 000–2 300 000 tonnes] (ChemView, 2016). According to another source, annual aniline production in the USA since 1990 has varied between 449 000 and 1 065 000 tonnes; in 2019, it was 845 000 tonnes (Statista, 2020a). The production volume of aniline in India during 2013–2019 varied between 34 470 and 48 230 tonnes (Statista, 2020b).

Table 1.1 Global production of aniline in 2006, by geographical region

Region	Aniline production (tonnes per year)
Western Europe	1 620 000
USA	1 380 000
Asia-Pacific (excluding Japan)	1 150 000
Japan	474 000
Eastern Europe	316 500
Latin America	70 000
Asia or Middle East	64 000

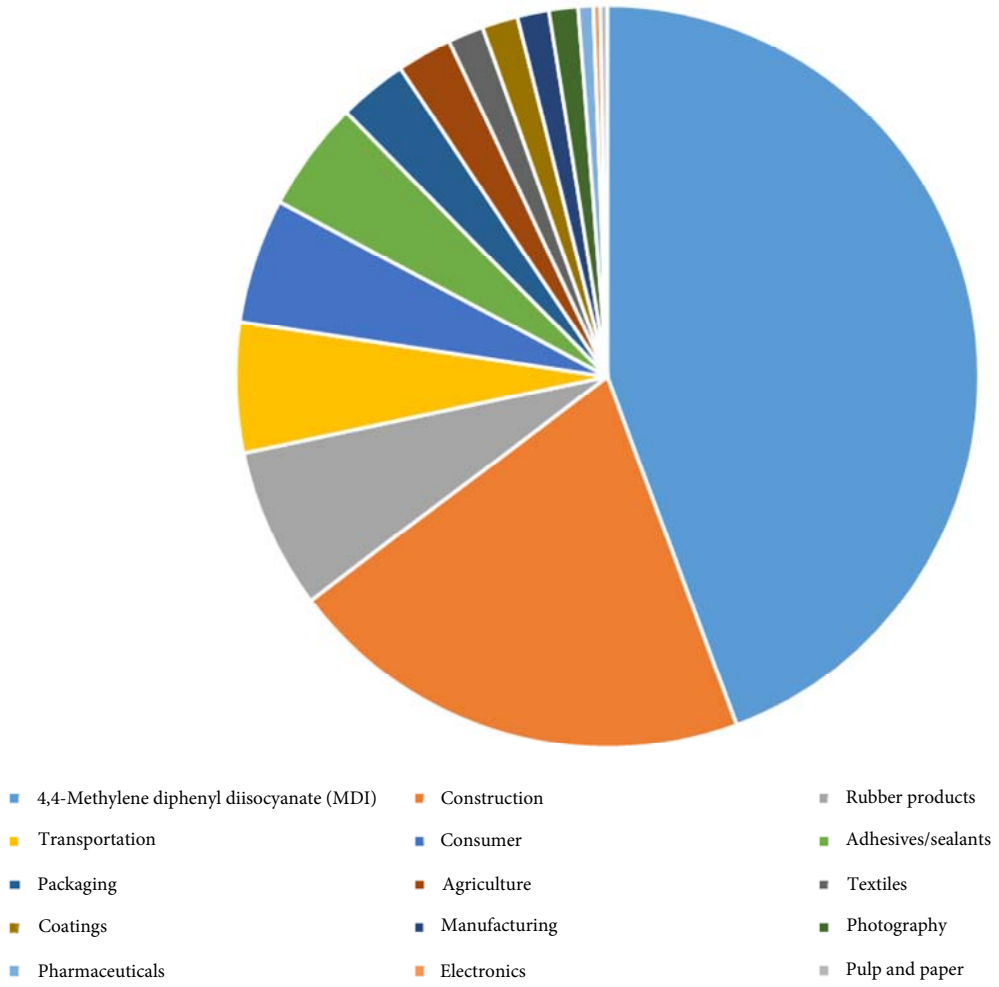
Data from ICIS (2008).

1.2.3 Uses

In the chemical industry, aniline is used as a parent substance for the synthesis of many compounds, including isocyanates, dyes and pigments, antioxidants, and accelerators in rubber processing, pharmaceuticals, varnishes, perfumes, photographic chemicals, herbicides, and fungicides (aniline is no longer approved for pesticide use in the European Union) (ICIS, 2008; European Commission, 2016; see Fig. 1.1).

China is the world's largest consumer of aniline and accounted for 37% of global demand in 2018. Western Europe is the second-largest consumer of aniline, accounting for 23% of global aniline demand in 2018. The USA follows as the third-largest consumer, accounting for 17% of global aniline consumption (IHS Markit, 2019). [The Working Group noted that, despite the major demand for aniline in Asia, few data

Fig. 1.1 Global consumption of aniline, by industry



From Independent Commodity Intelligence Service (ICIS, 2008). Reproduced with permission from www.icis.com.

on uses and exposure levels were available for this region.]

(a) 4,4-Methylene diphenyl diisocyanate

Production of 4,4-methylene diphenyl diisocyanate (MDI) accounts for more than 90% of aniline use (IHS Markit, 2019). Aniline and formaldehyde react in an acid-catalysed reaction, which is followed by liquid-phase phosgenation of diphenylmethane diamine to MDI (Lynch & Ryan, 2012). MDI is used to produce polyurethane foam. Rigid polyurethane is used in walls and roofs of new residential and commercial constructions as well as in the renovation of older buildings for insulating purposes. Flexible polyurethane foam is primarily used in furniture and transportation (IHS Markit, 2019; American Chemistry Council, 2020; GlobeNewswire, 2020).

(b) Rubber processing

Production of chemicals used in rubber processing is the second largest sector of aniline use (Amini & Lowenkron, 2003). Among the rubber-processing agents made from aniline are several important vulcanization accelerators and antioxidants, including aldehyde–aniline condensates (e.g. *N*-butyraldehyde condensate), guanidines (e.g. 1,3-diphenylguanidine), *N*-phenyl-2-naphthylamine (IARC, 1978), thiazoles (e.g. 2,2′-dithiobisbenzothiazole and other derivatives of 2-mercaptobenzothiazole, MBT), and a variety of derivatives of diphenylamine (which is made from aniline) (IARC, 1982). In new and used tyres, aniline was detected in concentrations near the detection limit of 100 mg/kg rubber (ECB, 2004).

(c) Dyes and colouring agents

Some 174 dyes can be prepared from aniline and more than 700 dyes can be prepared from aniline derivatives; however, very few are produced in commercially significant quantities (Northcott, 1978). Of 33 typical dyes derived from aniline (Kouris & Northcott, 1963), only 16 were reported to be produced commercially in the USA in 1979. Production data were published for six of these; those produced in the largest volumes were Basic Orange 2 (275 tonnes), Solvent Yellow 14 (159 tonnes), Acid Red 1 (128 tonnes) and Acid Black 1 (169 tonnes). Another dye made from aniline in significant quantities is Direct Orange 102: production by five companies in the USA in 1979 amounted to 192 tonnes (US International Trade Commission, 1980a; IARC, 1982). As of 2011, 50 000 tons [45 000 tonnes] of Indigo Blue have been synthesized per year, of which 95% was used to dye the more than 4 billion denim garments manufactured annually (Hsu et al., 2018; Nature, 2018). [The Working Group noted that no information was found on occupational or consumer-product exposure levels related to production and use of denim garments.]

(d) Pharmaceutical products

Aniline is used as an intermediate in the production of a variety of pharmaceutical products. A major derivative is acetanilide, which is used in the production of phenazopyridine and several sulfa drugs, including sulfafurazole and sulfamethoxazole (previously evaluated as IARC Group 3, *not classifiable as to its carcinogenicity to humans*, by the *IARC Monographs* programme; IARC, 1980), and also as a precursor in the synthesis of penicillin and other drugs (TMR, 2020).

(e) Consumer uses

Aniline is used in many products, including fabrics, textiles and apparel (e.g. clothing, mattresses, curtains or carpets, textile toys), leather (e.g. gloves, shoes, purses, furniture), paper (e.g. tissues, feminine hygiene products, diapers, books, magazines, wallpaper) and plastic (e.g. food packaging and storage, toys, mobile phones) (ECHA, 2020b). In a Danish survey of hobby products used by children, aniline was detected in samples of certain green and pink marker pens at concentrations of 0.22 and 0.11 mg/g (0.022% and 0.011%), respectively (Hansen et al., 2008). A study of inks in commonly used pens and markers conducted by Health Canada indicated that the level of aniline was below the limit of quantification (LOQ, 67 mg/kg) in all markers intended for children. Overall, inks in 94% of the 86 pens and markers sampled contained aniline at levels below the LOQ. Additionally, samples of black printer ink tested in Canada did not contain aniline at significant levels (Health Canada, 2011).

Table 1.2 Analytical methods for the quantitative determination of aniline

Sample matrix	Analytical technique	Limit of detection	Limit of quantification	Reference
Air	GC-FID	0.03 mg/m³	0.09 mg/m³	OSHA (1994)
Air	GC-FID	0.004 mg/sample	NR	NIOSH (1998)
Ground water	GC-MS	NR	10 µg/L	NEMI (1998)
Soil/sediment	GC-MS	NR	660 µg/kg	NEMI (1998)
Water	GC-MS	10 µg/L	NR	NEMI (2001)
River and tap water	Thin silica-coated and assembled silver nanoparticles-SERS	93 ppm [0.35 mg/L]	NR	Cha et al. (2017)
Water pipe smoke	LC-MS/MS	0.31 mg/m³	0.88 mg/m³	Schubert et al. (2011)
Mainstream cigarette smoke	HPLC-MS/MS	0.52 ng/cigarette	1.72 ng/cigarette	Xie et al. (2013)
Surface water	CZE	0.29 µg/L	NR	Liu et al. (2012)
River and sea water	HPLC-MS/MS	NR	0.016 µg/L	Furukawa et al. (2017)
Food contact materials	HPLC-MS/MS	0.8 µg/kg	2.6 µg/kg	Trier et al. (2010)
Urine	HPLC-EC	1.4 µg/L	NR	NIOSH (2003)
Blood	HPLC-FD	NR	3 µg/L	Chen et al. (2015)
Blood	GC-MS	1 ng/L	NR	Lewalter et al. (2012)

CZE, capillary zone electrophoresis; EC, electrochemical detector; FD, fluorescence detector; FID, flame ionization detector; GC, gas chromatography; HPLC, high-performance liquid chromatography; LC, liquid chromatography; MS, mass spectrometry; MS/MS, tandem mass spectrometry; NR, not reported; ppm, parts per million; SERS, surface-enhanced Raman scattering.

(f) Tattoo inks

Aniline is an ingredient in tattoo inks (Hauri et al., 2005). Most tattoo inks on the European Union market are manufactured in the USA (Piccinini et al., 2015). At present, since the tattoo ink market represents only a marginal fraction of the global production of colourants, the pigments used in tattoo inks are not specifically produced for such purposes. More than 100 colourants and 100 additives are in use in tattoo inks, with numerous impurities found (Piccinini et al., 2016). Approximately 162 800 L of tattoo inks and permanent make-up products are estimated to be placed on the market in the European Economic Area each year (ECHA, 2017).

1.3 Measurement and analysis

An overview of the analytical methods for quantitative determination of aniline in various sample types is provided in Table 1.2. Several methods have been reported for the analysis of aniline, its derivatives, and other primary aromatic amines in various matrices. The most common techniques applied currently for quantitative determination of aniline and its derivatives are based on gas chromatography (GC) or high-performance liquid chromatography coupled to different types of detectors, depending on the type of sample matrix and required sensitivity.

Primary aromatic amines and colourants have been determined in polyurethane, nylon, and textile toys by extraction in water for 30 minutes at 40 °C, followed by acidification and filtration. The filtered extract was analysed with liquid chromatography-tandem mass spectrometry and the reported detection limit was

0.2 µg/g (limit of quantification not reported). Residual aniline was detected in one toy (0.4 µg/g) (the number of toys tested was not stated) (Abe et al., 2016).

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

(a) Water and sediments

The available studies show that aniline is photolytically degraded within about 4–11 hours under spring or summer conditions in the top layer of surface waters (ECHA, 2019a).

Aniline can enter the environment from anthropogenic sources during any stage in the production, storage, transport, use, and disposal of aniline itself or of aniline-containing materials, or possibly by atmospheric and waterborne transport from other countries (Government Canada, 1994). No aniline release data were reported in the Canadian National Pollutant Release Inventory in 2000–2007, indicating that facilities were manufacturing, processing, or otherwise using less than 10 tonnes annually (Health Canada, 2011).

Data for aniline are available for drinking-water, groundwater, fresh surface water, and effluents. Aniline has been reported at concentrations of 0.5–3.7 µg/L in surface water samples taken in the 1970s from some rivers in Germany (Neurath et al., 1977) and in the Netherlands (Greve & Wegman, 1975; Meijers & van der Leer, 1976). The aniline concentrations found in Germany were attributed to industrial chemical waste (Herzel & Schmidt, 1977). Aniline has been identified in the USA in the late 1970–80s in industrial effluents from oil shale recovery and oil refineries, and from chemical and coal conversion plants (Shackelford & Keith, 1976; Hushon et al., 1980). In surveys of groundwater conducted in Ontario, Canada, in the 1980s and 1990s, the concentration of aniline in samples collected near a landfill site and in the vicinity of a chemical company was 0.01 mg/L and up to 300 mg/L, respectively. Levels of aniline ranged up to 20 000 mg/L in samples of dense non-aqueous–phase liquid collected from an area located beneath former containment areas of a chemical plant in Ontario, Canada (Government Canada, 1994). Point source pollution assessments for aniline in groundwater have been modelled for the United Kingdom to be 3.6 mg/L and 0.04 mg/L at 50 m and at 100 m, respectively. Concentrations of aniline ranged from 0 to 0.18 µg/L in drinking-water, 0 to 720 µg/L in groundwater, and 0 to 12 µg/L in fresh surface water, whereas aniline was identified but not quantifiable in effluents. No data were available for aniline in marine surface waters (Rockett et al., 2014). In samples collected from a Chinese reservoir polluted by aniline, maximum aniline content was 1.9 mg/L in water (13 samples) and 0.06 mg/kg in fish (12 samples) (He et al., 2014).

In sediment and soil, there are two competing processes: biodegradation and the formation of non-hydrolysable covalent bonds to humic substances. This binding leads to long biodegradation half-lives for bound aniline of 350 and 3500 days for soil and sediment, respectively. In the European Commission Risk Assessment Report, it was assumed that approximately 80% of aniline is covalently bound in soil (European Commission, 2004). Measurements in sediment tests gave a similar observation (ECHA, 2019a).

(b) Food, beverages, and animal feeds

Information on levels of aniline in food for the general population was available from a study conducted in Germany more than 40 years ago; aniline was quantified in samples of selected fruit and vegetables (range, < 0.1–30.9 mg/kg), with the highest concentration found in carrots (30.9 mg/kg) (Neurath et al., 1977). Among animal feeds, rapeseed cake contained aniline at 120 mg/kg (Neurath et al., 1977). In the early 1990s, average concentrations of aniline were reported for apples (0.16 mg/100 g), cabbage

(0.25 mg/100 g), carrots (3.1 mg/100 g), and garlic (1.00 mg/100 g) (Duke, 2004). Other food sources in which aniline was expected but was not quantified included: cows' milk, tea (black, green, herbal, red), and corn (Duke, 2004). Aniline has also been found as a volatile component of black tea (Vitzthum et al., 1975). No aniline was detected in the 23 vegetable samples from the 2005 Canadian Total Diet Study and, of the 16 fruit samples, it was detected only in apples at an average concentration of 0.278 mg/kg (Cao et al., 2009). In crops treated with the pesticide buprofezin, aniline is generated during specific food-processing methods. Aniline was below the LOQ (0.01 mg/kg) in all processed fractions containing tomato (juice, puree, ketchup, paste, and canned tomato). Tomato puree, ketchup, and paste contained aniline at levels above the limit of detection (LOD) (0.0029–0.0036 mg/kg). No data were available on the possible presence of aniline in other food commodities derived from fruit treated in accordance with authorized uses of buprofezin (e.g. citrus, pome fruits, stone fruits, grapes, strawberries). As a main degradation product of the herbicide desmedipham, aniline is a relevant impurity, with a maximum content of 0.5 g/kg. The European Food Safety Authority (EFSA) concluded, based on the available data, that dietary exposure of consumers and/or livestock to residues containing free and/or conjugated aniline could not be excluded (EFSA, 2015, 2018).

(c) Tobacco and tobacco smoke

Zhu & Aikawa (2004) investigated the presence of aniline in indoor air in selected homes in Canada, and recorded concentrations of 0.011 μg/m^3 for non-smokers and 0.034 μg/m^3 for smokers. [The Working Group noted that this is four orders of magnitude lower than mean levels found in occupational settings.] Luceri et al. (1993) reported aniline concentrations of 35 ng/m^3 in an office with one smoker, 75 ng/m^3 in an office with two smokers, 150 ng/m^3 in a card-playing room, 170 ng/m^3 in a non-smoking train compartment, and 190 ng/m^3 in a hairdresser's salon. Palmiotto et al. (2001) reported the highest values of aniline in two hospital wards (351 and 483 ng/m^3), and other air concentrations obtained were 400–500 ng/m^3 in hospital waiting rooms (n = 2), 60–1650 ng/m^3 in a bar, two clubs, and a discotheque (n = 4), and 10–100 ng/m^3 in office spaces (n = 7).

Average aniline concentrations from cigarettes of nine different brands were 430–9570 ng/cigarette in mainstream smoke, and 5250–15 930 ng/cigarette in sidestream smoke (Goniewicz & Czogała, 2005). Another analysis of aniline in cigarette smoke also showed that levels of aniline were about 20–80 times higher in sidestream smoke than in mainstream smoke (120–809 ng/cigarette for mainstream versus 9467–18 100 ng/cigarette for sidestream smoke) (Luceri et al., 1993). Xie et al. (2013) measured aniline in mainstream cigarette smoke and found aniline concentrations in the range of 129 to 838 ng/cigarette (depending on method used and cigarettes chosen).

1.4.2 Occupational exposure

The main scenarios for occupational exposure to aniline are during the production and distribution of aniline, when used as a chemical intermediate in the production of other chemicals and products, and when handling and using products containing residual aniline. The Working Group searched for information in publicly available exposure registries and identified data only for the USA and Finland. In the United States National Occupational Exposure Survey (NOES), nearly 42 000 workers (15 000 women) were identified as having potential aniline exposure in 1981–1983, with the majority in machine-operator occupations. In the NOES survey, 82 000 workers (47 000 women) were identified as potentially exposed to aniline hydrochloride in 1981–1983, the majority in assembly (notably electrical and

electronic equipment assembly), soldering, or janitorial and cleaning occupations (CDC, 2011). [The Working Group noted that aniline hydrochloride can be used as a flux in brazing and/or soldering operations (Horowitz & Hallock, 2019), but no information in the NOES was identified to support the reported uses of aniline hydrochloride. The Working Group found no information on current uses.] In Finland, fewer than 100 workers were identified as having exposure to aniline or its salts (Saalo et al., 2016). [The Working Group noted that Finland is not involved in aniline production; most of these workers were in research and development and other non-manufacturing settings.] The available quantitative data on aniline exposure were insufficient to draw any conclusions on air concentrations generated, the degree of dermal exposure, and amount absorbed, for all these exposure scenarios. These main scenarios together with the available data on human exposure to aniline are described below. [The Working Group noted that data on occupational exposure to aniline hydrochloride were lacking.]

(a) Manufacture and distribution of aniline

Aniline is synthesized in closed systems, but there is potential for exposure during sampling (mainly enclosed) and analysis of the product, and during checks on fill levels, as well as when the system is opened for cleaning, maintenance, and repair work (ECB, 2004). In the risk assessment of aniline performed by the European Chemicals Bureau, exposure measurements for aniline production were provided by the manufacturers from three production sites (countries not specified) (ECB, 2004). Some additional information was reported by the Health and Safety Executive in the United Kingdom (HSE, 1997). The exposure estimates are given in Table 1.3.

For the hydrogenation method of production of aniline, a geometric mean (GM) exposure to aniline for all jobs combined (personal, 8-hour time-weighted average, TWA) was reported as below 0.04 mg/m^3 in the unspecified western European countries (during 1990–96), whereas the arithmetic mean was 0.45 mg/m^3 (range, ≤ 2.8 mg/m^3) in the United Kingdom (1991–1996) (ECB, 2004; Table 1.3). Mean aniline exposure was highest in production and pilot plants (0.08 mg/m^3 and 0.07 mg/m^3, respectively), whereas the highest reported exposure measurement (2.7 mg/m^3) was measured in the workshop (no information on task was given). Whether this is representative of other production sites and time periods is not known. Nevertheless, aniline exposure measurements provided by the producers for operations performed during the 1980s indicate 8-hour TWA exposures ranging between non-detectable (detection limit not specified) and up to 1.4 ppm [5.3 mg/m^3] (Van Wagenen, 1985). The highest levels were reported for "maintenance" and "operations" (1.4 ppm [5.3 mg/m^3] and 0.86 ppm [3.3 mg/m^3], respectively). No short-term measurements were identified. [The Working Group noted that sampling times were infrequently reported in these publications. Whether any of the workers performed work with a potential for exposure as described above (e.g. opening of the vessels) was not reported.]

Urinary concentrations of aniline have been reported for 43 chemical-plant workers primarily synthesizing and processing aniline and 4-chloroaniline (Riffelmann et al., 1995; Table 1.4). The median urinary concentration of aniline was 7.7 µg/L and 9.6 µg/L for smokers and non-smokers, respectively. Although the range of aniline concentrations released from haemoglobin appeared to be wider among non-smokers (200–7000 ng/L) than smokers (320–1100 ng/L), the concentration did not differ statistically significantly between the two. The concentration of 4-aminobiphenyl released from haemoglobin differed statistically significantly between smokers and non-smokers (median, 17.5 ng/L versus 7.0 ng/L, $P = 0.0001$), being lowest among the non-smokers (Riffelmann et al., 1995).

Table 1.3 Occupational exposure to aniline in air: personal measurements

Reference	Collection year, country	Production process	Department, task, occupation	No. of samples	Sampling duration	Exposure concentration (mg/m³)	Exposure range (mg/m³)
Production of aniline							
ECB (2004)[a]	1990–1994, NR	Aniline production, reduction using H_2	Workplace or occupational group not defined	27	8-h TWA	NR	< 0.8
	1990–1996, NR	Aniline production, reduction using H_2	All workplaces	238		GM, < 0.04	< 1
			Production	53		GM, 0.08	0–0.9
			Pilot plants	9		GM, 0.07	0–0.56
			Filling area/store	3		NR	0.01–0.4
			Workshop	3		NR	0.02–2.7
ECB (2004), HSE (1997)	1991–1996, UK	Aniline production, reduction, using H_2	NR	152	8-h TWA	AM, 0.45	≤ 2.8
ECB (2004), HSE (1997)	1991–1996, UK	Maintenance	Maintenance	29	8-h TWA	AM, 0.4	≤ 1.8
ECB (2004)[a]	1990–1994, NR	Aniline production, reduction using Fe (Bechamp process)	NR	9	8-h TWA	NR	< 0.8
				6		NR	0.95–1.5
Van Wagenen (1985)[a]	1984, USA	Tank car loading	Operator (task-based)	1	83 min	< 0.4 ppm [< 1.52]	NR
			Area sample (1 ft [0.3 m] downward of the open tank hatch)	1		2 ppm [7.62]	NR
	1982, USA Data provided by USS Chemicals	Production of aniline (by high-pressure catalysed reaction of phenol with ammonia)	Aniline operator (field)	8	8-h samples	All non-detectable	NR
			Aniline technician	3			
			Aniline foreman	3			
			Aniline maintenance man (working in processing area)	12			
			Aniline chemist (laboratory)	3			
			Aniline analyst (laboratory)	3			

Table 1.3 (continued)

Reference	Collection year, country	Production process	Department, task, occupation	No. of samples	Sampling duration	Exposure concentration (mg/m³)	Exposure range (mg/m³)
Van Wagenen (1985)[a] (cont.)	1983, USA Data provided by SOCMA, 5 producers (1982 operations)		Operations Maintenance Quality control Warehouse/shipping Other (incl. supervisory and engineering personnel)	184 215 112 47 78	8-h TWA	NR	0.001–0.86 ppm [0.004–3.28] 0.001–1.4 ppm [0.004–5.33] 0.001–0.4 ppm [0.004–1.5] 0.01–0.6 ppm [0.04–2.3] 0.01 ppm [0.04]
Manufacture of rubber chemicals							
Hanley et al. (2012)	1990, USA	Rubber-chemical manufacturing plant Data collected by NIOSH	All jobs combined	45		GM (GSD), 0.032 (1.83) ppm [0.12 (6.97)]	0.014–0.19 ppm [0.053- 0.72]
			Antioxidant process	17		GM (GSD), 0.041 (2.00) ppm [0.16 (7.62)]	0.016–0.19 ppm [0.061–0.72]
			Maintenance	7		GM (GSD), 0.037 (1.60) ppm [0.14 (6.09)]	0.022–0.083 ppm [0.08–0.32]
			Recycle process	3		GM (GSD), 0.046 (2.72) ppm [0.18 (10.4)]	0.015–0.10 ppm [0.06–0.38]
			Accelerant process	18		GM (GSD), 0.023 (1.36) ppm [0.09 (5.18)]	0.014–0.042 ppm [0.05–0.16]
Hanley et al. (2012)[a]	1976–1979	Rubber-chemical manufacturing plant, USA Data collected by company	Rubber chemicals	36		GM (GSD), 0.081 (5.3) ppm [0.31 (20.2)]	0.026–1.7 ppm [0.10–6.48]
	1980–1994			200		GM (GSD), 0.015 (3.9) ppm [0.06 (14.9)]	0.0010–1.8 ppm [0.004–6.86]
	1995–2004			127		GM (GSD), 0.0021 (4.0) ppm [0.008 (15.2)]	0.000091–0.27 ppm [0.0003–1.03]
	1980–1994		Maintenance	43		GM (GSD), 0.0042 (3.5) ppm [0.016 (13.3)]	0.00069–0.39 ppm [0.003–1.49]
	1995–2004			63		GM (GSD), 0.00091 (4.7) ppm [0.003 (17.9)]	ND (< 0.00013) to 0.25 ppm [ND (0.0005) to 0.95]
	1980–1994		Laboratory	4		NR	0.0018–0.0038 ppm [0.007–0.014]
	1980–1994		Warehouse	2		NR	ND (< 0.096) ppm to ND (< 0.10) [< 0.366–0.38]
	1995–2004			11		NR	ND (< 0.0002) to 0.0020 ppm [< 0.0008–0.008]

Table 1.3 (continued)

Reference	Collection year, country	Production process	Department, task, occupation	No. of samples	Sampling duration	Exposure concentration (mg/m³)	Exposure range (mg/m³)
Hanley et al. (2012)[a] (cont.)	1980–1994		Vinyl chemicals	38		NR	ND (< 0.0005) to 0.00095 ppm [< 0.002–0.004]
Manufacture of dyes and colouring agent							
ECB (2004)[a, b]	NR	Further processing, initial dye products	NR	14	8-h TWA	NR	< 0.8
ECB (2004) HSE (1997)	NR, United Kingdom	Further processing of aniline to dyes	NR	NR	8-h TWA	NR	< 2 0.32 < 0.08
Further processing of aniline to other chemical agents							
ECB (2004)[a]	1990–1994, NR	Further processing to MDA	NR	20 15	8-h TWA	NR	< 0.8 < 0.08
	1993–1994, NR	Further processing to NaMBT	NR	5		NR	< 0.08
	1990–1994, NR	Further processing to organic products	NR	82		NR	< 0.8
	NR	Further processing to phenylhydrazine	NR	4 2		NR	< 0.8 < 0.1
	NR	Further processing to acetoacetic anilide	NR	13		NR	< 0.8
	1988–1994, NR	Further processing of aniline	NR	NR		NR	0.01–0.2
	1990–1996, NR	Further processing to dyes, plant protection and initial pharmaceutical products	NR	141		GM, 0.04	0–0.8
ECB (2004) HSE (1997)	1993, UK	Further processing of fine chemicals	NR	26	8-h TWA	AM, 1	0.16–3.6
	1992–1995, UK	Further processing of aniline to rubber chemicals		176 1 62	1–30 min	GM, 0.04–0.12	< 2 2.4 < 0.5–6
	1992–1996, UK			277 2	8-h TWA	GM, 0.04–0.12	< 2 2.4, 4.7
	NR, UK	Laboratory	NR	4		NR	< 0.64

Table 1.3 (continued)

Reference	Collection year, country	Production process	Department, task, occupation	No. of samples	Sampling duration	Exposure concentration (mg/m³)	Exposure range (mg/m³)
Use of products containing residual aniline							
Menichini et al. (1989)	NR, Italy	Production of remoulded tyres	Vulcanization of various tyres	10	6–7 h	AM, 0.005 [calculated from reported measurements]	0.0003–0.0098
Renman et al. (1986)	NR, Sweden Area samples (stationary)	Iron and steel foundries (use of polyurethane as core binder)	Casting, pouring station	8	NR	AM, 0.025	0.004–0.087
			Casting, operator's cabin	8		AM, 0.015	0.009–0.019
			Casting, manual raking of mould overflow	8		AM, 0.033	0.007–0.087
			Cooling	8		AM, 0.037	0.007–0.098
			Mould shake-out	8		AM, 0.032	0.009–0.078
		Aluminium foundry (use of polyurethane as core binder)	Manual pouring	2		NR	0.16–0.37
			Cooling, stationary sampling	2		NR	0.51–1.8
Westberg et al. (2001)	Sweden, 1992–1995	Aluminium foundry	All combined	33	Daily TWA	GM, 0.65	< 0.1–6.4
		Aluminium sand foundries	Moulding	4		GM, 2.0	1.3–2.6
			Pouring	4		GM, 2.2	2.0–0.6
			Shake-out	5		GM, 3.9	0.20–6.4
		Aluminium static die casting	Static die casting	10		GM, 0.31	0.1–1.3
			Core knock out	2		GM, 0.21; GSD, 1.1	< 0.27 to < 0.31

AM, arithmetic mean; GM, geometric mean; GSD, geometric standard deviation; MDA, 4,4′-methylenedianiline; NaMBT, sodium 2-mercaptobenzothiazole; ND, not detected; NIOSH, National Institute for Occupational Safety and Health; NR, not reported; ppm, parts per million; TWA, time-weighted average.

[a] Data provided by the producer.

[b] Workplace measurements from three production sites were submitted by industry. Workplaces, activities, durations of exposure and collectives of exposed persons were not described by all of the companies in a sufficiently differentiated manner.

Table 1.4 Studies on biological monitoring of aniline and its adducts after occupational exposure

Reference	Year, country	Occupational description	No. of participants	Aniline exposure (mg/m³)	Urinary aniline (µg/L)	Aniline–Hb (ng/L)	4-ABP–Hb (ng/L)	Met-Hb (%)
Käfferlein et al. (2014)	NR, Germany	Experimental study (volunteers; imitation of aniline manufacturing-industry conditions but without gloves or breathing masks; 4 × 20 min exercise to individually predetermined aerobic/anaerobic threshold during the 4 × 2 h exposure) Main study (volunteers; imitation of aniline manufacturing-industry conditions but without gloves or breathing masks; 3 × 20 min exercise to individually predetermined workload representing ventilation ~30 L/min during the 3 × 2 h exposure)	Pilot study: 4 volunteers	2 ppm [7.6]	Mean post-shift: 168.9 (SD, 80.2) Range, 138.9–305.6	NR	NR	1.21 (SD, 0.29) Range, 0.90–1.57 (maximum after 6 h, plateau)
			Main study: 19 volunteers	2 ppm [7.6]	Mean post-shift: 168.0 (SD, 51.8) Range, 79.5–418.3			1.21 (SD, 0.29) Range, 0.80–2.07 (maximum after 6 h, plateau)
Production of aniline								
Thier et al. (2001)	NR, Germany	Nitrobenzene reduction plant Low ambient exposure, potential for significant dermal exposure	80 workers	Previous mean concentrations 1987–1999: 1.20 (SD, 1.21)	NR	All (n = 75): 5180 (SD, 5192)	All (n = 75): 6.39 (SD, 5.48)	NR
			Non-smokers (n = 15)			4589 (SD, 4609)	3.96 (SD, 5.17)	

Table 1.4 (continued)

Reference	Year, country	Occupational description	No. of participants	Aniline exposure (mg/m³)	Urinary aniline (µg/L)	Aniline–Hb (ng/L)	4-ABP–Hb (ng/L)	Met-Hb (%)
Thier et al. (2001) (cont.)			Smokers (n = 18)			6872 (SD, 5003)	10.3 (SD, 3.66)	
Riffelmann et al. (1995)	NR, Germany	Primarily synthesis and processing of aniline and 4-chloroaniline	Smokers (n = 22)	NR	Mean, 13.9 (SD, 17.3); median, 7.7 (range, 1.1–58.7)	Mean, 680.9 (SD, 213.4); median, 700 (range, 320–1100)	Mean 19.9 (SD, 7.1); median, 17.5 (range, 10–35)	NR
			Non-smokers (n = 21)		Mean, 21.9 (SD, 31.4); median, 9.6 (range, 0.0–134.0)	Mean, 1025.2 (SD, 1432.7); median, 650 (range, 200–7000)	Mean, 7.3 (SD, 3.6); median, 7.0 (range, 3.0–20)	
Manufacture of rubber chemicals								
Ward et al. (1996)	1990, USA	Manufacture of rubber chemicals	Exposed (n = 42 post-shift)	Measurements on a subset of the workers during week before sampling: I. AM, 0.187 (SD, 0.181) II. AM, 0.153 (SD, 0.095)	Pre-shift: AM, 14.1 (SD, 16.6) Post-shift: AM, 29.8 (SD, 25.7)	AM, 17 441 (SD, 8867) pg/g Hb	AM, 81.7 (SD, 106.1) pg/g Hb	NR
			Non-smokers (n = 27)		Pre-shift: AM, 11.3 (SD, 11.9) Post-shift: AM, 22.6 (SD, 11.9)	AM, 16 072 (SD, 7422) pg/g Hb		
			Smokers (n = 15)		Pre-shift: AM, 19.4 (SD, 22.4) Post-shift: AM, 42.9 (SD, 37.3)	AM, 19 776 (SD, 10 748) pg/g Hb		

Table 1.4 (continued)

Reference	Year, country	Occupational description	No. of participants	Aniline exposure (mg/m^3)	Urinary aniline (µg/L)	Aniline–Hb (ng/L)	4-ABP–Hb (ng/L)	Met-Hb (%)
Ward et al. (1996) (cont.)			Non-exposed (n = 25 post-shift)	NR	Pre-shift: AM, 2.6 (SD, 2.4) Post-shift: AM, 3.9 (SD, 2.8)	AM, 3163 (SD, 1302) pg/g Hb	AM, 74.5 (SD, 63.8) pg/g Hb	
			Non-smokers (n = 16)		Pre-shift: AM, 1.6 (SD, 1.1) Post-shift: AM, 2.6 (SD, 1.8)	AM, 3118 (SD, 1513) pg/g Hb	AM, 48.2 (SD, 52.3) pg/g Hb	
			Smokers (n = 9)		Pre-shift: AM, 4.2 (SD, 3.1) Post-shift: AM, 6.2 (SD, 2.9)	AM, 3240 (SD, 905) pg/g Hb	AM, 119.3 (SD, 58.1) pg/g Hb	
Manufacturing of rubber and rubber goods								
Korinth et al. (2007)	NR, Germany	Supplier for the automobile industry Manufacturing of rubber products (mixing raw materials, semi-finishing/assembling, curing, deburring and final inspection of the products) 51 workers	Non-smokers (n = 15)	Range, 0.001–0.0374 Median, 0.0025 Mean, 0.0066 95th percentile, 0.0374	Range, 3.2–37.6 Median, 12.2 Mean, 12.7 95th percentile, 37.6	Range, 367–2662 Median, 1112 Mean, 1213 95th percentile, 2662	NR	NR
			Smokers (n = 36)	Range, 0.0003–0.0483 Median, 0.0033 Mean, 0.0067 95th percentile, 0.0335	Range, 2.2–37.0 Median, 10.2 Mean, 11.8 95th percentile, 36.4	Range, 351–2584 Median, 933 Mean, 1042 95th percentile, 2578		

Table 1.4 (continued)

Reference	Year, country	Occupational description	No. of participants	Aniline exposure (mg/m³)	Urinary aniline (µg/L)	Aniline–Hb (ng/L)	4-ABP–Hb (ng/L)	Met-Hb (%)
NIOSH (1981)	1980, USA	Production of sporting goods (baseball gloves and basketballs):		NR		NR	NR	NR
		Lacer	*n* = 13		Range, 1.0–5.8			
		Machine operator	*n* = 7		Range, 1–19.6			
		Sorter	*n* = 1		Range, 1.0			
		Cementer	*n* = 2		Range, 1–2.1			
		Packing	*n* = 1		Range, 1.2–2.1			
		Welder	*n* = 2		Range, 1.7–6.3			
		Inspector	*n* = 1		Range, 1.0–1.5			
Manufacture of dyes								
Beyerbach et al. (2006)	India, 1993	Manufacture of benzidine dihydrochloride (4 factories)	15 workers	NR	NR	Mean, 284 (SD, 221) pmol/100 mg Hb	Mean, 392 (SD, 452) pmol/100 mg Hb	NR
		Manufacture of Direct Black 38 (benzidine-dihydrochloride and aniline)	18 workers			Mean, 90.1 (SD, 87.6) pmol/100 mg Hb	Mean, 35.2 (SD, 43.9) pmol/100 mg Hb	
		Building construction company (reference)	15 workers			Mean, 1.37 (SD, 1.04) pmol/100 mg Hb	Mean, 0.12 (SD, 0.06) pmol/100 mg Hb	

4-ABP–Hb, 4-aminobiphenyl–haemoglobin adducts; AM, arithmetic mean; aniline–Hb, aniline–haemoglobin adducts; met-Hb, methaemoglobin; NR, not reported; ppm, parts per million; SD, standard deviation.

A second study reported on 75 workers having a mean concentration of aniline–haemoglobin adducts of 5180 ng/L (standard deviation, SD, 5192 ng/L), being somewhat higher in a subgroup of smokers than in non-smokers (Thier et al., 2001). The corresponding concentration for 4-aminobiphenyl–haemoglobin was 6.39 ng/L (SD, 5.48 ng/L). The study did not measure ambient aniline exposure, but aniline concentration in the workplace air of the aniline-production plant measured periodically between 1987 and 1999 was reported to be 1.20 mg/m^3 (SD, 1.21 mg/m^3). No information on the time lag between the exposure measurements and the biological monitoring was provided.

(b) Chemical industry

(i) Rubber-chemical manufacturing

Aniline is one of the agents used as a vulcanization accelerator and antioxidant during rubber processing. Although the accelerators and antioxidants are synthesized in confined systems, there is potential for inhalation and dermal exposure during several work tasks during which the processing equipment is opened. These tasks include changing sparkler filters, unclogging frozen recycle pipelines, repairing and maintaining pumps and pipes, collecting raw material and recycle samples, laboratory testing, and packing of the finished product (Hanley et al., 2012).

Although manufacture of rubber chemicals is the best-characterized scenario with respect to aniline exposure, information on exposure level is still scarce (Table 1.3). In a retrospective exposure assessment performed at a rubber-chemical manufacturing plant, mean (GM) exposure to aniline for all jobs combined was 0.032 ppm [0.12 mg/m^3] in 1990 (Hanley et al., 2012). The exposure assessment was performed by the National Institute for Occupational Safety and Health (NIOSH) and was based on historical exposure monitoring data provided by the company (1976–2004) and on data collected by NIOSH in 1990. The exposure was highest for the antioxidant process and recycle process (GM, 0.041 ppm [0.16 mg/m^3] and 0.046 ppm [0.18 mg/m^3], respectively). The highest measured value did not exceed 0.2 ppm [0.76 mg/m^3] for any of the processes. Historically, exposure measurements provided by the company indicated that the exposure level to aniline in the rubber-chemical departments (all jobs combined) declined over the decades: GM, 0.081 ppm [0.31 mg/m^3] (1976–1979); GM, 0.015 ppm [0.057 mg/m^3] (1980–1994); and GM, 0.0021 ppm [0.008 mg/m^3] (1995–2004). This is in line with the changes of procedures and implementation of exposure-reducing measures as outlined in Hanley et al. (2012). In 1992–1995, short-term exposure measurements (n = 62; sampling time, 1–30 minutes) indicated a potential for higher exposure over a shorter time period (range, < 0.5–6 mg/m^3; mean not reported) (ECB, 2004).

No data on exposure before 1976 were available to the Working Group.

Biological monitoring of workers employed in the manufacture of rubber additives in the 1980s and 1990s has demonstrated a statistically significant increase in urinary aniline levels from pre-shift to end of shift (Ruder et al., 1992; Stettler et al., 1992; Ward et al., 1996; Table 1.4). Furthermore, the exposed workers had a significantly higher post-shift mean level than the assumed non-exposed workers (32.3 versus 3.8 µg/L) (Ruder et al., 1992). Correspondingly, exposed workers had a significantly higher level of aniline–haemoglobin adducts than non-exposed workers (17 441 pg/g haemoglobin versus 3163 pg/g haemoglobin). There were no significant differences in respect to 4-aminobiphenyl–haemoglobin adducts between the two groups. The mean ambient aniline concentration measured the week before sampling for seven study participants who had personal air samplers was below 0.2 mg/m^3 (Ward et al., 1996).

[The Working Group noted that the studies by Ruder et al. (1992), Stettler et al. (1992), Teass et al. (1993), Ward et al. (1996), and Hanley et al. (2012) were performed in the same plant as a cohort study (Carreón et al., 2014) of incident cases of bladder cancer, as described in Sections 1.6.1 and 2.1.] Some of these studies also reported on the impact of tobacco smoking on biomarker levels (Ruder et al., 1992; Riffelmann et al., 1995; Ward et al., 1996; Thier et al., 2001; Korinth et al., 2007; Table 1.4). The results were conflicting with respect to the relative contribution of smoking to overall exposure to aniline.

(ii) Manufacture of rubber and rubber goods

Aniline is used as a vulcanization accelerator and antioxidant during rubber processing. Data on ambient air exposure stratified on job groups or work task during manufacture of rubber are lacking. Non-smoking workers manufacturing rubber products for the automobile industry (three plants, all jobs combined) had a median aniline exposure of 2.5 μg/m^3 (range, 1.0–37.4 μg/m^3) in the breathing zone, and a median concentration of aniline in urine post-shift of 12.2 μg/L (range, 3.2–37.6 μg/L). The work tasks included mixing raw materials, semi-finishing/assembling, curing, deburring, and final inspection of the products. Exposure did not differ significantly for smokers, with a median aniline exposure in the breathing zone of 3.3 μg/m^3 (range, 0.3–48.3 μg/m^3) and a median aniline concentration in urine post-shift of 10.2 μg/L (range, 2.2–37.0 μg/L) (Table 1.4; Korinth et al., 2007).

(iii) Processing of aniline to other chemical products, including dyes

Aniline is used as an intermediate in the production of other chemical products, including dyes and colouring agents, methylenediamine and related compounds (methylene diphenyl diisocyanate), pesticides, and pharmaceutical products.

For manufacturing of dyes and other chemical products, the data on aniline exposure are scarce. The limited available data provided by the manufacturers indicated that full-shift exposure is mainly below 1 mg/m^3 (ECB, 2004; Table 1.3).

Short-term exposure measurements (1990–1995) during further processing of aniline into organic products [not specified] involving tasks such as filling of drums, work at the filter press, sampling, container-closing work, and sieve cleaning (n = 96; duration, < 60 minutes) ranged between 0.8 and 12 mg/m^3 (ECB, 2004). [The Working Group noted that the lack of specificity about the organic products that were processed makes the utility of these reports uncertain.] In 1950, workers in the indigo-production area of an aromatic amine dye-manufacturing plant (Ott & Langner, 1983) were exposed to 8-hour TWA aniline concentrations of between 2.0 ppm [7.6 mg/m^3] and 8.4 ppm [32 mg/m^3] (this study is described further in Section 2).

Workers in India in 1993 had a mean aniline adduct level of 284 pmol/100 mg haemoglobin (SD, 221 pmol/100 mg haemoglobin) for those involved in the manufacture of benzidine dihydrochloride, and 90.1 pmol/100 mg haemoglobin (SD, 87.6 pmol/100 mg haemoglobin) for those working with Direct Black 38 dye (Beyerbach et al., 2006). [Ambient air or urinary aniline concentrations were not measured.]

(c) Use of products containing residual aniline

Exposures may occur during the handling of formulations with residual aniline contents, e.g. use of dyes containing residual aniline (e.g. textile industry), use of adhesives containing residual aniline (engineering, device- and tool-construction industries), or if aniline appears during further processing as a result of decomposition, e.g. in foundries where polyurethane is used as a core binder, during vulcanization of rubber plastics, and rubber processing and electrical engineering (ECB, 2004); however, there is a lack of data on exposure to aniline for all these exposure

scenarios, except for aniline exposure in foundries. For iron, steel, and aluminium foundries, reported 8-hour TWA exposure measurements between 1988 and 1995 ranged from 0.004 to 6.4 mg/m^3 (ECB, 2004), whereas all measurements collected during production of remoulded tyres in one study in Italy were below 0.01 mg/m^3 (Menichini et al., 1989).

One possible source of aniline exposure is through contact with synthetic turf made from crumb rubber. There are more than 12 000 synthetic turf fields in the USA. The United States National Toxicology Program (NTP) conducted a study to improve characterization of potential human exposure to and biological activity of aniline. A crumb rubber lot prepared by combining material from multiple commercial sources was analysed using a variety of techniques to generate information on chemical and physical characteristics. Data from a combination of analyses for volatile organic compounds identified 33 compounds totalling ~0.0007% by weight in crumb rubber, with an average contribution of aniline of 1 ppm [3.81 mg/m^3] by headspace gas chromatography-mass spectrometry (GC-MS) (NTP, 2019).

(d) Information gaps

Although aniline has been considered a potential occupational carcinogen since the 1970s, exposure data for aniline are scarce for all industries and scenarios where there is a significant potential for exposure to aniline. In particular, there is a lack of detailed exposure assessments stratified on production processes, job categories, and tasks. This information is needed to better define high-risk processes and individuals. Information on exposure to aniline hydrochloride was very sparse and inconsistent.

The database is insufficient to determine the magnitude of aniline exposure for workers with a potential exposure during: (i) production of aniline; (ii) manufacturing of rubber chemicals; (iii) manufacturing of rubber and rubber goods; and (iv) manufacturing of dyes from aniline. Data on aniline exposure could not be found for workers with a potential exposure during down-stream distribution of aniline, or further processing of aniline to other chemical products other than dye. Information on dermal exposure in any occupational exposure setting was not available to the Working Group. Although the production of MDI accounts for more than 90% of aniline use (see Section 1.2.3), the only available data on this scenario comprised 35 exposure measurements from 1990–1994 for processing of a starting material for production of polyurethane plastics (4,4′-methylenedianiline, MDA), provided by the industry (Table 1.3; ECB, 2004).

The majority of the available data on aniline exposure are reported in the USA and in European countries. Except for a study on haemoglobin adducts in a group of Indian workers producing azo dyes and benzidine (Beyerbach et al., 2006), no data were retrieved from the Asia-Pacific region, one of the major producers and consumers of aniline. No data on occupational exposure to aniline were reported from Africa, South America, Canada, or Australia.

Studies investigating occupational exposure to aniline have mainly included men, and hence the database is insufficient to conclude whether sex-specific differences exist with respect to aniline exposure burden; however, in a controlled study with 19 volunteers of which 9 were women, no statistically significant sex-specific differences in the urinary concentration of aniline or methaemoglobin were found after environmental exposure to aniline at 2 ppm [7.6 mg/m^3] for 6 hours (Käfferlein et al., 2014).

1.4.3 Co-exposure in the workplace

Table 1.5 summarizes the agents that have been reviewed by the *IARC Monographs* Working Group and are found in the same industries as aniline. There are nine agents for which there is *sufficient* or *limited* evidence for bladder

Table 1.5 Chemicals co-occurring occupationally with aniline that have *limited* or *sufficient* evidence of bladder carcinogenicity in humans (or structural similarity to such chemicals)

Chemical (CAS No.) or process	Use				IARC Group (Volume, year)	Cohort studies	Case series and reports[a]
	Aniline production	Rubber chemicals	Rubber goods	Dye production			
Chemicals that have limited *or* sufficient *evidence of bladder carcinogenicity in humans*							
2-Mercaptobenzothiazole (149-30-4)		✓			2A (Vol. 115, 2018)	Sorahan (2008)	
2-Naphthylamine (91-59-8)	✓			✓	1 (Vol. 100F, 2012)	Case et al. (1954)	Anon. (1921); Gehrmann (1936); Hueper (1938); Aboulker & Smagghe (1953); Vigliani & Barsotti (1961)
4-Aminobiphenyl (92-67-1)				✓	1 (Vol. 100F, 2012)		
4-Chloro-*ortho*-toluidine (95-69-2)				✓	2A (Vol. 99, 2010)		
Auramine production				✓	1 (Vol. 100F, 2012)	Case et al. (1954)	
Benzidine (92-87-5)	✓			✓	1 (Vol. 100F, 2012)	Case et al. (1954)	Anon (1921); Gehrmann (1936); Hueper (1938); Aboulker & Smagghe (1953); Vigliani & Barsotti (1961)
Magenta production				✓	1 (Vol. 100F, 2012)	Case et al. (1954)	Rehn (1895)
ortho-Toluidine (95-53-4)	✓	✓	✓	✓	1 (Vol. 100F, 2012)	Ott & Langner (1983); Sorahan (2008); Hanley et al. (2012); Carreón et al. (2014)	Rehn (1895); Hueper (1938); Aboulker & Smagghe (1953); Nakano et al. (2018)
Rubber production			✓		1 (Vol 100F, 2012)		
Other chemicals (structurally similar to known bladder carcinogens)							
Magenta (632-99-5)				✓	2B (Vol 100F, 2012)	Case et al. (1954); Case & Pearson (1954)	
Nitrobenzene (98-95-3)	✓	✓			2B (Vol. 65, 1996)	Hanley et al. (2012); Carreón et al. (2014)	Rehn (1895)
N-Phenyl-2-naphthylamine (135-88-6)		✓			3 (Suppl. 7, 1987)	Sorahan (2008)	
ortho-Anisidine (90-04-0)				✓	2B (Vol. 99, 2012)		
2,4-Xylidine (95-68-1)				✓	3 (Suppl. 7, 1987)		Hueper (1938)

Table 1.5 (continued)

Chemical (CAS No.) or process	Use				IARC Group (Volume, year)	Cohort studies	Case series and reports[a]
	Aniline production	Rubber chemicals	Rubber goods	Dye production			
ortho-Chloroaniline (95-51-2)				✓	1 (Vol. 100F, 2012)		
1-Naphthylamine (134-32-7)				✓	3 (Suppl. 7, 1987)	Case et al. (1954)	Gehrmann (1936); Aboulker & Smagghe (1953); Vigliani & Barsotti (1961)
Acenaphthene (83-32-9)				✓	3 (Vol. 92, 2010)	Ott & Langner (1983)	
2-Amino benzoic acid (118-92-3)				✓	3 (Suppl. 7, 1987)	Ott & Langner (1983)	

CAS, Chemical Abstracts Service; Suppl., Supplement; Vol., Volume.

[a] These case series and reports mention co-exposure of aniline with the listed agent, and are further described in Section 2.3.

carcinogenicity from studies in humans, and a further nine agents that have been previously reviewed by the *IARC Monographs* programme, but do not have *sufficient* or *limited* evidence for bladder carcinogenicity from studies in humans. For each agent, the industrial circumstances in which they could occur as co-exposures with aniline, the *IARC Monographs* classification, and the cohort or case-series publications in which they have been mentioned are reported. [The Working Group noted that it was not always clear whether the participants in the studies were co-exposed to aniline and these agents.]

Table 1.6 summarizes environmental and biological monitoring of occupational co-exposure to other bladder carcinogens among workers with aniline exposure.

(a) Primary aniline production

As noted in Section 1.4.2 above, work in primary production of aniline has been reported to be associated with increases in urinary concentrations of *ortho*-toluidine, 2-naphthylamine, and benzidine (Riffelmann et al., 1995). Workers in primary production of aniline are also exposed to nitrobenzene, which has not been determined to be a bladder carcinogen by the *IARC Monographs* programme.

(b) Manufacture of rubber chemicals

Workers in rubber-chemical production have potential co-exposure to two known or suspected bladder carcinogens: 2-mercaptobenzothiazole (IARC Group 2A) and *ortho*-toluidine (IARC Group 1). These workers may also be exposed to nitrobenzene (IARC Group 2B) and *N*-phenyl-2-naphthylamine (IARC Group 3) (Teass et al., 1993; Ward et al., 1996; Hanley et al., 2012). Reported levels of *ortho*-toluidine are given in Table 1.6.

In the retrospective exposure assessment performed at a rubber-chemical manufacturing plant by Hanley et al. (2012), the mean (GM) exposure to *ortho*-toluidine for all jobs combined in 1990 was 0.070 ppm [0.41 mg/m^3] (range, 0.020–0.37 ppm [0.18–2.17 mg/m^3]) (Table 1.6). The exposure was highest in the antioxidant process and recycle process (GM, 0.096 ppm [0.56 mg/m^3] and 0.086 ppm [0.50 mg/m^3], respectively). In a biomonitoring study on workers manufacturing rubber chemicals (n = 43), an exposure to *ortho*-toluidine the week before collection of biological samples was reported to be 0.412 mg/m^3 (SD, 0.37 mg/m^3). Among the exposed workers, the urinary *ortho*-toluidine concentration increased from 15.4 µg/L (SD, 27.1 µg/L) to 98.7 µg/L (SD, 119.4 µg/L) during the work shift (Ward et al. 1996). In a second study on rubber-chemical producers (n = 46), the mean urinary concentration of *ortho*-toluidine increased from 18 µg/L (SD, 27 µg/L) to 104 µg/L (SD, 111 µg/L) during the work shift (Teass et al., 1993). [In the latter study, the concentration of *ortho*-toluidine in the working atmosphere was not measured.] Co-exposure to nitrobenzene (in 1990) in the rubber-chemicals department was reported to range between 0.067 and 0.076 ppm [0.34 and 0.39 mg/m^3] for the antioxidant process but was not detected in the recycle process or the accelerant process (Hanley et al., 2012).

(c) Manufacturing of rubber and rubber goods

Workers manufacturing rubber and rubber goods are potentially exposed to *ortho*-toluidine. Non-smoking workers manufacturing rubber products for the automobile industry (three plants, all jobs combined) had a median *ortho*-toluidine exposure of 26.3 µg/m^3 (range, 0.1–524.0 µg/m^3) in the breathing zone, and a median *ortho*-toluidine concentration in urine post-shift of 6 µg/L (range, < LOD to 294.4 µg/L) (Korinth et al., 2007; Table 1.6). No quantitative exposure data were found for other co-exposures for this scenario.

Table 1.6 Studies on environmental and biological monitoring of occupational co-exposure to other bladder carcinogens among workers with aniline exposure

Reference	Country, year	Occupational description	No. of participants	TWA breathing zone exposure to *ortho*-toluidine (mg/m³)	Urinary arylamine concentrations (µg/L)			
					ortho-Toluidine	4-Chloro-*ortho*-toluidine	2-Naphthylamine	Benzidine
Production of aniline								
Riffelmann et al. (1995)	Germany, NR	Primarily synthesis and processing of aniline and 4-chloroaniline	22 smokers	NR	Mean, 0.6 (SD, 1.0); median, 0.0 (range, 0.0–2.8)	Mean, 1.6 (SD, 3.1); median, 0.4 (range, 0.0–14.2)	Mean, 3.9 (SD, 2.2); median, 3.9(range, 0.0–9.8)	Mean, 0.3 (SD, 0.6); median, 0.0 (range, 0.0–2.2)
			21 non-smokers		Mean, 0.4 (SD, 1.1); median, 0.0 (range, 0.0–4.2)	Mean, 2.6 (SD, 3.5); median, 0.0 (range, 0.0–10.4)	Mean, 2.1 (SD, 2.8); median, 1.7 (range, 0.0–11.6)	Mean, 0.1 (SD, 0.5); range, 0.0–0.0
Manufacture of rubber chemicals								
Hanley et al. (2012) [Same plant as in Ruder et al. (1992); Stettler et al. (1992); Teass et al. (1993); Ward et al. (1996)]	USA, 1990	Rubber-chemical manufacturing plant Data collected by NIOSH			NR	NR	NR	NR
		All jobs combined	45	GM, 0.070 (SD, 1.84) ppm [0.41 (10.8) mg/m³] Range, 0.020–0.37 ppm [0.12–2.17 mg/m³]				
		Antioxidant process	17	GM, 0.096 (SD, 1.74) ppm [0.56 (10.2) mg/m³] Range, 0.035–0.35 ppm [0.21–2.05 mg/m³]				
		Maintenance	7	GM, 0.086 (SD, 2.02) ppm [0.50 (11.9) mg/m³] Range, 0.054–0.37 ppm [0.32–2.17 mg/m³]				

Table 1.6 (continued)

Reference	Country, year	Occupational description	No. of participants	TWA breathing zone exposure to *ortho*-toluidine (mg/m³)	Urinary arylamine concentrations (µg/L)			
					ortho-Toluidine	4-Chloro-*ortho*-toluidine	2-Naphthylamine	Benzidine
Hanley et al. (2012) (cont.)		Recycle process	3	GM, 0.052 (SD, 2.35) ppm [0.31 (13.8) mg/m³] Range, 0.020–0.10 ppm [0.12–0.59 mg/m³]				
		Accelerant process	18	GM, 0.051 (SD, 1.50) ppm [0.30 (8.80) mg/m³] Range, 0.029–0.099 ppm [0.17–0.58 mg/m³]				
		Rubber-chemical manufacturing plant, USA Data collected by company			NR	NR	NR	NR
	1976–1979	Rubber chemicals	30	GM, 0.10 (GSD, 5.9) ppm [0.59 (34.6) mg/m³] Range, ND (< 0.023) to 1.8 ppm [ND (< 0.14) to 10.6 mg/m³]				
	1980–1994		200	GM, 0.015 (GSD, 3.2) ppm [0.09 (18.78) mg/m³] Range, 0.0025–1.5 ppm [0.015–8.80 mg/m³]				
	1995–2004		127	GM, 0.0028 (GSD, 3.8) ppm [0.016 (22.31) mg/m³] Range, 0.00021–0.22 ppm [0.001–1.29 mg/m³]				

Table 1.6 (continued)

Reference	Country, year	Occupational description	No. of participants	TWA breathing zone exposure to *ortho*-toluidine (mg/m³)	Urinary arylamine concentrations (µg/L)			
					ortho-Toluidine	4-Chloro-*ortho*-toluidine	2-Naphthylamine	Benzidine
Hanley et al. (2012) (cont.)	1980–1994	Maintenance	43	GM, 0.0049 (GSD, 4.4) ppm [0.03 (25.8) mg/m³] Range, 0.00051–0.12 ppm [0.0030–0.70 mg/m³]				
	1995–2004		63	GM, 0.0014 (GSD, 5.6) ppm [0.008 (32.9) mg/m³] Range, ND (< 0.0001) to 0.24 ppm [< 0.0006–1.41 mg/m³]				
	1980–1994	Laboratory	4	NR Range, 0.0018–0.002 ppm [0.011–0.012 mg/m³]				
	1995–2004		1	NR Range, ND (< 0.0020 ppm) [ND (< 0.012 mg/m³)]				
	1980–1994	Warehouse	2	NR ND (< 0.008 ppm) [0.047 mg/m³]				
	1995–2004		11	NA Range, 0.00020–0.0020 ppm [< 0.0012–0.012 mg/m³]				
	1980–1994	Vinyl chemicals	39	NA Range, < 0.0004–0.056 ppm [< 0.002–0.33 mg/m³]				

Table 1.6 (continued)

Reference	Country, year	Occupational description	No. of participants	TWA breathing zone exposure to *ortho*-toluidine (mg/m³)	Urinary arylamine concentrations (µg/L)			
					ortho-Toluidine	4-Chloro-*ortho*-toluidine	2-Naphthylamine	Benzidine
Ward et al. (1996)	USA, 1990	Manufacture of rubber chemicals	43 exposed	Measurements on a subset of the workers during the week before sampling: I. AM, 0.412 (SD, 0.366) II. AM, 0.516 (SD, 0.513)	Pre-shift: AM, 15.4 (SD, 27.1) Post-shift: AM, 98.7 (SD, 119.4)	NR	NR	NR
			Non-smokers (*n* = 28)		Pre-shift: AM, 16.1 (SD, 33.0) Post-shift: AM, 80.1 (SD, 94.0)			
			Smokers (*n* = 15)		Pre-shift: AM, 14.3 (SD, 10.2) Post-shift: AM, 132.1 (SD, 153.1)			
Teass et al. (1993) [Same data set and results in Stettler et al. (1992)]	USA, 1990	Manufacture of rubber additives (rubber antioxidant and rubber accelerator)	Unexposed (*n* = 31)	NR	Pre-shift: 1.1 (SD, 1.0) Post-shift: 2.7 (SD, 1.4)	NR	NR	NR
			Exposed (*n* = 46)		Pre-shift: 18 (SD, 27) Post-shift: 104 (SD, 111)			
Ruder et al. (1992) [Same dataset as in Teass et al. (1993) and Stettler et al. (1992)]			Exposed non-smokers (*n* = 29) Exposed smokers (*n* = 19)		Pre-shift: 17.5 Post-shift: 83.9 Pre-shift: 20.0 Post-shift: 135.6	NR	NR	NR

Table 1.6 (continued)

Reference	Country, year	Occupational description	No. of participants	TWA breathing zone exposure to *ortho*-toluidine (mg/m³)	Urinary arylamine concentrations (µg/L)			
					ortho-Toluidine	4-Chloro-*ortho*-toluidine	2-Naphthylamine	Benzidine
Manufacturing of rubber and rubber goods								
Korinth et al. (2007)	Germany, NR	Supplier for the automobile industry Manufacturing of rubber products (mixing raw materials, semi-finishing/ assembling, curing, deburring and final inspection of the products) 51 workers	Non-smokers (n = 15)	Range, 0.0001–0.524 Median, 0.0263 Mean, 0.0614 95th percentile, 0.524	Range, < LOD to 292.4 Median, 6.0 Mean, 38.6 95th percentile, 292.4	NR	NR	NR
			Smokers (n = 36)	Range, < LOD to 0.0939 Median, 0.0004 Mean, 0.011 95th percentile, 0.0725	Range, < LOD to 242.9 Median, 0.6 Mean, 14.5 95th percentile, 100.0			

AM, arithmetic mean; GM, geometric mean; GSD, geometric standard deviation; LOD, limit of detection; ND, not detected; NIOSH, National Institute for Occupational Safety and Health; NR, not reported; ppm, parts per million; SD, standard deviation; TWA, time-weighted average.

(d) Manufacturing of dye and pigments

In addition to aniline, workers in the manufacturing of dye and pigment intermediates are potentially exposed to bladder carcinogens including 2-naphthylamine, 4-aminobiphenyl, auramine, benzidine, magenta, and *ortho*-toluidine. These workers may also be exposed to *ortho*-anisidine, 2,4-xylidine, *ortho*-chloroaniline, 1-naphthylamine, and acenaphthene. No study on aniline-exposed workers reported any quantitative exposure data for these agents.

(e) Foundries

In foundry workers there is a potential for exposure to soot, which (in the setting of exposures among chimney sweeps) shows *limited* evidence of bladder carcinogenicity (IARC, 1984).

1.4.4 Exposure of the general population

The general population may be exposed to aniline via the environmental release of industrial effluents to air (Käfferlein et al., 2014), water, land, or groundwater (US EPA, 1994; ATSDR, 2002). Available quantitative information on concentrations of aniline in drinking-water in Canada was restricted to the results of a survey conducted in Québec in which this substance was not detected (i.e. concentrations were < 0.5 µg/L) in samples from 17 municipalities (Government Canada, 1994). The estimated aniline concentrations in drinking-water in England and Wales, UK, using the most effective and the least effective removal technique, were 0.0224 µg/L and 0.2245 µg/L, respectively (Rockett et al., 2014). A detailed study on 24 amines in a drinking-water treatment plant in Spain reported average aniline concentrations of 9.2 ng/L [0.009 µg/L] in the distribution system. These levels were subject to seasonal variation, increasing during high rainfall or cold temperature events to 13 ng/L [0.013 µg/L] and 11 ng/L [0.011 µg/L] on average, respectively (Jurado-Sanchez et al., 2012). Data from 15 dug wells in a coal industry area in Burnpur, West Bengal, India, showed that the average aniline concentration in groundwater of the study area was 0.242 mg/L [242 µg/L] (Mohanta & Mishra, 2020).

Cigarette smoking is one of the main contributors to aniline exposure in non-occupational environments. Most measurements showed considerable contamination with aromatic amines derived from sidestream tobacco smoke, which was detected also in parts of the buildings in which tobacco smoking was not allowed (Luceri et al. 1993). Air from aniline emissions from materials and products attains a high concentration in heavily contaminated indoor environments, due to tobacco smoking and poor ventilation. High concentrations of aniline in outdoor air (120–340 ng/m^3) were also measured in the centre of Florence and in the Brindizi industrial zone, in Italy (Palmiotto et al., 2001).

Aniline is a component of tobacco smoke. Smoking or inhaling sidestream smoke (environmental tobacco smoke) will therefore lead to aniline exposures (Luceri et al., 1993; Goniewicz & Czogała, 2005; Xie et al., 2013). A few studies have reported that the concentration of urinary aniline in smokers (3.1 µg/24-hour urine sample) is higher than in non-smokers (2.8 µg/24-hour urine sample) (el-Bayoumy et al., 1986). Among workers employed in manufacture of rubber additives but not exposed to aniline, urinary aniline concentrations were statistically significantly higher in smokers – pre-shift, 4.2 µg/L (SD, 3.1 µg/L); post-shift, 6.2 µg/L (SD, 2.9 µg/L) – than in non-smokers – pre-shift, 1.6 µg/L (SD, 1.1 µg/L); post-shift, 2.6 µg/L (SD, 1.8 µg/L) (Ward et al., 1996). Urinary concentrations of aniline in the two studies were approximately equivalent. [The Working Group noted that the study by Ward et al. (1996) was performed in the same plant in which a cohort study (Carreón et al., 2014) of incident cases of bladder cancer was conducted, as described in Section 1.6.1 and Section 2.1.]

Aniline can be released to the environment from products (described below) and building materials.

Indoor use of products includes, for example, automotive care products, paints and coatings or adhesives, and fragrances and air fresheners. Aniline was shown to migrate from polyamide cooking utensils (Brede & Skjevrak, 2004), and shoe polish (Zhu & Aikawa, 2004; Health Canada, 2011). In one study in Canada, air samples were collected during a shoe-polishing activity at home, and indoor aniline concentration increased sharply from 0.016 μg/m^3 to 0.53 μg/m^3 (Zhu & Aikawa, 2004). Although information from the European Union's Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) dossiers provided by registrants indicates the presence of aniline in a broad range of articles and consumer products, including fabrics, detergents, diapers, and feminine hygiene products (ECHA, 2020b), the Working Group was not able to identify data that would characterize and quantify these exposures.

Indoor use in long-life materials with low release rate includes, for example, flooring, furniture, toys, construction materials, curtains, footwear, leather products, paper and cardboard products, and electronic equipment. A residual aniline migration concentration of 0.4 μg/g was determined in a polyurethane toy (Abe et al., 2016).

Outdoor use in long-life materials with low release rate includes, for example, metal, wooden, and plastic construction and building materials.

Aniline content in tattoo inks ranges from 5 to 61 mg/kg (ECHA, 2019b). The European Chemicals Agency (ECHA) estimated that 12% of European citizens are tattooed and that this prevalence may be doubled in the younger generations (age, 18–35 years). [The Working Group noted that, as aniline is a constituent in cigarettes and tattoo colourants, individuals smoking tobacco and having their bodies tattooed will have a greater aniline exposure than others in the general population.]

Biomonitoring of aniline exposures has been reported (Dierkes et al., 2014). In 1986 in New York, USA, among healthy men aged 25–45 years, the urinary aniline concentration range was from 3.1 μg/24-hour urine sample in smokers to 2.8 μg/24-hour urine sample in non-smokers (el-Bayoumy et al., 1986). In a cross-sectional population-based survey in Germany, in which 93.9% of 1004 individuals had detectable urinary aniline concentrations, the mean urinary aniline concentration was 5.44 μg/L (range, 0.1–384.04 μg/L) (Kütting et al., 2009). Breast milk samples from smokers and non-smokers had quantifiable concentrations of aniline (0.05–5.2 μg/L). There was no statistically significant difference in the mean concentration of aniline in breast milk between smokers and non-smokers (DeBruin et al., 1999).

1.5 Regulations and guidelines

ECHA has classified aniline as carcinogenic (Category 2), mutagenic (Category 2), skin sensitizing (Category 1), damaging to eyes (Category 1), and causing acute toxicity (Category 3). Aniline and its salts are banned from use in any cosmetic products marketed for sale or use in the European Union. Workers who are under age 18 years, pregnant, or breastfeeding, may not be exposed to aniline. Employers are obliged to minimize worker exposure to aniline as far as possible, and must arrange for medical surveillance of exposed workers (ECHA, 2020c).

The United States Environmental Protection Agency (US EPA) has listed aniline as a hazardous air pollutant (HAP) (US EPA, 2018); its emissions are subject to regulation under the Clean Air Act Amendments. Under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), the reportable quantity for aniline is 5000 pounds [2.27 tonnes]. Releases of CERCLA hazardous substances, in quantities

equal to or greater than their reportable quantity, are subject to reporting to the National Response Center under CERCLA. Under the Emergency Planning and Community Right-To-Know Act (Section 313), or EPA's Toxics Release Inventory (TRI), emissions, transfers, and waste management data must be reported annually. Discarded commercial-grade aniline or container and spill residues are listed as a toxic waste under the U012 code. Additionally, aniline has a threshold planning quantity (TPQ) of 1000 pounds [0.45 tonnes] which triggers development of emergency response plans within communities and localities (US EPA, 2019). As a chemical that is known or anticipated to occur in public water systems and which may require regulation under the United States Safe Drinking-water Act, aniline is listed on the Contaminant Candidate List 3 (US EPA, 2009); however, it is currently not subject to any proposed or promulgated national primary drinking-water standard. As of 28 September 1979, notification must be given to the US EPA of any discharge into waterways of mixtures containing 454 kg or more of aniline (US EPA, 1979). As of 6 February 2020, the reportable quantity was 2270 kg (Office of the Federal Register, 2019b).

In the state of Washington, USA, aniline was added to the Children's Safe Product Act Reporting Rule as a chemical of high concern to children (CHCC) (Washington State Department of Ecology, 2018) which must be reported in children's products (Washington State Chemicals of High Concern Reporting Rule; Department of Ecology (2020)).

In the USA, the colour additives in tattoo pigments are subject to premarket approval under the United States Federal Food, Drug, and Cosmetic Act. However, the Food and Drug Administration (FDA) traditionally has not exercised regulatory authority for colour additives on the pigments used in tattoo inks (FDA, 2019).

In the European Union, tattoo inks are covered primarily by the General Product Safety Directive. Seven European Union Member States have developed specific tattoo legislation, three Member States have drafts in place, and others are developing various legislative instruments (Piccinini et al., 2016; ECHA, 2019b). These are informed by concentration limits proposed by ECHA in 2017; the limit for aniline is 0.0005% w/w (ECHA, 2017).

Under the Association of Southeast Asian Nations (ASEAN) Harmonised Cosmetic Regulatory Scheme, aniline, its salts, and its halogenated and sulfonated derivatives are on the list of substances which must not form part of the composition of cosmetic products. The colouring agent Solvent Red 23 (Colour Index number 26100), allowed for use exclusively in cosmetic products intended not to come into contact with the mucous membranes may contain ≤ 0.2% aniline (ASEAN, 2019). [The Working Group noted that these colouring agents would include tattoo ink.]

1.5.1 Occupational exposure limits

Occupational exposure limits for aniline exist in several countries, as shown in Table 1.7 (European Commission, 2016; ILO, 2011).

NIOSH considers aniline to be a potential occupational carcinogen as defined by the Occupational Safety and Health Administration (OSHA) carcinogen policy (NIOSH, 2012). No recommended exposure limit (REL) has been established (NIOSH, 2018).

Since the US EPA has identified aniline as a toxic waste, as of 19 November 1980, persons who generate, transport, treat, store, or dispose of aniline must comply with the regulations of the federal hazardous waste management programme. Included in the list of hazardous wastes are: distillation bottoms from aniline production, process residues from aniline extraction from the production of aniline, and combined wastewater streams generated from

Table 1.7 Occupational 8-hour exposure limits for aniline

Country	Year[a]	Limit value, 8 h			Status
		(mg/m³)	(ppm)	Interpretation	
Australia	2020	7.6	2	TWA	Safe Work Australia guideline
Austria	2011	8	2	TWA	Regulation
Belgium	2018	7.7	2	TWA	Royal decree
Canada, Ontario	2016		2	TWA	Ontario Ministry of Labour regulation
Canada, Québec	2020	7.6	2	TWA	Regulation
China	2019	3			
Denmark	2016	4	1	TWA	
European Union	2019	7.74	2	TWA	SCOEL Recommendation, Commission Directive
Finland	2019	1.9	0.5	TWA	Regulation
France[b]	2016	10	2	TWA	
Germany	2019	7.7	2	MAK	DFG Recommendation
Hungary	2000	8			Regulation
Ireland	2011	3.8	1		
Japan	2018	3.8	1	TWA	Guideline
Latvia	2019	0.1	1	TWA	WES
Mexico		10	2		
New Zealand	2020	4	1	TWA	Regulation
Poland	2011	1.9	0.8	TWA	
Romania	2018	3	0.8	TWA	
Singapore[c]	2020	7.6	2	PEL (long term)	Regulation
South Africa		10	2		
Republic of Korea		10			
Spain	2018	7.7	2		
Sweden	2020	4	1	TWA	Regulation
Switzerland	2020	8	2	TWA	
United Kingdom	2020	4	1	TWA	Regulation
USA	2020	19	5	PEL (TWA)	OSHA Regulation
		7.6	2	TLV	ACGIH recommendation

ACGIH, American Conference of Governmental Industrial Hygienists; DFG, Deutsche Forschungsgemeinschaft, German Research Foundation; MAK, maximale Arbeitsplatz-Konzentration, maximum workplace concentration; PEL, permissible exposure limit; OSHA, Occupational Safety and Health Administration; SCOEL, Scientific Committee on Occupational Exposure Limits; TLV, threshold limit value; TWA, time-weighted average; WES, workplace exposure standard.

[a] Year is either the year of latest update or if this is not given, the year is 2020 when this was accessed.

[b] In France, an 8-hour occupational exposure limit value of 7.6 mg/m³ has been established for aniline salts.

[c] In Singapore, an 8-hour occupational exposure limit value of 0.5 mg/m³ has been established for aniline salts.

From IRSST (2010), ILO (2011); IFA (2019).

nitrobenzene/aniline production (Office of the Federal Register, 2019c).

Additionally, as of 20 November 1980, shipments of aniline in the USA are subject to a variety of labelling, packaging, quantity, and shipping restrictions consistent with the designation of aniline as a hazardous material (IARC, 1982).

1.5.2 Reference values for biological monitoring of exposure

Nine Member States of the European Union (Croatia, Germany, Hungary, Ireland, Poland, Romania, Slovakia, Slovenia, and Spain) have adopted biological limit values (BLV) for aniline. In Romania and Slovenia, they are statutory or obligatory, whereas in the other countries they are facultative. In blood, BLVs vary between 0.015 and 0.05 mol methaemoglobin/mol haemoglobin (1.5–5%, at the end of the work shift), or < 100 µg/L of aniline in the erythrocyte fraction. The statutory urine BLVs are: *para*-aminophenol [4-aminophenol], 10 mg/g creatinine; or *para*-aminophenol-methaemoglobin, 10 µg/L, at end of shift (European Commission, 2019).

Table 1.8 provides reference values for biomarkers of exposure established by ACGIH (2018) and DFG (2018).

1.6 Quality of exposure assessment in key epidemiological studies

1.6.1 Exposure assessment in cohort studies

Occupational cohorts with potential exposure to aniline have been studied in aromatic amine dye-manufacturing plants in the United Kingdom (Case et al., 1954; Case & Pearson, 1954), and the USA (Ott & Langner, 1983), and in rubber-chemical manufacturing plants in the United Kingdom (Sorahan et al., 2000; Sorahan, 2008) and the USA (Hanley et al., 2012; Carreón et al., 2014). A review and critique of the exposure assessments conducted in these studies is provided in Table S1.9 (Annex 1, Supplementary material for Section 1, web only; available from: https://publications.iarc.fr/599).

(a) Exposure assessment methods used in cohort studies

(i) Manufacture of aromatic amine dyes

In an early study by the Association of British Chemical Manufacturers in the dye-manufacturing industry, "firms participating in the scheme were asked to provide a nominal roll of all workers known to have had any contact with aniline, benzidine, α-naphthylamine [1-naphthylamine] or β-naphthylamine [2-naphthylamine]" (Case et al., 1954). Exposure to aniline was not clearly defined. No exposure measurements were taken, and potential exposure to aniline (as "ever/never") was estimated from process records and work history. It was possible to identify aniline-exposed workers who were not also exposed to magenta, auramine, benzidine, or α- or β-naphthylamine (Case & Pearson, 1954). The reference group for statistical analyses was the general population of England and Wales, on the reasonable assumption that the general population during this period was not exposed to these substances.

A company conducted a small cohort study of mortality in employees who had worked at an aromatic amine dye-manufacturing plant in the USA (Ott & Langner, 1983). Employment history and process records were used to determine potential exposure to aniline and derivatives of *ortho*-toluidine, with aniline exposure considered to have been present in the indigo- and acetanilide-production processes. Other exposures in the indigo-production process were to chloroacetic acid, phenyl glycine, aniline tars, and indoxyl. Workers in the acetanilide-production process were also potentially exposed to acetic acid, acetic anhydride, and acetanilide. Reference was made to a 1950 industrial hygiene survey that measured levels of aniline exposure in the indigo-production area. This survey found that operators in the area were exposed to aniline concentrations of 2.0–8.4 ppm [7.6–32.0 mg/m^3]; however, this information was not used to assess

Table 1.8 Reference values for biomarkers of exposure to aniline[a]

Organization	Biomarker	Sampling time	Biological value	Value
ACGIH	Aniline in urine	End of shift	BEI	–
	Aniline released from haemoglobin in blood	End of shift	BEI	–
	para-Aminophenol in urine	End of shift	BEI	50 mg/L
Germany (DFG)	Aniline in urine after hydrolysis	End of shift	BAT	500 µg/L
	Aniline released from aniline–haemoglobin conjugate in the erythrocyte fraction of whole blood	After exposure for at least 3 months	BLW	100 µg/L
Switzerland (SUVA)	Aniline in urine after hydrolysis	End of shift	VBT	1 mg/L urine
	Aniline released from aniline–haemoglobin conjugate in the erythrocyte fraction of whole blood	After exposure for at least 3 months	VBT	100 µg/L
	para-Aminophenol in urine	End of shift	VBT	50 mg/g creatinine

ACGIH, American Conference of Governmental Industrial Hygienists; BAT, Biologische Arbeitsstoff-Toleranzwerte, biological tolerance value; BEI, biological exposure index; BLW, Biologische Leit-Werte, biological guidance value; DFG, Deutsche Forschungsgemeinschaft, German Research Foundation; SUVA, Schweizerische Unfallversicherung, Swiss National Accident Insurance Fund; VBT, valeurs biologiques tolérables, biologically tolerable values.

[a] Established by ACGIH (2018) and DFG (2018).

intensity of exposure, and assessment was limited to "ever/never" and duration of exposure. Workers with potential exposure to arsenic, vinyl chloride, or asbestos were not included in the analyses of risk associated with aniline exposure. The general population was used as the comparison group.

(ii) Rubber-chemical manufacturing

Sorahan et al. (2000) investigated mortality and cancer incidence in a cohort of 2160 male production workers from a manufacturer of vulcanization inhibitors and accelerators, antioxidants, and other proprietary products for the rubber industry, in Wales. Employment history and process records, reviewed by a former occupational hygienist employed in this factory, were used to estimate the potential for exposure to aniline as well as to *ortho*-toluidine, phenyl-β-naphthylamine [*N*-phenyl-2-naphthylamine] (PBN), and MBT. Aniline exposure was judged initially (Sorahan et al., 2000) to occur in four departments, but in the later update (Sorahan, 2008) aniline exposure was found in six departments, including a department that manufactured PBN. Quantitative estimates of exposure by different time periods were used to develop a detailed job-exposure matrix (JEM) for MBT; however, for aniline it was not possible to derive estimates more specific than duration of employment by department with potential for exposure. Periods of employment in the aniline department were classified separately from period of employment in the department in which there was potential exposure to *ortho*-toluidine; however, a significant proportion of workers had been exposed to more than one of the four agents of interest (Sorahan, 2008). [The Working Group noted that although 44% (266/611) of workers were members of more than one of the four subcohorts, it was impossible to calculate how many of the aniline-exposed workers had also been exposed to one of the other agents of interest.] In the updated analysis of mortality and cancer incidence in this cohort with revised exposure estimates, the analyses were performed using "ever/never" exposure status and duration of employment (0.1–4.9 years, ≥ 5 years),

adjusting for exposure to the other three agents of interest.

Carreón et al. (2014) conducted an updated investigation of the incidence of bladder cancer at a rubber-chemical manufacturing plant in the USA, using a comprehensive retrospective exposure-assessment methodology (Hanley et al., 2012). The exposure assessment incorporated reviews of historical process records, as well as company breathing-zone exposure-monitoring data for aniline, *ortho*-toluidine, and nitrobenzene covering the period 1976–2004, and exposure-monitoring data from a survey by NIOSH in 1990. The investigators described conducting a site visit and plant walk-through, and interviews with current and former employees, company management, and union representatives. While this comprehensive retrospective exposure assessment methodology was used to gain insight into exposures in the plant, (Hanley et al., 2012), subsequent analyses classified job title and department into one of four ordinal categories of exposure to an amalgamated exposure factor consisting of aniline, *ortho*-toluidine, and nitrobenzene combined (probably not exposed, probably exposed low and irregularly/occasionally, probably exposed low and regularly, definitely exposed moderate/high) (Carreón et al., 2014). Each department and job-title combination was assigned a relative rank (0–10) for these exposures within period of employment. The relative ranks were used to estimate the cumulative exposure rank defined as the product of the number of days in each department/job-title and the assigned rank, summed over all jobs worked. The study could not differentiate the effect of aniline alone or aniline adjusted for other exposures.

(b) Quality of exposure assessment methods in cohort studies

The exposure assessment methods of the key cohort studies cited in this monograph are evaluated according to five principal considerations: exposure opportunity, carcinogenic co-exposures, completeness of exposure history data, accuracy of exposure intensity measurement, and appropriateness of exposure metrics used in the epidemiological models of risk of cancer.

(i) Exposure opportunity

In an ideal cohort study of the carcinogenicity of aniline, workers with known exposure to aniline would be identified and differentiated from those who are clearly not exposed. Aniline exposure was specifically identified on an individual worker level by employers in Case et al. (1954), and by detailed process review in Sorahan (2008). In Ott & Langner (1983), the aniline exposure was measured only on a department level, and in Carreón et al. (2014), aniline exposure was not specifically identified.

(ii) Carcinogenic co-exposures

As reviewed in Section 1.4.3, there are several agents other than aniline used in both the dye and rubber industries (see Table 1.5 and Table 1.6). Some of these have been previously evaluated by the *IARC Monographs* programme as having *sufficient* or *limited* evidence for bladder carcinogenicity in humans: MBT, 2-naphthylamine, 4-aminobiphenyl, 4-chloro-*ortho*-toluidine, auramine production, benzidine, magenta production, rubber production, and *ortho*-toluidine. Others are similar chemicals for which the *IARC Monographs* programme has previously determined there is *inadequate* evidence for bladder carcinogenicity in humans: magenta, nitrobenzene, PBN, *ortho*-anisidine, 2,4-xylidine, *ortho*-chloroaniline, 1-naphthylamine, acenaphthene, and 2-amino benzoic acid.

Each of the cohort studies evaluated above identified co-exposure to some carcinogenic aromatic amines; however, only Sorahan (2008) was able to adjust for exposure to other carcinogens (*ortho*-toluidine, PBN, and MBT). That study also excluded workers with potential exposure to arsenic, vinyl chloride or asbestos. Case et al.

(1954) was able to divide the aniline-exposed subjects into those with or without exposure to magenta, leaving the number with exclusive exposure to aniline too small to observe a statistically significant effect if one were there. Neither of the other studies (Ott & Langner, 1983; Carreón et al., 2014) could conclusively differentiate the effects of the different substances.

(iii) Completeness of exposure histories

In each of the cohort studies evaluated, the work history records by job and department were provided by the employers. It is not possible to confirm the completeness of these records. The minimum period of employment required for an individual to be included in each cohort was not stated (Case & Pearson, 1954) not clear (Ott & Langner, 1983) or varied from 1 day (Carreón et al., 2014) to 6 months (Sorahan et al., 2000).

(iv) Accuracy of exposure intensity measurement

None of the cohort studies reviewed included quantitative estimates of aniline exposure. The chemical manufacturing study in the USA (Ott & Langner, 1983) and the rubber-chemical manufacturing study in the United Kingdom (Sorahan et al., 2000) both used the duration of employment in departments that used or produced aniline as a proxy for exposure intensity. Although the rubber-chemical manufacturing industry study in the USA included an intensity ranking, this was for aniline, *ortho*-toluidine, and nitrobenzene combined.

(c) Overall summary of exposure assessment in key cohort studies

The Working Group noted that the cohort studies reviewed all suffer from limitations in exposure assessment and/or in their ability to differentiate aniline-related effects from the effects of co-exposures. As described above, the exposure classifications are either: (i) ever/never employed in departments using aniline, with cumulative exposure assessment based on duration of employment; or (ii) ranked exposure to a combination of agents. The Working Group considered that in none of the studies was the exposure assessment of a sufficient standard to provide clear evidence that aniline was the responsible agent for the bladder cancer excesses observed.

1.6.2 Exposure assessment in case–control studies

See Table S1.10 (Annex 1, Supplementary material for Section 1, web only; available from: https://publications.iarc.fr/599).

(a) Exposure assessment methods used in case–control studies

Preti et al. (1988) conducted a hospital-based case–control study of bronchogenic carcinoma and volatile organic compounds in lung air in Pennsylvania, USA. [The Working Group noted that the selection criteria for controls included that they did not have chronic or acute lung diseases, had no industrial dust exposure, and had normal chest X-rays. These selection criteria resulted in the probability of exposure to aniline among the controls being less than that of the cases and thus may have biased the study.] Cases and controls were asked to exhale end-expiratory air into a Tedlar bag. The bags were immediately returned to the laboratory and the contents transferred to an adsorbent tube using a vacuum pump. The tubes were frozen until analysis, which was within 1 week of detection. Analyses were performed by desorbing the volatiles from the tubes onto the capillary column of the GC-MS system. [The Working Group noted that detection of aniline in exhaled breath at the time of diagnosis of lung cancer is not likely to be a good measure of exposures in the past that are likely to be more etiologically relevant given the long latency (i.e. > 15–20 years) of most occupational

and environmental carcinogens, and the short half-life of aniline in the body.]

Nizamova (1991) reported findings from a case–control study on bladder cancer in the Tambov manufacturing region of the Russian Federation. This region is described as having an advanced chemical industry, including aniline dyes. No details were provided on how exposure to aniline dyes and other chemicals was identified.

Feingold et al. (1992) conducted a population-based case–control study on childhood cancer in Denver, Colorado, USA. Parental work history information was obtained from a questionnaire that was administered mostly at home by a trained interviewer (not blinded to case status) to the parents (mostly mothers). The work histories included job title, industry, and dates of employment for each job held for at least 6 months between the year before the child's birth and the year of diagnosis. Occupational histories were coded and linked with a JEM that was previously developed by the National Cancer Institute (Hoar et al., 1980). The JEM allowed for assignment of exposures based on the combination of job title and industry that was derived from previous industrial hygiene surveys and knowledge of industrial processes. The JEM also provided an estimate as high, medium, low, or unknown. All maternal and paternal jobs held for a period of 6 months or longer during the year before birth were linked to the JEM to identify chemicals associated with a particular job. All exposures estimated using the JEM were included for individuals who had had two jobs during the year before the child's birth.

To reduce the large number of chemicals identified by the JEM, the researchers limited the study to 13 parental occupations that had been previously associated with childhood cancer: carpenter, dyer, electrician, lumberman, machine repairman, machinist, miner, motor vehicle driver, motor vehicle mechanic, painter, printer, service station attendant, and welder. The JEM identified 220 chemicals associated with one or more of these occupations. Further restriction was made to include only those chemicals associated with four or more of the occupations (45 chemicals). Exposure was assigned to the highest level for groups of exposures (e.g. solvents). Exposed (yes/no) was assigned when the specific chemical exposure was high or medium. Only 10 cases of childhood cancer (acute lymphocytic leukaemia, 5 cases; brain cancer, 2 cases; and other cancers, 3 cases) were determined to be exposed to aniline (Feingold et al., 1992).

Alguacil et al. (2000) conducted a case–control study on pancreatic cancer in Spain. Occupational histories were obtained by direct interviews and were available for approximately 90% of the cases and controls. Most interviews (88%) were performed with the patient, 6% involved interviewing a relative, and the interviews for the remaining 6% were not described. A sample of the relatives was concurrently and separately interviewed and agreement between the responses was compared. [The Working Group noted that the results of this comparison were not presented; the authors refer to another publication by Gavaldà et al. (1995), which also does not present the results of this comparison of occupational exposures.]

Participants were asked if they had ever worked in any of 10 activities or industries believed to be potentially associated with risk of pancreatic or biliary cancer based on a literature review. These activities/industries included pesticide use, handling of petroleum derivatives, and working in the chemical industry, metal industry, rubber industry, graphic arts, jewellery, manufacture or repair of automobiles, leather tanning, or textile industry. Individuals who reported having worked in one of these activities were asked about the duration of time worked, specific activities, and products to which they were exposed. Two additional questionnaire sections were reserved for reporting any other job activities performed for at least 6 years. Two

industrial hygienists evaluated the potential for exposure to 22 suspected carcinogens based on a review of the work histories. [The Working Group noted that the authors do not describe how these carcinogens were chosen.] Cases and controls were classified as being exposed, unexposed, or unknown for each of these agents. Only 6 cases and 5 controls were determined to have exposure to aniline derivatives. The intensity of exposure was also coded as high, low, unknown, or none, and analyses were performed with and without a 10-year lag (Alguacil et al., 2000). [The Working Group noted that the authors do not mention whether the industrial hygienists were blinded as to the case status or whether the exposures changed over calendar time.] In addition, analyses were performed using a JEM called FINJEM (Kauppinen et al., 1998), but aniline was not on the list of chemicals evaluated using FINJEM.

(b) Quality of exposure assessment methods in case–control studies

The exposure assessment of the key epidemiological studies cited in this monograph are evaluated according to five principal considerations: exposure opportunity, carcinogenic co-exposures, completeness of exposure history data, accuracy of exposure intensity measurement, and appropriateness of exposure metrics used in the epidemiological models of risk of cancer.

(i) Exposure opportunity

An ideal epidemiological study for investigating the carcinogenicity of aniline would evaluate a population exposed to a high concentration over a long period of time. The Working Group evaluated each study against this ideal. This first consideration of quality does not strictly concern the exposure assessment, but rather the exposure to the chemical of interest and its distribution across the population and over time.

In general, the available case–control studies do not provide much information on the intensity and duration of exposures to aniline. The case–control study on bronchogenic lung cancer by Preti et al. (1988), which was based on measurements of exhaled breath, did not present any data on level or duration of exposure to aniline. Nizamova (1991) did not provide any information on duration or intensity of exposure. The case–control study by Feingold et al. (1992) on childhood cancer did not present any analyses stratified by aniline intensity or duration of exposure. The case–control study on pancreatic cancer by Alguacil et al. (2000) only examined the risk of those "highly" exposed to aniline compared with those non-exposed, and separate analyses were conducted for those exposed for at least 10 years and those exposed for only 6 months.

(ii) Carcinogenic co-exposures

Although the case–control studies (Preti et al., 1988; Nizamova, 1991; Feingold et al., 1992; Alguacil et al., 2000) examined other potentially carcinogenic exposures, none of the studies provided information or controls for other exposures when examining the association between aniline and cancer. [The Working Group noted that these publications do not report whether there was co-exposure to aniline and these carcinogenic exposures, but there most probably was co-exposure for industries such as the rubber and dye industries.]

(iii) Completeness of exposure histories

Preti et al. (1988) did not consider occupational or environmental exposure histories in their bronchogenic lung cancer case–control study in which they measured aniline in expired air. Nizamova (1991) did not provide any information on how work histories were obtained, except that "sometimes" it was possible to contact workers and conduct detailed interviews. In the study on childhood cancer by Feingold et al. (1992), one parent was interviewed (usually the mother), who might not have been aware of all

details of the other parent's job. Work histories in the case–control study on pancreatic cancer by Alguacil et al. (2000) were based on interviews of patients and their controls or of a relative. It is difficult to know how complete these histories were, but of particular concern is the completeness of the information obtained from the relatives. Alguacil et al. (2000) mention a comparison of responses from a sample of relatives who were separately interviewed but did not provide the results of this comparison.

(iv) Accuracy of exposure intensity measurement

None of the case–control studies reported information on the intensity of exposure to aniline.

(v) Appropriateness of the exposure metrics

The use of analysis of lung air for aniline in the bronchogenic cancer case–control study by Preti et al. (1988) is not an appropriate measure given that aniline is a highly volatile chemical and measurements in breath do not reflect past exposures, and that occupational and environmental causes of lung cancer are generally related to exposures that occurred at least 10–20 years before diagnosis. Nizamova (1991) did not provide any information on how exposure was assessed and thus it is not possible to judge whether the method used was appropriate. The case–control study on childhood cancer developed estimates of exposure (yes/no) by linkage of the self-reported work histories with a JEM (Feingold et al., 1992). It is well-recognized that these qualitative methods for estimating exposures have a high degree of exposure misclassification. The exposure measure in the case–control study on pancreatic cancer was based on a review of the occupational histories by two industrial hygienists who coded the exposure as exposed, unexposed, or unknown (Alguacil et al., 2000).

(c) Overall summary of exposure assessment in key case–control studies

The Working Group noted that the case–control studies reviewed in this monograph suffer from several limitations in their exposure assessment for aniline. The studies do not provide information on duration, intensity, or cumulative exposures to aniline. They are fundamentally based on work histories derived from interviews of the study participants or a family member who may have an incomplete recollection, with potential for recall bias. One study is based on analysis of aniline in lung air after a diagnosis of lung cancer, which is a poor measure of past exposures. These studies do not account for the potential for confounding by co-exposures. Considering these limitations, the Working Group considered that these case–control studies should be given low weight in the review of the epidemiological evidence of cancer in humans.

2. Cancer in Humans

This section comprises a review of the evidence from studies of cancer in humans. The epidemiological database for the evaluation of aniline is quite limited, comprising only four cohort studies and four case–control studies. It is noteworthy that in the present review the Working Group also included consideration of case reports and case series (hereafter described as "case reports"). In many instances, case reports may not greatly contribute to our understanding of causality since no information is provided on the number of expected cases. However, there are some important exceptions, such as the discovery of unusually high numbers of cases of specific cancer types among workers in some occupations, for example, mesothelioma among South African asbestos miners (Wagner et al., 1960) and angiosarcoma among workers

exposed to vinyl chloride monomer (Creech & Johnson, 1974). In these examples, the fact that these are very rare cancers with few known risk factors made these initial case reports credible, and the association between these exposures and cancer was confirmed in subsequent formal epidemiological investigations.

Bladder cancer is the 10th most common cancer in the world (Bray et al., 2018). Although far more common than mesothelioma or angiosarcoma, bladder cancer is still sufficiently rare that a few cases occurring in a small industrial facility may be suggestive of an occupational etiology. The interpretation of clusters of cases of bladder cancer is complicated by the fact that there are many known risk factors with *sufficient* evidence in humans, including tobacco smoking, aluminium production, 4-aminobiphenyl, arsenic and inorganic arsenic compounds, auramine production, benzidine, chlornaphazine, cyclophosphamide, magenta production, 2-naphthylamine, painting, the rubber manufacturing industry, *Schistosoma haematobium*, *ortho*-toluidine, and X- and gamma-radiation (see IARC, 2020). Case reports have, however, played an important role in identifying the carcinogenic hazards associated with occupational exposures to aromatic amines. Rehn (1895) was the first to report an unusually large number of incident bladder cancer cases among workers exposed to aromatic amines in the aniline dye industry in Germany.

Some studies included in this review are based on bladder cancer incidence, whereas others are based on bladder cancer mortality; the two approaches may yield different results. For bladder cancer, survival rates are relatively high. For example, the 5-year survival rate for bladder cancer between 2009 and 2015 in the USA was 77% for all stages combined (American Cancer Society, 2020), and the 5-year survival rate in three regions of China during the 1990s ranged from 43% to 75% (Sankaranarayanan et al., 2011). Thus studies that rely on mortality from bladder cancer may omit the large proportion of cases that are not fatal, which would reduce the statistical power of these studies to detect an effect of exposure, if one exists. Furthermore, studies that examine mortality may tend to overrepresent more aggressive tumours, which could have a different etiology to that of less aggressive forms. Although incidence is generally preferable to mortality for epidemiological investigations of cancer, there may be a potential for bias in some studies in which routine occupational screening for bladder cancer has been conducted. This bias may result in inflated rates of cancer in the workplace when compared with national or local rates in the general population.

2.1 Cohort studies

See Table 2.1.

Studies of exposure to aniline were carried out in aromatic amine dye-manufacturing plants and in rubber-chemical manufacturing plants. Four cohort studies were identified that investigated cancer risks in workers occupationally exposed to aniline. Two of the cohorts were followed-up repeatedly, and the earlier follow-up publications (e.g. Ward et al., 1991; Sorahan et al., 2000; Carreón et al., 2010) are not described in detail below. One series of additional studies of workers in the aniline-dye industry within the Russian Federation was excluded because it did not mention exposure to aniline specifically (Bul'bulian & Goldfarb, 1991; Bul'bulian, 1991; Bulbulyan et al., 1995).

Tumours of the urinary bladder in workers engaged in the manufacture and use of certain dyestuff intermediates in the British chemical industry have been examined (Case et al., 1954). The firms participating in the study were asked to provide a "nominal roll" of all workers known to have had any contact with aniline, benzidine, α-naphthylamine [1-naphthylamine] or β-naphthylamine [2-naphthylamine]. The authors judged the roll to be reasonably complete from

Table 2.1 Cohort studies on cancer and exposure to aniline

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Case et al. (1954) UK 1921–1949	4622 male workers employed ≥ 6 mo in the British chemical industry and known to have had contact with aniline, benzidine, α-naphthylamine [1-naphthylamine], or β-naphthylamine [2-naphthylamine] Exposure assessment method: records; ever/never exposure assessment, based on reports from companies as to workers' exposure to each chemical	Urinary bladder	Exposed to aniline, but without any of the following contacts: magenta, benzidine, α-naphthylamine [1-naphthylamine], β-naphthylamine [2-naphthylamine]	1	–	Age, sex, year	*Exposure assessment critique*: Poorly defined exposure and poorly characterized exposure assessment with no details on total cohort, only of cases Other comments: 4 cases (2 alive; 2 dead) exposed to aniline, but not magenta (or benzidine, α-naphthylamine [1-naphthylamine], or β-naphthylamine [2-naphthylamine]), who may also have been exposed to auramine. The single case reported here is for mortality. Strengths: exhaustive search for deaths Limitations: the exhaustive search for deaths may have biased the SIRs upwards

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Ott & Langner (1983) USA 1940–1975	275 men identified from workplace census lists who were working as of (or hired after) 1940 in one of three dye-production areas of a chemical manufacturer, without high exposure to arsenicals, vinyl chloride, or asbestos. Exposure assessment method: records; exposure was based on years working in an area with exposure to aniline; no other details of exposure levels; some workers worked in other areas with potential exposure to *ortho*-toluidine	All cancers combined, mortality	Indigo- and potassium phenyl glycine-production area (SMR)	10	[1.16 (0.56–2.14)]	Age, sex, race, calendar period	*Exposure assessment critique*: Moderately well-defined exposure, but poorly characterized exposure assessment. Not adjusted for other potential exposures, no information on intensity of exposure. Strengths: occupational study of workers with known aniline exposure Limitations: small cohort; inadequate description of mortality outcome assessment; mortality study inadequate for bladder cancer; prevalent hire bias; inadequately described; short follow-up
			Acetanilide production area (SMR)	4	[1.29 (0.35–3.30)]		
		Colon and rectum (includes all digestive organs and peritoneum), mortality	All production areas combined (SMR)	10	[1.75 (0.84–3.23)]		
		Lung (includes all respiratory system), mortality	All production areas combined (SMR)	6	[1.18 (0.43–2.56)]		
		Urinary bladder (includes all urinary tract), mortality	All production areas combined (SMR)	0	[0 (0–3.07)]		
		Lymphatic and haematopoietic system, mortality	All production areas combined (SMR)	1	[1 (0.03–5.57)]		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Sorahan (2008) Wales, UK 1955–2005 (mortality), 1971–2005 (cancer incidence)	2160 male production workers, with ≥ 6 mo of employment at the chemical factory from 1955 to 1984 Exposure assessment method: questionnaire; cohort-specific JEM of ever/never worked in department with potential exposure to aniline applied to job history; exposure assigned as years with potential exposure to aniline with no further details	All causes of death (ICD 001–999)	Overall cohort (SMR)	1334	1.02 (0.97–1.08)	Age, sex, year	*Exposure assessment critique*: Moderately well-defined exposure, but poorly characterized exposure assessment. Adjusted for other potential exposures, but no information on intensity of exposure. Strengths: availability of job histories Limitations: aniline-exposed subcohort quite small, exposures not assessed
		Lung, mortality	Overall cohort (SMR)	120	0.91 (0.75–1.09)		
		Urinary bladder, mortality	Combined exposed subcohort (potential exposure to one or more of MBT, aniline, *ortho*-toluidine, or PBN) (SMR)	11	2.78 (1.39–4.97)		
			Remainder of cohort (SMR)	11	1.05 (0.52–1.88)		
			Overall cohort (SMR)	22	1.52 (0.96–2.31)		
		Urinary bladder, mortality	Aniline-exposed subcohort (SMR)	8	2.77 (1.19–5.45)		
		Urinary bladder, incidence	Combined exposed subcohort (potential exposure to one or more of MBT, aniline, *ortho*-toluidine, or PBN) (SIR)	18	2.14 (1.27–3.37)		
			Remainder of cohort (SIR)	21	1.06 (0.65–1.62)		
		Urinary bladder, incidence	Aniline-exposed subcohort (SIR)	15	2.45 (1.37–4.05)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Sorahan (2008) (cont.)		Urinary bladder, incidence and mortality	Duration of employment in aniline-exposed department (RR):			Age, sex, year, exposure to MBT, PBN, and *ortho*-toluidine	
			None	37	1		
			0.1–4.9 yr	8	1.24 (0.52–3.00)		
			≥ 5 yr	11	1.35 (0.56–3.25)		
			Trend-test *P* value, 0.44				
Carreón et al. (2014) USA Follow-up, 1976–2007	1875 workers at a rubber-chemical manufacturing plant that used *ortho*-toluidine, aniline, and nitrobenzene combined; employed for ≥ 1 day in 1946–2006 Exposure assessment method: records; cohort-specific JEM to combined exposures was based on measurements and detailed understanding of process; applied to job history and multiplied by no. of days in that job	Urinary bladder, incidence	Category of *ortho*-toluidine, aniline, and nitrobenzene exposure (SIR):			Age, sex, race, year	*Exposure assessment critique*: Intensive work to develop JEM for combined exposure but impossible to determine exposure to aniline alone Strengths: large exposure contrast Limitations: small number of cases; despite the adequate exposure assessment, exposures to aniline, *ortho*-toluidine, and nitrobenzene could not be separately discerned
			Probably not exposed	< 5	0.59 (0.07–2.12)		
			Probably exposed (low, irregularly/ occasionally)	< 5	2.47 (0.67–6.33)		
			Probably exposed (low, regularly)	< 5	4.21 (1.15–10.8)		
			Definitely exposed (moderate/high)	27	3.90 (2.57–5.68)		
			Overall	37	2.87 (2.02–3.96)		
		Urinary bladder, incidence	Duration of exposure to *ortho*-toluidine, aniline, and nitrobenzene among definitely exposed (moderate/high) workers (SIR):				
			< 5 yr	< 10	1.98 (0.80–4.08)		
			5 to < 10 yr	< 5	4.52 (0.93–13.2)		
			≥ 10 yr	17	6.24 (3.63–9.99)		
		Urinary bladder, incidence	Duration of exposure to *ortho*-toluidine, aniline, and nitrobenzene among definitely exposed (moderate/high) workers (SRR):				
			< 5 yr	< 10	1		
			5 to < 10 yr	< 5	2.56 (0.60–10.9)		
			≥ 10 yr	17	4.50 (1.74–11.6)		
			Trend-test *P* value, < 0.001				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Carreón et al. (2014) (cont.)		Urinary bladder, incidence	Time since first exposure to *ortho*-toluidine, aniline, and nitrobenzene among definitely exposed (moderate/high) workers (SIR):			Age, sex, race, year	
			< 10 yr	< 5	1.74 (0.04–9.68)		
			10 to < 20 yr	< 5	3.41 (0.93–8.72)		
			20 to < 30 yr	9	4.75 (2.17–9.02)		
			≥ 30 yr	13	3.97 (2.11–6.79)		
		Urinary bladder, incidence	Time since first exposure to *ortho*-toluidine, aniline, and nitrobenzene among definitely exposed (moderate/high) workers (SRR):				
			< 10 yr	< 5	1		
			10 to < 20 yr	< 5	7.09 (0.76–66.2)		
			20 to < 30 yr	9	13.4 (1.59–[113[a]])		
			≥ 30 yr	13	8.61 (1.11–67.0)		
			Trend-test *P* value, < 0.001				
		Urinary bladder, incidence	Cumulative rank quartile, 10-yr lag (SIR):				
			< 11 000 unit-days	9	1.32 (0.61–2.51)		
			11 000 to < 27 000 unit-days	10	3.37 (1.62–6.20)		
			27 000 to < 48 000 unit-days	9	5.44 (2.49–10.3)		
			≥ 48 000 unit-days	9	6.13 (2.80–11.6)		
		Urinary bladder, incidence	Cumulative rank quartile, 10-yr lag (SRR):				
			< 11 000 unit-days	9	1		
			11 000 to < 27 000 unit-days	10	3.05 (1.13–8.22)		
			27 000 to < 48 000 unit-days	9	6.37 (2.30–17.7)		
			≥ 48 000 unit-days	9	7.34 (2.44–22.1)		
			Trend-test *P* value, < 0.001				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Carreón et al. (2014) (cont.)		Urinary bladder, incidence	Cumulative rank quartile, 10-yr lag (HR):			Attained age, sex, race, birth year	
			< 11 000 unit-days	9	1		
			11 000 to < 27 000 unit-days	10	3.76 (1.37–10.7)		
			27 000 to < 48 000 unit-days	9	4.65 (1.55–14.0)		
			≥ 48 000 unit-days	9	8.94 (3.57–24.6)		
		Urinary bladder, incidence	Cumulative rank quartile, 20-yr lag (SRR):			Age, sex, race, year	
			< 2800 unit-days	9	1		
			2800 to < 11 000 unit-days	10	2.95 (1.00–8.70)		
			11 000 to < 28 000 unit-days	9	2.22 (0.78–6.37)		
			≥ 28 000 unit-days	9	6.70 (2.09–21.5)		
			Trend-test *P* value, 0.037				
		Urinary bladder, incidence	Cumulative rank quartile, age < 60 yr, 10-yr lag (HR):			Attained age, sex, race, birth year	
			< 11 000 unit-days	< 5	1		
			11 000 to < 27 000 unit-days	< 5	6.07 (1.33–31.0)		
			27 000 to < 48 000 unit-days	7	10.2 (2.16–54.3)		
			≥ 48 000 unit-days	5	23.6 (6.66–113)		
		Urinary bladder, incidence	Cumulative rank quartile, age ≥ 60 yr, 10-yr lag (HR):			Attained age, sex, race, birth year	
			< 11 000 unit-days	5	1		
			11 000 to < 27 000 unit-days	6	1.73 (0.45–7.09)		
			27 000 to < 48 000 unit-days	< 5	1.63 (0.32–7.45)		
			≥ 48 000 unit-days	< 5	2.46 (0.64–10.0)		

CI, confidence interval; HR, hazard ratio; ICD, International Classification of Diseases; JEM, job-exposure matrix; MBT, 2-mercaptobenzothiazole; mo, month; PBN, *N*-phenyl-2-naphthylamine; RR, relative risk; SIR, standardized incidence ratio; SMR, standardized mortality ratio; SRR, standardized rate ratio; yr, year.

[a] This value was incorrectly reported in the original publication as 11.3, but was verified by the Secretariat with the authors (Carreón et al., 2014).

1920 onwards. A total of 4622 male workers were included in the cohort who had worked in the industry for at least 6 months. Cases of bladder cancer were identified from several sources: reported by firms; hospital records confirmed by firms, or patients, or relatives of patients; death certificates with mentions of occupation in the chemical industry; and coroners' records. As reported in Section 1.6.1, in the absence of exposure measurements, potential exposure to aniline (as "ever/never") was estimated from process records and work histories. Follow-up was from 1921 to early 1952 (1921–1949 for deaths) during which time 341 cases of bladder cancer were identified, 298 (87.4%) of which were in workers deemed to have had contact with benzidine, α-naphthylamine, or β-naphthylamine, and 32 (9.4%) in workers who had not had contact with any of these agents. In total there were 13 cases of bladder cancer in workers exposed to aniline: 9 workers who had possible contact with magenta and 4 who did not. There was a single death among those exposed to aniline but not magenta, for which "bladder tumour" was mentioned on the death certificate, with 0.54 deaths expected. A companion study examined bladder cancer risk in 1223 workers involved in the manufacture of magenta (which included exposure to aniline) and in the manufacture of auramine (with no exposure to aniline) (Case & Pearson, 1954). Follow-up for this analysis was to the end of 1952. Among those men who had contact with aniline but not with magenta, auramine, benzidine, α-naphthylamine [1-naphthylamine], or β-naphthylamine [2-naphthylamine], 3 cases of bladder tumour were found, and there was a single death, with 0.83 expected. [The Working Group noted that although this was a well-conducted study for its time, case ascertainment is likely to have been incomplete and the reference rates are likely to be subject to error. There was only one death in the cohort exposed to aniline and not to other known or suspected occupational bladder carcinogens and this, together with the lack of control for tobacco smoking, means that this study is fairly uninformative in terms of bladder cancer risk after aniline exposure.]

Ott & Langner examined mortality among 342 white male workers assigned to three aromatic amine dye-production areas at a facility in the USA (Ott & Langner, 1983). Exposure to aniline occurred during the production of indigo dye, the operation dating back to approximately 1914 and discontinued by 1958. The plant had four production areas for: (i) acetanilide; (ii) indigo; (iii) bromindigo; and (iv) thioindigo. Indigo production comprised two steps, both with exposure to aniline: (i) the manufacture of potassium phenyl glycine from aniline and chloroacetic acid; and (ii) the manufacture of indigo from potassium phenyl glycine. The acetanilide production area involved exposure to aniline, whereas the bromindigo and thioindigo production areas did not involve exposure to aniline. The acetanilide process was operated from 1934 to 1958. The cohort was identified from yearly census lists available from the mid-1920s onward. All employees working for the company as of 1 January 1940 or hired after this date were included. The authors state that "nearly all employees who worked for at least one year would have been identified; however, some employees who worked for less than one year may not have been included in the cohort." As indicated in Section 1.6.1, employment history and process records were used to determine the potential for exposure to aniline and other known or suspected occupational bladder carcinogens. Work histories were obtained for all identified employees. Employees lost to direct company follow-up (n = 124) were followed-up via the social security administration. Deaths were coded according to the seventh or eighth revision of the International Classification of Diseases and were followed-up until 31 December 1975. Standardized mortality ratios (SMRs) were computed based on mortality data for USA white males in five-year age groups and five-year calendar year groups. Of the 342

employees, analyses were done excluding 56 workers who had worked in the past at an arsenicals-formulating plant and 11 who had worked with vinyl chloride or asbestos. The standardized mortality ratio for all causes for the 275 dye employees without high exposure to arsenicals, vinyl chloride, or asbestos was 0.98 [95% CI, 0.80–1.20], based on 98 observed deaths. For all malignant neoplasms for the indigo- and potassium phenyl glycine-production area, the standardized mortality ratio was 1.16 [95% CI, 0.56–2.14], and for the acetanilide production area the standardized mortality ratio was 1.29 [95% CI, 0.35–3.30]. For the indigo- and potassium phenyl glycine-production area, only a single death occurred from respiratory cancer with a latency of 15 years or more and for a duration of employment of 5 years or more, and for the acetanilide production area there was a single death from respiratory cancer with a latency of 15 years or more but for a duration of employment of less than 1 year. There were no observed deaths from cancer of the urinary tract, 1.2 were expected. There were no data on tobacco smoking in this study. [The Working Group noted that this study was relatively small and therefore limited in its informativeness in relation to bladder cancer risk.]

The bladder cancer risk in workers from a factory in Wales, UK, manufacturing chemicals for the rubber industry was examined (Sorahan, 2008). Mortality follow-up was from 1955 to 2005 and cancer incidence follow-up was from 1971 to 2005. The cohort consisted of 2160 male production workers with at least 6 months employment at the factory between 1955 and 1984. Job histories were available for the period 1930–1988. Altogether 611 exposed workers were categorized into overlapping subcohorts with exposure to aniline (n = 442), PBN [*N*-phenyl-2-naphthylamine] (n = 94), *ortho*-toluidine (n = 53), and/or MBT (n = 363) (an agent with limited evidence of bladder carcinogenicity in humans; see Section 1.4.3). As noted in Section 1.6.1, quantitative estimates of exposure were used to develop a detailed JEM for MBT and its derivatives, but for aniline, PBN, and *ortho*-toluidine, it was not possible to derive estimates more specific than duration of employment by department with potential for exposure. Mortality from all causes combined in the overall cohort was close to expected, as was mortality from lung cancer. In the overall cohort, for bladder cancer, the standardized mortality ratio was 1.52 (95% CI, 0.96–2.31) based on 22 observed deaths. In the combined exposed subcohort with potential exposure to one or more agents among MBT, aniline, *ortho*-toluidine, or PBN, the bladder cancer standardized mortality ratio was 2.78 (95% CI, 1.39–4.97; 11 deaths). In the remainder of the cohort, the bladder cancer standardized mortality ratio was 1.05 (95% CI, 0.52–1.88; 11 deaths). In the subcohort exposed to aniline and potentially other exposures listed above, the standardized mortality ratio was 2.77 (95% CI, 1.19–5.45; 8 deaths). The standardized incidence ratio for bladder cancer in the subcohort with potential exposure to MBT, aniline, *ortho*-toluidine, or PBN was 2.14 (95% CI, 1.27–3.37; 18 cases), and in the remainder of the cohort the standardized incidence ratio was 1.06 (95% CI, 0.65–1.62; 21 cases). In the aniline-exposed subcohort, the standardized incidence ratio was 2.45 (95% CI, 1.37–4.05; 15 cases). A Poisson regression analysis examined duration of employment (in years) in aniline-exposed and *ortho*-toluidine-exposed departments. Without adjustment for other chemicals, a significant positive trend ($P < 0.05$) was found for aniline exposure and a highly significant positive trend ($P < 0.001$) was found for *ortho*-toluidine; however, when adjusted for other chemical exposures, the trend for duration of aniline exposure was not significant ($P = 0.44$), although a significantly increasing trend with duration of *ortho*-toluidine exposure ($P < 0.05$) remained. No adjustment was made for tobacco smoking. [The Working Group noted that lung cancer standardized

mortality ratios were not elevated compared with the general population, suggesting that tobacco smoking confounding was unlikely in the standardized mortality ratio estimates for bladder cancer. The Working Group also noted that since the subcohorts analysed are overlapping, a considerable proportion of the aniline-exposed workers were also exposed to *ortho*-toluidine and other potentially carcinogenic occupational chemicals, possibly resulting in overadjustment in models with multiple occupational exposures included, which may have reduced the precision of the estimates.]

A study in the USA examined bladder cancer incidence among workers at a rubber-chemical manufacturing plant where *ortho*-toluidine, aniline, and nitrobenzene were used (Ward et al., 1991; Carreón et al., 2010, 2014; Hanley et al., 2012). Among other chemicals, aniline was used in antioxidant production until 1992. This cohort has been analysed using three different exposure assessment methods. First, the study by Ward et al. (1991) evaluated exposure based on department. Second, Carreón et al. (2010) presented a reanalysis of the Ward et al. (1991) study data that reclassified exposure for some departments. Third, Carreón et al. (2014) extended follow-up and used a revised exposure metric, as described in Section 1.6.1, in which work history records were assigned to one of four exposure categories (probably not exposed; probably exposed low and irregularly/occasionally; probably exposed low and regularly; definitely exposed moderate/high and regularly) representing combined exposures to *ortho*-toluidine, aniline, and nitrobenzene, and a relative rank based on job, department, and era was used to estimate a cumulative exposure rank. None of the three studies, therefore, could estimate the effect of aniline alone or aniline adjusted for other occupational exposures (Hanley et al., 2012). The third cohort included 1875 workers ever employed at the plant between 1946 and 2006 (Carreón et al., 2014). Incident cancers were identified from state cancer registries. Standardized incidence ratios and standardized rate ratios (SRRs) for bladder cancer were derived by exposure category and cumulative rank quartiles for different lag periods. Cox regression was used to model bladder cancer incidence by estimated cumulative exposure rank, adjusting for confounders. Indirect methods were used to adjust for tobacco smoking (Steenland & Greenland, 2004). Relative risks were not estimated separately for aniline or *ortho*-toluidine. Overall, the standardized incidence ratio for bladder cancer was 2.87 (95% CI, 2.02–3.96; 37 observed cases). For those probably exposed to low levels and regularly, the standardized incidence ratio was 4.21 (95% CI, 1.15–10.80; fewer than 5 cases). For those definitely exposed to moderate/high levels, the standardized incidence ratio was 3.90 (95% CI, 2.57–5.68; 27 cases). Examination of duration of exposure among those definitely exposed to moderate/high levels revealed a trend towards increasing risk with increasing duration of exposure (P for trend, < 0.001). A trend with increasing time since first exposure in this same group of workers was also observed (P for trend, < 0.001). A significant trend was also observed for cumulative rank across all exposed workers for an unlagged analysis and for a lag of 10 or 20 years, but not 30 years. Cox regression, where the lag of 10 years was deemed to provide the best fit, was used to fit a variety of models. Cumulative rank was significantly associated with bladder cancer hazard rate in categorical models using quartiles, quintiles, and deciles, based on the exposure distribution among all cases and in continuous models including log–linear, log–square root and log–log as well as restricted cubic spline models. The bias factor for tobacco smoking was 1.08 and so made little difference to the inferences. [The Working Group noted that in this study it was not possible to separate any aniline-specific effect due to the limited available records on worker exposure to the different chemicals used.]

Table 2.2 Case–control studies on cancer and exposure to aniline

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Preti et al. (1988) USA NR	Cases: 10 hospital-based cases confirmed by X-ray, bronchoscopy, and biopsy Controls: 16, in two groups; 8 controls were younger (age, 22–41 yr) and possibly had non-cancer lung diseases; 8 controls were healthy hospital employees matched by age (age, 57–66 yr) Exposure assessment method: quantitative measurements; aniline and other compounds measured by GC-MS in exhaled air	Lung (ICD10 C34), incidence	Aniline detected above detection limit (0.1 ng per 20 L lung air) in exhaled air (OR): No Yes	 5 5	 1 [7.0 (1.0–48.3)]	None	*Exposure assessment critique*: Exposure not well-defined nor well assessed. Current exposure unsuitable for cancers due to long latency. Mass spectrometry in 1988 may not have identified aniline correctly. Exposure assessed after disease for cases. Controls selected to not have industrial exposure. Other comments: aniline was detected in exhaled air of 5 of the cases, none of the older controls, and 2 of the younger controls; measures of association were not reported, but crude ORs were calculated using information from table, but could not be stratified or adjusted due to small study size Strengths: case diagnoses were medically confirmed. Limitations: small study size; one group of controls was hospital-based; all cases were heavy smokers; no adjustment for confounders was conducted; although exposure was measured quantitatively, exhaled air is not an indicator of long-term exposure

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Nizamova (1991) the Russian Federation NR	Cases: 258 bladder cancer cases; no other information provided. Controls: 454 healthy controls, matched by sex, place of residence, and age in a 1:2 ratio Exposure assessment method: exposure assessment by interview but not otherwise described	Urinary bladder (ICD10 C67), incidence	Aniline dye production (OR): Never exposed Ever exposed	 3 4	 1 2.4 (0.1–69.5)	Sex, residence, age	*Exposure assessment critique*: Exposure not well-defined. Quality is therefore uncertain and probably poor. Other comments: chance, bias or confounding could explain the findings Limitations: no information on case or control selection; no information on exposure assessment; small sample size; no adjustment for confounders
Feingold et al. (1992) USA 1976–1983	Cases: 252 cases of childhood cancer (age, 0–14 yr), ascertained through cancer registry, supplemented by hospital and pathology records, and reviewed by paediatric oncologists Controls: 222 controls identified through random-digit dialling and matched individually by age (± 3 yr), sex, and telephone exchange area Exposure assessment method: expert judgement; parental occupation obtained by interview and exposure assigned on the basis of job title and industry with a JEM; exposure was defined as those whose parent had aniline (and other chemical) exposure at a medium or high level for at least 6 mo in the year before the birth of the child	All cancers combined, incidence Leukaemia (acute lymphoblastic/ lymphocytic leukaemia; ICD10 C91), incidence Brain (childhood cancer, ICD10 C71), incidence	Father's exposure to aniline in the year before the child's birth (OR): Never Ever exposed Father's exposure to aniline in the year before the child's birth (OR): Never Ever exposed Father's exposure to aniline in the year before the child's birth (OR): Never Ever exposed	 NR 10 NR 3 NR 2	 1 1.8 (0.6–6.0) 1 2.1 (0.4–10.5) 1 1.4 (0.2–10.9)	Age, sex, area, father's education	*Exposure assessment critique*: Exposure was defined but not well-assessed. Overall quality assessed as poor. Other comments: response rate was approximately 70%; high degree of correlation among exposures and small study size prevented adjustment for other exposures; interviewers assigned occupation and were not blinded to case ascertainment, but were trained to be objective and were not aware of the study hypotheses Strengths: diagnostic confirmation of case status; adjustment for paternal education Limitations: small study size; one parent usually reported both parents' occupation, which could lead to exposure misclassification

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Alguacil et al. (2000) Spain 1992–1995	Cases: 164 hospital-based incident cases Controls: 238 hospital-based controls free of pancreatic cancer, admitted under suspicion of pancreatic cancer, biliary cancer, or chronic pancreatitis. Exposure assessment method: expert judgement; interviews of subjects or next of kin about employment in different industries, with industrial hygiene expert review about potential exposure to 22 agents, including aniline, and exposure categorized as high, low, unknown, or none	Pancreas (ICD10 C25), incidence	Exposure to aniline (OR):			Sex, age, hospital, smoking (status and pack-years), alcohol consumption	*Exposure assessment critique*: Exposure was defined but not well assessed. Overall quality assessed as poor. Strengths: diagnostic confirmation of cases; high percentage (90%) of subjects with occupational histories; occupational exposures were assessed by expert judgement Limitations: small study size; potential for non-differential misclassification of exposure; potential for selection bias since controls had chronic pancreatitis and other cancers, but sensitivity analyses found only small decreases in risk estimates
			Never	NR	1		
			Ever exposed	6	1.35 (0.36–5.11)		
		Pancreas (ICD10 C25), incidence	High exposure to aniline for ≥ 6 mo (OR):				
			Never	158	1		
			Ever exposed	5	1.77 (0.36–8.57)		
		Pancreas (ICD10 C25), incidence	High exposure to aniline for ≥ 10 yr, 10 yr before diagnosis (OR):				
			Never	158	1		
			Ever exposed	5	2.58 (0.43–15.3)		

CI, confidence interval; GC-MS, gas chromatography-mass spectrometry; ICD10, International Classification of Diseases, 10th edition; JEM, job-exposure matrix; mo, month; NR, not reported; OR, odds ratio; yr, year.

2.2 Case–control studies

See Table 2.2.

Four case–control studies investigated the association between exposure to aniline and various cancers. Each study focused on a different cancer: bladder, lung, pancreatic, or childhood cancer.

A case–control study by Preti et al. (1988) used exhaled air measurements of volatile compounds among newly diagnosed cases of lung cancer and two groups of controls in Pennsylvania, USA. The study included 10 hospital-based cases (seven men, three women; age range, 59–77 years). The diagnosis of lung cancer (squamous cell carcinoma, 6 cases; undifferentiated large cell cancer, 2 cases; and adenocarcinoma, 2 cases) was confirmed by X-ray, bronchoscopy, and biopsy. One control group included eight individuals who were younger than the cases (range, 22–41 years) and who were recruited from a lung disease programme at the same hospital as the cases and were free of lung cancer. [The Working Group noted that the article did not mention whether these individuals had another health condition apart from lung cancer for which they were receiving treatment.] The second group of controls included eight healthy hospital employees of similar age as the cases (range, 57–66 years). The selection criteria for these controls were: (i) absence of chronic or acute lung disease; (ii) no industrial dust exposure; (iii) normal chest X-ray; and (iv) no medication use at the time of the study. [The Working Group noted that the first three control selection criteria likely reduced the probability of exposure to aniline among the controls to be less than that of cases and thus may have biased the study.] Tobacco smoking and occupational histories were obtained from all study participants. All cases were in patients who were or had been smokers (consumed more than a half pack of cigarettes per day).

No measures of association were reported in the study; however, based on data from a table, the Working Group calculated a crude odds ratio (OR) for aniline in exhaled air above the limit of detection versus none-detected of 7.0 (95% CI, 1.0–48.3) using all controls. [The Working Group considered this study uninformative for several reasons: that aniline was measured after disease occurrence, that aniline in exhaled breath likely reflects only very recent exposure because of its demonstrated short half-life (see Section 4.1), the very small study size, and the potential bias due to control selection criteria.]

Nizamova (1991) conducted a case–control study on bladder cancer among the population of the industrial region of Tambov in the Russian Federation. The study included 258 patients with bladder cancer and 454 controls matched by sex, place of residence, and age. [The Working Group noted that no case definition or method of case ascertainment was provided, and the source of controls and whether they were free of disease were not reported. The time frame in which the study was conducted was not reported. No details were provided on how the exposure assessment was conducted.] Overall, people with any contact with aromatic amines showed an increased risk of bladder cancer (OR, 4.7; 95% CI, 1.2–19.2). For exposure in aniline dye production, the odds ratio was 2.4 (95% CI, 0.1–69.5) (Nizamova, 1991). [The Working Group did not find the study informative; as noted above, the study did not provide information on case or control ascertainment, aniline exposure, or occupational co-exposures. The study size was underpowered, hence the imprecise confidence intervals, and the risk estimates were not adjusted for any confounders.]

A case–control study on parental occupation and childhood cancer was conducted in the USA by Feingold et al. (1992). The study included 252 cases of childhood cancer diagnosed between 1976 and 1983 that were ascertained through the Colorado Cancer Registry and supplemented by

record review at area hospitals, and that were confirmed microscopically (95%) or through direct visualization or radiography (3%). In addition, case records were reviewed by paediatric oncologists for diagnostic accuracy. Controls (n = 222) were identified through random-digit dialling and were individually matched to cases by age (± 3 years), sex, and telephone exchange area.

As described in Section 1.6.2, information on parental work history was obtained from a questionnaire that was administered mostly to the mothers. Occupational histories were coded and linked with a JEM that was previously developed by the National Cancer Institute (Hoar et al., 1980). The JEM contained information on 220 chemicals associated with one or more parental occupations that had been previously associated with childhood cancer. Only 10 childhood cancer cases were in children whose fathers were considered to have any history of occupational exposure to aniline.

Since only a small number of mothers reported an occupational history with any exposure to hydrocarbons (which included aniline), no results were reported for mothers. For fathers' exposure to aniline, the odds ratio, adjusted for father's education, was 1.8 (95% CI, 0.6–6.0; 10 exposed cases) for all childhood cancers, 2.1 (95% CI, 0.4–10.5; 3 exposed cases) for acute lymphocytic leukaemia, and 1.4 (95% CI, 0.2–10.9; 2 exposed cases) for brain tumours. Although maternal tobacco smoking during pregnancy confounded the association in stratified analyses, it was no longer a confounder in unconditional logistic regression models when paternal education was included (Feingold et al., 1992).

[The Working Group noted that although analyses were adjusted by father's education, the high degree of correlation between exposures and the small number of cases prevented adjustment for other exposures. In addition, one parent usually reported both parents' occupation, which could lead to exposure misclassification.]

The PANKRAS II study, a case–control study of pancreatic cancer associated with occupational exposures, was conducted between 1992 and 1995 at five hospitals in eastern Spain (Alguacil et al., 2000). The study included 164 hospital-based incident cases of pancreatic cancer for which diagnoses were reviewed by a panel of experts. Controls (n = 238) were admitted to the same hospitals as cases and were free of pancreatic cancer, but had chronic pancreatitis (n = 93), acute pancreatitis (n = 34), other benign pathologies (n = 70), or other cancers (n = 41). Trained interviewers administered a questionnaire that obtained clinical history, symptoms before admission, occupational history, and lifestyle information. [The Working Group noted that information regarding whether the interviewers were blinded as to case status was not provided but it seems unlikely that they were.]

As described in Section 1.6.2, occupational histories were obtained by direct interviews and were available for approximately 90% of cases and controls. Participants were asked if they had ever performed any of 10 workplace activities that were believed to be potentially associated with pancreas and biliary cancer risk. Odds ratios were adjusted for sex, age, hospital, tobacco smoking (status and pack-years), and alcohol consumption using unconditional logistic-regression models. The risk of pancreatic cancer associated with ever exposure to aniline derivatives was 1.35 (95% CI, 0.36–5.11; 6 exposed cases). For high exposure to aniline derivatives for at least 6 months, the odds ratio was 1.77 (95% CI, 0.36–8.57; 5 exposed cases), and for high exposure to aniline derivatives for at least 10 years, 10 years before diagnosis, the odds ratio was 2.58 (95% CI, 0.43–15.3; 5 exposed cases) (Alguacil et al., 2000).

[The Working Group considered that the study had strengths, including diagnostic confirmation of cases, a high percentage (> 90%) of participants with occupational histories, and occupational exposures assessed by expert

judgement. Limitations of the study included the small numbers of exposed cases. Although the authors reported that the potential for misclassification of exposure is non-differential since cases were interviewed in the same way as controls, differential misclassification of exposure is possible since the interviewers were most likely not blinded as to case status. There is also potential for selection bias since controls had chronic pancreatitis and other cancers; however, sensitivity analyses excluding controls with pancreatitis (chronic or acute) found only small changes in risk estimates.]

2.3 Case reports and case series

See Table S2.3 (Annex 2, Supplementary material for Section 2, web only; available from: https://publications.iarc.fr/599).

Altogether 17 case reports or series on aniline exposure and cancer risk are reported in this section, 16 on bladder tumours and one on lung cancer. Several other potentially relevant case reports on cancers of the bladder or other organ sites were identified from a literature search but that were considered ineligible because the title or abstract did not indicate likely aniline exposure, they were not actual case reports, or they were replicated elsewhere. The Working Group, in reviewing these case reports and case series, also noted when the study mentioned co-exposures to other chemicals for which there is *sufficient* or *limited* evidence that they cause bladder cancer in humans (see Section 1.4.3; and also IARC, 2020).

(a) Bladder cancer

Ludwig Rehn was the first to report on the occurrence of bladder tumours in workers exposed to aniline who were engaged in fuchsine dye production (Rehn, 1895). He noted 3 cases among 45 workers who had all worked in the same room of a factory in Frankfurt, Germany, where toluidine, aniline, nitrobenzene, and iron chloride were mixed and heated to produce "raw" fuchsine. Case No. 1 was a worker diagnosed with papillary fibroma of the bladder at age 40 years, after 15 years of employment at the factory. Case No. 2 was a worker aged 29 years when diagnosed with a papillary fibroma of the bladder; the duration of employment was not known. Case No. 3 was a worker aged 49 years who had worked at the plant for 20 years when he was diagnosed with what appeared to be a bladder carcinoma, by cystoscopy, but histologically was classified as a bladder sarcoma. [The Working Group noted that workers were exposed not only to aniline, but also to other known occupational bladder carcinogens such as toluidine (isomer not specified).]

At a surgical congress in Berlin, Germany, in 1904, a series of 23 cases of bladder tumours was reported that had been observed in factories (in Germany and England) where aniline and "its homologues and allied substances" were produced (Anon., 1904). There were five papillomas (of which two became malignant), one sarcoma, and 17 carcinomas. The shortest latency period observed was 9 years. [The Working Group noted that there was no information on individual cases, specific exposures, duration of exposure, or observation period, and that co-exposure to known occupational bladder carcinogens (e.g. 2-naphthylamine, benzidine, *ortho*-toluidine), is probable in the aniline production industry.]

In 1901–1910, 6 deaths resulting from bladder tumours were reported among 840 workers employed in aniline factories in Basel, Switzerland (Anon., 1921). During the same period, 12 additional deaths from bladder tumours occurred among 56 500 male workers in the city. In 1861–1900, before the chemical industry was established in the city, 6 cases of bladder tumours were seen at the surgical clinic; while during the following 10 years (1901–1910), 16 cases appeared at the clinic, 10 of which were

in workers exposed to aniline, 2 were involved in dye manufacturing. A large proportion of the bladder tumours were malignant and were diagnosed mainly in men older than 40 years and employed for "many years" in the aniline industry. Benzidine and β-naphthylamine [2-naphthylamine] are mentioned as suspected co-exposure bladder carcinogens in these factories (Anon., 1921). [The Working Group noted that no information on individual cases is given and co-exposure to other occupational bladder carcinogens was reported.]

In a review discussing several aspects of the "aniline tumours", Berenblum (1932) referred to three case reports (Curshmann, 1920; Schwerin, 1920; Oppenheimer, 1927) on bladder tumours in which 8, 5, and 24 cases, respectively, were in workers who had been exposed to aniline only.

Gehrmann (1934) reported findings from two plants within the dye industry in the USA where workers had been systematically screened for bladder tumours by cystoscopy since 1931. In a group of 577 workers, 27 bladder tumours were observed. Of these, 13 workers had been exposed to benzidine and β-naphthylamine [2-naphthylamine], 10 to β-naphthylamine only, 2 to benzidine only, and 2 to α-naphthylamine [1-naphthylamine] only. In a group of workers [number not stated] exposed to aniline only, also subject to regular cystoscopic examinations, no bladder tumours were observed. [The Working Group noted that no bladder tumours occurred in the aniline-only exposed group. The absence of information on the size of this group, however, renders this finding uninformative.]

In the USA, aniline production started in 1915; the first bladder tumour among workers was diagnosed in 1931. Routine cystoscopy among workers at one plant identified 63 bladder tumours, of which 24 were carcinomas and 39 were papillomas (Gehrmann, 1936). The average duration of exposure to aniline was 13.2 years for the group diagnosed with carcinoma. In addition to aniline, the workers were exposed to the bladder carcinogens β-naphthylamine [2-naphthylamine], and benzidine, as well as α-naphthylamine [1-naphthylamine]. [The Working Group noted that no information on individual cases was given and that co-exposure to occupational bladder carcinogens was reported.]

By 1938, approximately 550 so-called "aniline tumours" had been reported in the literature, according to a review by Hueper (1938). Of these, more than 300 cases were reported in Germany, more than 80 (since 1905) in Switzerland, and about 40 in England. In the USA, approximately 100 cases were reported, all in the same company. In the former Soviet Union, three cases were reported in 1926, and more cases [numbers not stated] in 1932. In Austria, cases [no numbers given] were reported in 1926, 1927, and 1932. Reports from Italy in 1936 and 1937 stated that 12 aniline tumours had occurred during the past few years among 86 workers in one dye factory. The review notes that the occurrence of bladder tumours is often associated with prolonged occupational contact with phenylamine [aniline), its isomers and homologues (toluidine, isomer not specified, and xylidine) and other amino derivatives; diphenylamine (benzidine), its isomers and derivatives (e.g. toluidine, isomer not specified, or dianisidine); and naphthylamines and related compounds (e.g. naphthylene diamines) (Hueper, 1938). [The Working Group noted that no data on individual cases were given and occupational co-exposure to known bladder carcinogens occurred.]

Orts (1948) reported on 2 cases of bladder cancer in Galicia, Spain, where aniline was widely used as a colourant in red wine. The author describes how wine was served in big cups, which remained stained a strong red colour after emptying. Both cases were in men who were heavy drinkers, and who were diagnosed at ages 60 and 75 years, respectively. The author suspected that the cancers were caused by aniline exposure. No occupational or other source of aniline exposure is mentioned. [The Working

Group considered the study uninformative, as exposure information is very limited.]

Goldblatt (1949) reported details of 99 cases of bladder tumour diagnosed between 1934 and 1947 in some 4000 men employed in two chemical factories in the United Kingdom. Three papillomas arose in a subgroup of men exposed only to aniline. Larger numbers of tumours, comprising more carcinomas than papillomas, arose in men exposed only to α-naphthylamine [1-naphthylamine] (n = 11 tumours), to β-naphthylamine [2-naphthylamine] (n = 22 tumours), or to benzidine (n = 6 tumours), or to the combination of two or more of these substances (n = 34 tumours). [The Working Group noted that no information was given on the number of workers exposed to aniline only.]

Pujol (1950) reported one case of a bladder tumour in a worker employed for 26 years in a hat-manufacturing factory where several aniline-based dyes were used, in Spain. Exposure through dermal contact and inhalation had been nearly continuous. No protective equipment was used. One additional worker at the plant had unspecified bladder issues but was not examined. The other workers had been at the plant for shorter periods and were asymptomatic. [The Working Group noted that no data on specific exposures or tumour characteristics were given and co-exposure to other occupational bladder carcinogens is probable.]

Aboulker & Smagghe (1953) reported on 21 cases of bladder tumour occurring in workers in the dye industry in France between 1941 and 1952. Three of the cases were exposed to aniline. [The Working Group noted that for all three cases, no occupational co-exposure to any bladder carcinogen was reported.] Case No. 5 of the series was diagnosed in 1947 at the age of 57 years, had worked for 17 years in a dye-production factory [The Working Group noted that no further exposure details were provided]; latency was unknown; tumour histology was unknown. Case No. 6 was diagnosed in 1947 at the age of 47 years, had worked for 24 years in a dye-production factory, and had been exposed to aniline and α-naphthylamine [1-naphthylamine] for 13 years [the Working Group noted that no further exposure details were provided]; latency was 5 years; histology was "benign tumour". Case No. 9 was diagnosed in 1949 at the age of 69 years, had worked for 38 years in a dye-production factory, and had been exposed to aniline and α-naphthylamine [1-naphthylamine] for 26 years [the Working Group noted that no further exposure details were provided]; latency duration was unknown; histology was "malignant tumour". The 18 other cases were exposed to α-naphthylamine, β-naphthylamine, benzidine, *ortho*-toluidine (these latter three are bladder carcinogens) and/or α-aminoanthraquinone. [Information on tobacco smoking was not given.]

Pujol (1954) reported on a second bladder tumour in Spain. The case was in a worker aged 52 years employed for 30 years in the preparation of tints who had used synthetic dyes made of water/alcohol- and oil-based anilines, potassium bichromate, and other series of tints. The worker never used personal protective equipment. The tumour was described as an infiltrating epithelioma; the patient had local recurrence, spreading to the pelvic wall, 1 year after operation. [The Working Group noted that co-exposure to other occupational bladder carcinogens could not be excluded.]

In an autopsy study on multiple primary cancers, Link (1961) noted one case of bladder cancer in a man who had worked in dye production, where he had been specifically exposed to aniline and toluidine. [The Working Group noted that this bladder cancer case was also exposed to toluidine, a known bladder carcinogen.]

Vigliani & Barsotti (1961) reported on bladder tumours in workers in six dyestuff factories in Italy in 1931–1960. Among the 42 cases of carcinoma and 36 cases of papilloma reported, only 1 case of papilloma occurred in a worker exposed to aniline. No co-exposure to bladder carcinogens

was reported for this case. The remaining cases were tumours diagnosed in workers exposed to benzidine only (31 carcinomas, 16 papillomas), β-naphthylamine [2-naphthylamine] only (7 carcinomas, 12 papillomas), benzidine and β-naphthylamine [2-naphthylamine] in combination (2 carcinomas, 1 papilloma), α-naphthylamine [1-naphthylamine] only (1 carcinoma, 3 papillomas), and to azo-dyes in production [the Working Group noted that no information on specific exposure is given for this group] (1 carcinoma, 3 papillomas). [The Working Group noted that no information on the number of workers in each exposed group, age at diagnosis, duration of exposure, or latency was given.]

Temkin (1963) reported on 208 cases of bladder disease related to occupational exposure observed during 25 years in the aniline dye industry in the former Soviet Union. Among workers who had undergone cystoscopy once or twice each year, 125 cases of bladder disease occurred; of these, 5 were malignant tumours. In a group of workers not systematically examined by cystoscopy, 83 workers presented with bladder tumours, of which 32 were malignant. [The Working Group noted that there was no information on specific exposures, duration of exposure, or number of workers in the screened and unscreened groups; the numbers of malignant/benign and examined/not examined cases did not add up correctly; and co-exposure to occupational bladder carcinogens other than aniline is probable.]

Nakano et al. (2018) reported on 10 cases of bladder cancer in male Japanese workers exposed primarily to *ortho*-toluidine and employed at two plants producing organic dye and pigment intermediates. No exposure measurements were available, but four jobs or production processes were identified, and levels of exposure to *ortho*-toluidine and other aromatic amines were estimated based on number of years and proportion of time spent on each of the four processes each month. In most of the 10 cases, there was a higher level of exposure to *ortho*-toluidine than to the other amines. Co-exposure to aniline occurred in 9 out of 10 cases. The 10 identified cases were in workers hired between 1987 and 1997. Duration of exposure to aniline ranged from 3 to 21 years (mean, 13.6 years), estimated latency from the initial exposure to aniline to diagnosis ranged from 15 to 27 years (mean, 21.7 years). Eight out of the nine cases were tobacco smokers and the mean number of pack-years for the smokers was 29.9 (range, 10–45). All the affected workers had been primarily engaged in drying and packing the product made from *ortho*-toluidine, a known bladder carcinogen. [The Working Group noted that all aniline workers were co-exposed to *ortho*-toluidine. Eight of the nine cases were also tobacco smokers.]

(b) Lung cancer

Thiess et al. (1969) reported on a series of lung cancer cases diagnosed between 1957 and 1967 at BASF (Badische Anilin und Soda Fabrik) plants in Germany. In the group of workers belonging to the company's dye warehouses, including the aniline book-binding department, the department for tri-colouring, the indigo, aniline, and alizarin departments, and the engineering department, 2 cases of lung cancer occurred among 185 workers. Both cases were in workers aged 53 years at diagnosis, and their smoking status was unknown. [The Working Group concluded that the study is uninformative.]

2.4 Evidence synthesis for cancer in humans

Epidemiological studies available for the evaluation of the carcinogenicity of aniline are scarce. Since the publication of *IARC Monographs* Volumes 4 and 27 and Supplement 7 (IARC, 1974, 1982, 1987), only four case–control studies and two new cohort studies have been published. Cohort studies examined bladder

cancer incidence and mortality, as well as mortality from cancers of the digestive organs, lung, and haematopoietic malignancies. Each case–control study examined a different cancer site in association with aniline: lung, bladder, and pancreas in adults, and leukaemia and brain cancer in children. Seventeen case reports and case series were reviewed; 16 of these reported on bladder tumour cases and one reported on lung cancer cases. These case series and case reports provided historical perspective but were mostly uninformative.

2.4.1 Exposure assessment and misclassification of exposure

Quality of exposure assessment was a major consideration in the evaluation of the studies by the Working Group. Detailed reports on the strengths and limitations of exposure evaluations in cohort and case–control studies are provided in Sections 1.6.1 and 1.6.2, respectively.

In the three cohort studies with moderate to well-defined exposure assessments (Ott & Langner, 1983; Sorahan, 2008; Carreón et al., 2014), the information was collected retrospectively. This could have resulted in non-differential misclassification of exposure (bias due to inaccurate reporting independent of disease status) that may result in an underestimate of the true strength of an association between exposure and disease. The Working Group also noted that none of the cohort studies could provide clear evidence that aniline was the agent responsible for any bladder cancer excesses observed.

The Working Group noted that the case–control studies did not provide information on duration, intensity, or cumulative exposure to aniline. These studies were fundamentally based on work histories derived from interviews of the study participants or a family member who may have had an incomplete recollection, and thus have the potential for recall bias. One study was based on analysis of aniline in exhaled breath after a diagnosis of lung cancer, which is a poor measure of past exposures.

2.4.2 Co-exposures to other agents with sufficient or limited evidence of bladder carcinogenicity in humans

The main factor that affected most studies was their inability to control for concurrent exposures to other agents for which there is *sufficient* (e.g. *ortho*-toluidine, 2-naphthylamine) or *limited* (e.g. MBT) evidence of bladder carcinogenicity in humans and therefore to differentiate aniline-related effects from the effects of co-exposures. Only two case series (Aboulker & Smagghe, 1953; Vigliani & Barsotti, 1961) reported 3 cases and 1 case, respectively, that may not have had exposure to other occupational bladder carcinogens; however, no information was given on tobacco smoking history for these cases. None of the case–control studies accounted for the potential for confounding by co-exposures. Each of the cohort studies identified concurrent occupational exposure to some carcinogenic aromatic amines, but only Sorahan (2008) was able to estimate risk of bladder cancer in aniline-exposed workers while adjusting for duration of work in a department where *ortho*-toluidine was present, work in a department where PBN was present, and cumulative exposure to MBT.

2.4.3 Tobacco smoking

Tobacco smoking is the most important risk factor for bladder cancer, and smokers are at least three times more likely to develop bladder cancer than are never-smokers (Cumberbatch et al., 2018). In addition, aniline is a component of tobacco smoke, but the contribution of tobacco smoking to aniline exposure among aniline workers is considered to be negligible to minimal (see Section 1.4.1). Only one case series (Nakano et al., 2018) informed on tobacco smoking status; 8 out of 9 aniline-exposed cases were smokers.

Only one case–control study (of pancreatic cancer) controlled for potential cofounding by tobacco smoking (Alguacil et al., 2000). Tobacco smoking was indirectly adjusted for in one of the cohort studies (Carreón et al. 2014); in this study, the estimated bias factor was small, compared with the risk estimate due to exposure to aniline, *ortho*-toluidine, and nitrobenzene combined. The Working Group considered that studies that did not evaluate the contribution of tobacco smoking to their risk estimates were of low quality.

2.4.4 Bladder cancer

In total, four cohort studies (Case et al., 1954; Case & Pearson, 1954; Ott & Langner, 1983; Sorahan, 2008; Carreón et al., 2014) and one case–control study (Nizamova, 1991) evaluated the association between aniline exposure and bladder cancer risk. The case–control study is not considered informative and therefore will not be further discussed. Of the four cohort studies, two were considered to be potentially informative for the evaluation. Sorahan (2008) found significant excesses of both bladder cancer mortality and incidence in aniline-exposed workers, in analyses that did not control for co-exposure to other known or suspected bladder carcinogens. They also found a non-significant increased risk by duration of employment in the aniline-exposed department, after adjusting for other known or suspected occupational bladder carcinogens. Carreón et al. (2014) found increased bladder cancer incidence associated with exposure to aniline, *ortho*-toluidine, and nitrobenzene combined. Statistically significant trends were also observed by duration of exposure, time since first exposure, and cumulative rank exposure lagged 10 and 20 years; these trends are unlikely to have occurred by chance. Both studies were methodologically sound, and both had reasonable power; however, the study by Carreón et al. (2014) could not separate any aniline-specific effect from the effects of *ortho*-toluidine and nitrobenzene. Overall, the Working Group considered that these available studies in humans are of good quality, but they are not sufficiently informative to permit a conclusion to be drawn about the presence of a causal association between aniline and bladder cancer.

2.4.5 Other cancers

Other cancer sites (lung and pancreas, and brain and leukaemia in children), were evaluated each in a different case–control study (Preti et al., 1988; Feingold et al., 1992; Alguacil et al., 2000). Ott & Langner (1983) also reported findings for mortality of cancer of the digestive organs, lung, and haematopoietic system in their cohort study. Sorahan (2008) reported a small non-significant deficit in lung cancer mortality compared with the national population in its cohort study. Some positive associations were observed in other studies. Overall, exposures were not well assessed, and there was a small number of exposed cases among other limitations, therefore these studies are of low to moderate quality and informativeness.

3. Cancer in Experimental Animals

Aniline was evaluated previously by the *IARC Monographs* programme in 1981 and 1987 (IARC, 1982, 1987). In its evaluation in 1987 (IARC, 1987), the Working Group concluded that there was *limited evidence* in experimental animals for the carcinogenicity of aniline.

See Table 3.1.

Table 3.1 Studies of carcinogenicity with aniline and aniline hydrochloride in experimental animals

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (M) 6 wk 107 wk (lower and higher dose) or 109 wk (control) NCI (1978)	Oral Aniline hydrochloride, NR ["high purity"; see comments] Feed 0, 0.6%, 1.2% in feed for 103 wk 50, 50, 50 33, 43, 41	*Thyroid gland*: follicular-cell adenoma or carcinoma (combined) 0/38, 3/43, 1/43	NS	Principal strengths: appropriate statistics; sufficient number of animals and survival; use of males and females; adequate durations of exposure and observation Chemical analysis performed by the authors suggests a compound of high purity. Seven control males died and were found autolysed in wk 11–13. On the basis of survival data, adequate numbers of male mice were at risk for late-developing tumours (82% at the higher dose, 86% at the lower dose, and 66% of the control group survived until the end of the study). All mice were necropsied regardless of whether they died early, were killed when moribund, or were killed at the end of the study. Statistical tests were performed on mice that survived at least 52 wk, unless a tumour was found at the anatomical site of interest before wk 52; when such an early tumour was found, comparisons were based on mice that survived at least as long as the animal in which the first tumour was found
Full carcinogenicity Mouse, B6C3F$_1$ (F) 6 wk 107 wk (lower and higher dose) or 109 wk (control) NCI (1978)	Oral Aniline hydrochloride, NR ["high purity"; see comments] Feed 0, 0.6, 1.2% for 103 wk 50, 50, 49 30, 37, 41	*Liver*: hepatocellular carcinoma 1/46, 5/48, 5/48	NS	Principal strengths: appropriate statistics; sufficient number of animals and survival; use of males and females; adequate durations of exposure and observation Chemical analysis performed by the authors suggests a compound of high purity. On the basis of survival data, adequate numbers of female mice were at risk for late-developing tumours (84% at the higher dose, 74% at the lower dose, and 60% of the control mice survived until the end of the study). All mice were necropsied regardless of whether they died early, were killed when moribund, or were killed at the end of the study. Statistical tests were performed on mice that survived at least 52 wk, unless a tumour was found at the anatomical site of interest before wk 52; when such an early tumour was found, comparisons were based on mice that survived at least as long as the animal in which the first tumour was found

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (M) 12 days 9.5 mo Delclos et al. (1984)	Intraperitoneal injection Aniline hydrochloride, NR Sodium acetate buffer 0 (control), 0.15 µmol/g bw Single injection of 10 µL of a 50 mM sodium acetate buffer solution (pH, 5.0), ± aniline hydrochloride 33, 26 NR	*Liver*: hepatoma Tumour incidence, 3/33 (9%), 2/26 (8%) Tumour multiplicity (mean ± standard deviation), 0.1 ± 0.3, 0.1 ± 0.3	 NS NS	Principal limitations: no body-weight data; not a long-term carcinogenicity study No. of animals at start is the number of weaned animals (at 28 days)
Co-carcinogenicity Mouse, CBA × C57/Bl6 (F) NR [weight, 10–12 g] 26 or 39 wk Litvinov et al. (1984)	Oral Aniline, NR Drinking-water 0 (for 26 wk), 1.0 (for 26 wk), 0 (for 39 wk), 1.0 (for 39 wk) mg/L + NDMA (10 mg/L) 50, 50, 50, 50 30, 15, 25, 32	*Liver* Tumours (epithelial) 0/30, 0/15, 2/25, 4/32 Tumours (endothelial [vascular]) 0/30, 1/15, 11/25, 5/32 Tumours (all) 0/30, 1/15, 13/25, 9/32 *Lung*: tumours 16/30, 12/15, 20/25, 14/32	 [NS] [NS] [NS] [NS]	Principal limitations: no body-weight data. Total doses/animal: NDMA (26 wk), 5.050 mg; NDMA (39 wk), 6.825 mg; aniline (26 wk), 0.505 mg; aniline (39 wk), 0.683 mg. Histopathological examination of liver, lung, kidney, spleen, and all gross lesions. Pairwise comparison between NDMA + aniline-treated group versus NDMA-only group at 26 wk and at 39 wk. No results were provided for an aniline-only and an untreated group

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
Co-carcinogenicity Mouse, CBA × C57/Bl6 (F) NR [weight, 10–12 g] 12 mo Litvinov et al. (1986)	Oral Aniline, NR Drinking-water 0, 1.0 mg/L + NDEA (10.0 mg/L) 100, 100 NR	*Liver* Haemangioma 4/79, 19/88* Angiosarcoma 1/79, 0/88 Adenoma 1/79, 2/88 Carcinoma 3/79, 2/88 Tumours (all) 9/79, 23/88* *Lung*: adenoma 22/79, 55/88*	 *[$P = 0.003$; 2-tail Fisher exact test] [NS] [NS] [NS] *[$P = 0.02$; 2-tail Fisher exact test] *[$P < 0.0001$; 2-tail Fisher exact test]	Principal strengths: high number of mice per group Principal limitations: no body-weight data, no survival data, lack of aniline-only or untreated control groups Total doses: aniline, 1.09 mg; NDEA, 10.95 mg. No group treated with aniline only was available. Histopathological examination of liver, lung, kidney, oesophagus, stomach, spleen, and all gross lesions. The effective number of mice was the number of animals surviving after identification of the first tumour. No haemangiosarcoma of the liver was observed in any group. Potential differences between the current histopathological classification and that used when the study was performed

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
Full carcinogenicity Rat, F344 (M) 6 wk 107 wk (lower dose), 108 wk (higher dose), or 110 wk (control) NCI (1978)	Oral Aniline hydrochloride, NR ["high purity"; see comments] Feed 0, 0.3, 0.6% for 103 wk 25, 50, 50 17, 34, 27	*Spleen* Fibrosarcoma or sarcoma (NOS) (combined) 0/25, 7/50, 9/46*	*P* = 0.020, Cochran–Armitage trend-test; **P* = 0.015, Fisher exact test	Principal strengths: appropriate statistics; use of males and females; sufficient survival; adequate duration of exposure and observation Chemical analysis performed by the authors suggests a compound of high purity. On the basis of survival data, adequate numbers of male rats were at risk for late-developing tumours (54% at the higher dose, 68% at the lower dose, and 68% of the control rats survived until the end of the study). All rats were necropsied regardless of whether they died early, were killed when moribund, or were killed at the end of the study. Statistical tests were performed on rats that survived at least 52 wk, unless a tumour was found at the anatomical site of interest before wk 52; when such an early tumour was found, comparisons were based on rats that survived at least as long as the animal in which the first tumour was found
		Haemangiosarcoma 0/25, 19/50*, 20/46*	*P* = 0.001, Cochran–Armitage trend-test; **P* < 0.001, Fisher exact test	
		Body cavities, multiple organs (other than spleen): fibrosarcoma or sarcoma (NOS) (combined) 0/25, 2/50, 9/48*	*P* = 0.004, Cochran–Armitage trend-test; **P* = 0.017, Fisher exact test	
		Spleen or body cavities, multiple organs (other than spleen) (combined) Fibrosarcoma or sarcoma (NOS) (combined) 0/25, 5/50, 18/48*	*P* < 0.001, Cochran–Armitage trend-test; **P* < 0.001, Fisher exact test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
NCI (1978) (cont.)		Haemangiosarcoma		
		0/25, 19/50*, 21/48*	$P = 0.001$, Cochran–Armitage trend-test; *$P < 0.001$, Fisher exact test	
		Spleen: fibroma		
		0/25, 7/50, 6/46	NS	
		Adrenal gland: benign or malignant (combined) pheochromocytoma		
		2/24, 6/50, 12/44	$P = 0.022$, Cochran–Armitage trend-test	
Full carcinogenicity Rat, F344 (F) 6 wk 107 wk (lower dose), 108 wk (higher dose), or 110 wk (control) NCI (1978)	Oral Aniline hydrochloride, NR ["high purity"; see comments] Feed 0, 0.3, 0.6% for 103 wk 25, 50, 50 16, 44, 41	*Spleen or body cavities, multiple organs (other than spleen) (combined)*: fibrosarcoma or sarcoma (NOS) (combined)		Principal strengths: appropriate statistics; use of males and females; sufficient survival; adequate duration of exposure and observation Chemical analysis performed by the authors suggests a compound of high purity. On the basis of survival data, adequate numbers of female rats were at risk for late-developing tumours (82% at the higher dose, 88% at the lower dose, and 64% of the control rats survived until the end of the study). All rats were necropsied regardless of whether they died early, were killed when moribund, or were killed at the end of the study. Statistical tests were performed on animals that survived at least 52 wk, unless a tumour was found at the anatomical site of interest before week 52; when such an early tumour was found, comparisons were based on rats that survived at least as long as the animal in which the first tumour was found
		0/24, 1/50, 7/50	$P = 0.009$, Cochran–Armitage trend-test	
		Spleen: sarcoma (NOS)		
		0/23, 0/50, 3/50	NS	
		Uterus: endometrial stromal polyps		
		2/24, 15/48*, 7/50	*$P = 0.027$, Fisher exact test	
		Body cavities, multiple organs (other than spleen): fibrosarcoma or sarcoma (NOS) (combined)		
		0/24, 1/50, 4/50	NS	
		Adrenal gland: benign or malignant (combined) pheochromocytoma		
		1/24, 0/50, 5/48	NS	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
Full carcinogenicity Rat, Wistar (M) NR [weight, ~150 g] 80 wk Hagiwara et al. (1980)	Oral (feed) Aniline, NR Feed 0, 0.03, 0.06, 0.12% 28, 28, 28, 28 NR	*Forestomach*: papilloma 0/15, 1/10, 1/18, 0/16 *Pituitary gland*: adenoma 0/15, 1/10, 0/18, 0/16	NR, [NS] NR, [NS]	Principal strengths: multiple-dose study Principal limitations: limited reporting of survival data; not a long-term carcinogenicity study
Full carcinogenicity Rat, F344 albino (CD-F) (M) 4–5 wk 104 wk US EPA (1982)	Oral Aniline hydrochloride, assumed to be 100% pure Feed 0, 10, 30, 100 mg/kg bw per day for 104 wk 130, 130, 130, 130 114, 116, 115, 104	*Spleen* Stromal sarcoma 0/123, 0/129, 1/128, 21/130* Haemangiosarcoma 0/123, 0/129, 0/128, 6/130* Fibrosarcoma 0/123, 0/129, 0/128, 3/130 Osteogenic sarcoma	 NR [$P < 0.001$, Cochran–Armitage trend-test]; *[$P < 0.0001$, Fisher exact test] NR [$P < 0.001$, Cochran–Armitage trend-test]; *[$P = 0.03$, Fisher exact test] NR [NS]	Principal strengths: adequate duration of exposure and observation; multiple-dose study; use of males and females; high number of rats per group; sufficient survival Principal limitations: data are not clearly presented throughout the study report, no statistics reported in the study report This report is difficult to follow especially in terms of clarity of incidence numbers. All major tissues and organs from the killed animals in the control and highest-dose groups, and only the spleen and any unusual lesions from the lowest- and intermediate-dose groups, were examined microscopically. There was a significant lower survival rate in high-dose males compared with controls; however, adequate numbers of male rats were at risk for late-developing tumours (80% at the highest dose, 88% at the intermediate dose, 89% at the lowest dose, and 88% of the control male rats survived until the end of the study). Food consumption during the first 50 wk was lower for treated male rats than for controls; however, the authors stated that "these differences were not considered to be treatment-related and were within the range of normal biological variability"

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
US EPA (1982) (cont.)		0/123, 0/129, 0/128, 3/130	NR [NS]	
		Mesothelioma, metastatic		
		0/123, 2/129, 4/128, 2/130	NR [NS]	
		Stromal hyperplasia		
		1/123, 2/129, 0/128, 31/130*	NR [$P < 0.001$, Cochran–Armitage trend-test]; *[$P < 0.0001$, Fisher exact test]	
		Testes: mesothelioma, tunica vaginalis		
		1/114, 4/130, 9/130*, 1/104	NR [$P = 0.019$, Cochran–Armitage trend-test]; *[$P = 0.022$, Fisher exact test]	
Full carcinogenicity Rat, F344 albino (CD-F) (F) 4–5 wk 104 wk US EPA (1982)	Oral Aniline hydrochloride, assumed to be 100% pure Feed 0, 10, 30, 100 mg/kg bw per day for 104 wk 130, 130, 130, 130 110, 109, 116, 117	*Spleen*: stromal hyperplasia		Principal strengths: adequate duration of exposure and observation; multiple-dose study; use of males and females; high number of rats per group; sufficient survival Principal limitations: data are not clearly presented throughout the study report; no statistics reported in the study report This report is difficult to follow especially in terms of clarity of incidence numbers. All major tissues and organs from the killed animals in the control and highest-dose groups, and only the spleen and any unusual lesions from the lowest- and intermediate-dose groups, were examined microscopically. Adequate numbers of female rats were at risk for late-developing tumours (90% at the highest-dose, 89% at the intermediate dose, 84% at the lowest-dose, and 85% of the control female rats survived until the end of the study). No significantly increased incidence of tumours was observed in treated female rats
		0/129, 0/129, 0/130, 9/130*	NR [$P < 0.001$, Cochran–Armitage trend-test]; *[$P = 0.003$, Fisher exact test]	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
Co-carcinogenicity Rat, albino outbred (M) NR [weight, 150–200 g] 19 mo Litvinov et al. (1982)	Oral Aniline, NR Drinking-water 0, 0.5 mg/L + NDMA (10 mg/L, 0.5 mg/kg bw per day) 50, 50 42, 44	*Liver*: tumours [mainly hepatocellular] 15/42, 34/44*	*[$P < 0.0002$; 2-tail Fisher exact test]	Principal limitations: strain NR; no body-weight data. Histopathological examination of the liver, lung, kidney, spleen, and all gross lesions. No liver, kidney, or lung tumours occurred in 38 untreated rats (number at start, 50). No group treated with aniline alone was available. The effective number of animals was the number of surviving animals
Full carcinogenicity Hamster, Syrian golden (M) 8 wk 87 wk Hecht et al. (1983)	Subcutaneous injection Aniline, NR Peanut oil 0, 99 mmol/kg bw total dose 52 weekly injections of 1.9 mmol/kg bw 15, 15 NR	*Any tumour type* 0/15, 0/15	NA	Principal limitations: limited reporting of survival and body-weight data; only gross lesions and representative samples of all major organs were processed for microscopic evaluation Mean survival time was shorter in the treated group: 67.7 wk for treated males (compared with 75.5 wk for male controls). Unconventional route of exposure
Full carcinogenicity Hamster, Syrian golden (F) 8 wk 87 wk Hecht et al. (1983)	Subcutaneous injection Aniline, NR Peanut oil 0, 99 mmol/kg bw total dose 52 weekly injections of 1.9 mmol/kg bw 15, 15 NR	*Any tumour type* 0/15, 0/15	NA	Principal limitations: limited reporting of survival and body-weight data; only gross lesions and representative samples of all major organs were processed for microscopic evaluation Mean survival time was shorter in the treated group: 62.1 wk for treated females (compared with 68.7 wk for female controls). Unconventional route of exposure
Full carcinogenicity Rabbit, NR (M) NR (weight, ~2 kg) Lifetime (up to 362 days) Yamazaki & Sato (1937)	Urinary bladder instillation Aniline, NR Water 0.6 g/wk 10 mL of 1% aniline (0.1 g) given by daily instillation (0.6 g/wk) 30 0	*Urinary bladder*: papillomas 12/30	NA	Principal strengths: high quality of gross descriptions and microscopic examinations Principal limitations: lack of adequate control group A control group of 20 rabbits was instilled with water only or olive oil only, but the number of animals instilled with water only was unspecified. No rabbits developed urinary bladder tumours in this control group. Unconventional route of exposure

bw, body weight; F, female; M, male; mo, month; NA, not applicable; NDEA, *N*-nitrosodiethylamine; NDMA, *N*-nitrosodimethylamine; NOS, not otherwise specified; NR, not reported; NS, not significant; wk, week.

3.1 Mouse

3.1.1 Oral administration (feed)

Groups of 50 male and 49–50 female B6C3F$_1$ mice (age, 6 weeks) were given feed containing aniline hydrochloride [purity not reported; chemical analysis performed by the authors suggested a compound of high purity] at 0% (controls), 0.6% (lower dose) or 1.2% (higher dose) for 103 weeks (NCI, 1978). Mice at the higher dose, lower dose, and in the control group were killed at 107, 107, and 109 weeks, respectively. There was no significant effect on survival in groups of treated male and female mice. Seven control males died and were found autolysed in weeks 11–13. Adequate numbers of mice were at risk of late-developing tumours: for males, 82% (41/50) at the higher dose, 86% (43/50) at the lower dose, and 66% (33/50) of the controls survived until the end of the study; for females, 84% (41/49) at the higher dose, 74% (37/50) at the lower dose, and 60% (30/50) of the controls survived until the end of the study. Significant mean body-weight decreases occurred in both groups of treated males. An anatomopathological study with histopathological examination was performed on gross lesions and all major organs and tissues. There was no significant increase in the incidence of tumours in the groups of treated male and female mice compared with their respective controls (NCI, 1978). [The Working Group noted the principal strengths of the study: there was a sufficient number of mice at start and adequate survival, appropriate statistics were performed, males and females were used, and the durations of exposure and observation were adequate.]

3.1.2 Intraperitoneal injection

Two groups of male B6C3F$_1$ mice [number of mice per group at start unspecified] (age, 12 days) were given a single intraperitoneal injection of aniline hydrochloride [purity not reported] at a dose of 0 (vehicle control) or 0.15 μmol/g body weight (bw) in 10 μL/g bw of 50 mM sodium acetate buffer solution (pH, 5.0) (Delclos et al., 1984). Mice were weaned at age 28 days (26 treated mice and 33 controls), and the study was terminated when mice were aged 10 months. On death or at the termination of the study, gross routine autopsies were performed for all mice; this included inspection of the skin, subcutaneous tissues, and the organs of the abdominal and thoracic cavities. In the liver, hepatic nodules were enumerated to determine the incidence and multiplicity of hepatoma. Representative liver tumours from each mouse were evaluated by microscopic examination. The hepatic tumours were diagnosed histologically as type A hepatoma, type B hepatoma, or as mixed type A–type B hepatoma. In addition, all gross tumours in other tissues were examined histologically. [The Working Group noted the high quality of the gross description and microscopic examination, with meticulous care for detail.] Neither the incidence of hepatoma nor the average number of hepatomas per mouse was significantly higher in the treated group compared with vehicle controls. [The Working Group noted that this was not a long-term carcinogenicity study, and that body-weight data were lacking.]

3.1.3 Subcutaneous injection

In a 24-month study, two groups of 10 Stock female mice and one group of 10 strain D female mice were given repeated [no further details provided] subcutaneous injections of 0.25 mL of lard containing 0.5% aniline [purity not reported]. Five mice per group survived at the end of the study. No tumours were detected at necropsy (Shear & Stewart, 1941). [The Working Group noted the lack of controls and the limited experimental details. The study was considered inadequate for the evaluation.]

One group of 20 strain C female mice was given eight subcutaneous injections of 5 mg of aniline [purity not reported] in olive oil in a

15-month experiment, and one group of 11 strain C female mice was given 13 subcutaneous injections of 4 mg of aniline hydrochloride [purity not reported] in aqueous solution in a 12-month experiment. No tumours were detected at necropsy (Hartwell & Andervont, 1951). [The Working Group noted the lack of controls and the limited experimental details. The study was considered inadequate for the evaluation.]

3.1.4 Co-carcinogenicity

In a study by Litvinov et al. (1984), four groups of 50 female CBA × C57/Bl6 mice [age not reported] (body weight, 10–12 g) were given drinking-water containing *N*-nitrosodimethylamine (NDMA) at a concentration of 10 mg/L plus aniline [purity not reported] at a concentration of 0 (control group) or 1.0 mg/L for either 26 weeks (total dose, aniline, 0.505 mg; NDMA, 5.050 mg) or 39 weeks (total dose, aniline, 0.683 mg; NDMA, 6.825 mg). Two additional groups of 50 female mice received drinking-water only or aniline only (total dose, 0.683 mg) for 39 weeks. Necropsy was performed on surviving mice, and the liver, lung, kidney, spleen, and all gross lesions were examined histologically. The effective number of animals was the number of surviving mice. There was no increase in the incidence of tumours of the liver, kidney, or lung after 26 or 39 weeks in the groups treated with NDMA plus aniline compared with their respective NDMA-only controls. [The Working Group noted the lack of body-weight data. No results were provided for the aniline-only treated group and the untreated group.]

In a study by Litvinov et al. (1986), two groups of 100 female CBA × C57/Bl6 mice [age not reported], (body weight, 10–12 g), were given drinking-water containing *N*-nitrosodiethylamine (NDEA) at a concentration of 10.0 mg/L (total dose, 10.95 mg) plus aniline [purity not reported] at a concentration of 0 (control group) or 1.0 mg/L (total dose, 1.09 mg). There was no group treated with aniline alone. The study lasted 12 months. Necropsy was performed on all mice, and the liver, lung, kidney, oesophagus, stomach, spleen, and all gross lesions were examined histologically. The effective number of animals was the number of mice surviving after identification of the first tumour. The incidence of liver tumours was significantly higher in the group treated with NDEA plus aniline than in the NDEA-only control group (23/88 versus 9/79; [$P = 0.02$]). The incidence of liver haemangioma was significantly higher in the group treated with NDEA plus aniline than in the NDEA-only control group (19/88 versus 4/79; [$P = 0.003$]). The incidence of lung adenoma was significantly higher in the group treated with NDEA plus aniline than in the NDEA-only control group (55/88 versus 22/79; [$P < 0.0001$]). There was no increase in the incidence of tumours of the oesophagus, kidney, or forestomach, or other types of tumours in the group treated with NDEA plus aniline when compared with NDEA-only controls. [The Working Group noted the high number of mice per group, the lack of body-weight and survival data, the absence of aniline-only or untreated control groups, and the potential differences between the current histopathological classification and that used when the study was performed.]

3.2 Rat

3.2.1 Oral administration (feed)

In a lifetime study, a group of 43 male and female Osborne-Mendel rats [age not reported; body weight, 75–85 g] were given feed containing aniline hydrochloride [purity not reported] at a concentration of 0.033% for 420–1032 (average, 654) days (White et al., 1948). Hepatomas in the liver (4/43) and fibrosarcomas of the spleen (3/43) were reported. [These tumours were considered by the authors not to be spontaneous because hepatomas had never been seen before in this

strain of rats, including in rats aged 2 years or older, and all tumours were present before day 700.] [The Working Group noted the principal limitations of the study: the use of only one dose group; the lack of controls; and that it was not clear whether the number of animals per group (43) was the starting, effective, or surviving number. The Working Group considered the study inadequate for the evaluation due to the absence of controls.]

Groups of 50 male and 50 female Fischer 344 rats (age, 6 weeks) were given feed containing aniline hydrochloride [purity not reported; chemical analysis performed by the authors suggested a compound of high purity] at a concentration of 0.3% (lower dose) or 0.6% (higher dose) for 103 weeks (NCI, 1978). Groups of 25 male and 25 female Fischer 344 rats (control groups) were given feed only for 103 weeks. Rats at the higher dose, lower dose, and in the control groups were killed at 108, 107, and 110 weeks, respectively. There was no significant effect on survival and body weight in groups of treated male and female rats. Adequate numbers of rats were at risk of late-developing tumours: for males, 54% (27/50) at the higher dose, 68% (34/50) at the lower dose, and 68% (17/25) of the controls survived until the end of the study; for females, 82% (41/50) at the higher dose, 88% (44/50) at the lower dose, and 64% (16/25) of the controls survived until the end of the study. An anatomopathological study with histopathological examination was performed on gross lesions and all major organs and tissues. Reference was made to neoplasms of "multiple organs within the body cavities"; such a neoplasm was observed in more than one of the organs [other than spleen] located in the pleural or the abdominal cavity (or both).

The incidence of fibrosarcoma or sarcoma (not otherwise specified, NOS) (combined) of the spleen was significantly higher in treated male rats than in controls (control group, 0/25; lower dose, 7/50; and higher dose, 9/46) with a significant positive trend ($P = 0.020$, trend test; $P = 0.015$ for the group at the higher dose compared with controls). The incidence of haemangiosarcoma of the spleen was significantly higher in treated male rats than in controls (control group, 0/25; lower dose, 19/50; and higher dose, 20/46), with a significant positive trend ($P = 0.001$, trend test; $P < 0.001$ for the groups at the lower and higher doses compared with controls). The incidence of fibrosarcoma or sarcoma NOS (combined) in multiple organs other than spleen within the body cavities was significantly higher in treated male rats than in controls (control group, 0/25; lower dose, 2/50; and higher dose, 9/48) with a significant positive trend ($P = 0.004$, trend test; $P = 0.017$ for the group at the higher dose compared with controls). The incidence of fibrosarcoma or sarcoma NOS (combined) of the spleen or of multiple organs other than spleen within the body cavities (combined) was significantly higher in treated male rats than in controls (control group, 0/25; lower dose, 5/50; and higher dose, 18/48), with a significant positive trend ($P < 0.001$, trend test; $P < 0.001$ for the group at the higher dose compared with controls). The incidence of haemangiosarcoma of the spleen or of multiple organs other than spleen within the body cavities (combined) was significantly higher in treated male rats than in controls (control group, 0/25; lower dose, 19/50; and higher dose, 21/48), with a significant positive trend ($P = 0.001$, trend test; $P < 0.001$ for the groups at the lower and higher doses compared with controls). There was a significant positive trend in the incidence of benign or malignant (combined) pheochromocytoma of the adrenal gland in treated male rats compared with controls (control group, 2/24; lower dose, 6/50; and higher dose, 12/44; $P = 0.022$, trend test).

There was a significant positive trend in the incidence of fibrosarcoma or sarcoma NOS (combined) of the spleen or of multiple organs other than spleen within the body cavities (combined) in treated female rats compared with controls (control group, 0/24; lower dose, 1/50;

and higher dose, 7/50; $P = 0.009$, trend test). The incidence of endometrial stromal polyp of the uterus was significantly higher in treated female rats than in controls (control group, 2/24; lower dose, 15/48; and higher dose, 7/50; $P = 0.027$ for the group at the lower dose compared with controls).

Regarding non-neoplastic lesions, only treated male and female rats developed fibrosis of the splenic capsule and trabeculae, with the presence of scattered large fat cells in the splenic parenchyma. Many treated male and female rats also developed papillary hyperplasia of the splenic capsule surface (NCI, 1978). [The Working Group noted the principal strengths of the study: there was adequate survival, appropriate statistical analyses were performed, males and females were used, and the durations of exposure and observation were adequate.]

Four groups of 28 male Wistar rats [age not reported; weight, approximately 150 g] received feed containing aniline [purity not reported] at a concentration of 0 (controls), 0.03%, 0.06%, or 0.12% for 80 weeks (Hagiwara et al., 1980). No marked adverse effects on body weight were observed. A complete analysis was conducted on all rats found dead or killed when moribund, as well as on rats surviving up to 80 weeks. The effective numbers of rats were 15, 10, 18, and 16 in the control group and at the lowest, intermediate, and highest dose, respectively. Papilloma of the forestomach was observed in 0/15, 1/10, 1/18, and 0/16 rats in the four groups, respectively; pituitary adenoma was observed only in 1/10 rats at the lowest dose. No tumours were observed in the urinary bladder and subcutaneous tissues. [The Working Group noted the multiple doses tested, limited reporting of survival data, and that this was not a long-term carcinogenicity study.]

Groups of 130 male and 130 female Fischer 344 albino (CD-F) rats (age, 4–5 weeks) were given feed containing aniline hydrochloride [assumed by the authors to be 100% pure] for 104 weeks at a dose of 0 (controls), 10, 30, or 100 mg/kg bw per day (US EPA, 1982). There was no significant effect on body weight in the treated groups. Adequate numbers of male rats were at risk of late-developing tumours, although the survival rate was significantly lower in males at the highest dose than in controls: 104/130 (80%) at the highest dose, 115/130 (88%) at the intermediate dose, 116/130 (89%) at the lowest dose, and 114/130 (88%) in the control group survived until the end of the study. Adequate numbers of female rats were at risk of late-developing tumours: 117/130 (90%) at the highest dose, 116/130 (89%) at the intermediate dose, 109/130 (84%) at the lowest dose, and 110/130 (85%) in the control group survived until the end of the study. Food consumption during the first 50 weeks was lower for treated male rats than for controls; however, these differences were not considered to be treatment-related and were within the range of normal biological variability. All major tissues and organs from the rats in the control group and at the highest dose, and only the spleen and any unusual lesions from rats at the lowest and intermediate doses, were examined microscopically.

The incidence of stromal sarcoma of the spleen was significantly higher in treated male rats than in controls (control group, 0/123; lowest dose, 0/129; intermediate dose, 1/128; and highest dose, 21/130), with a significant positive trend [$P < 0.001$, trend test; $P < 0.0001$ for the group at the highest dose compared with controls]. The incidence of haemangiosarcoma of the spleen was significantly higher in treated male rats than in controls (control group, 0/123; lowest dose, 0/129; intermediate dose, 0/128; and highest dose, 6/130), with a significant positive trend [$P < 0.001$, trend test; $P = 0.03$ for the group at the highest dose compared with controls]. The incidence of mesothelioma of the tunica vaginalis of the testis was significantly higher in treated male rats than in controls (control group, 1/114; lowest dose, 4/130; intermediate dose, 9/130; and highest dose, 1/104), with a significant positive trend [$P = 0.019$, trend test; $P = 0.022$ for the

group at the intermediate dose compared with controls].

Regarding non-neoplastic lesions, the incidence of stromal hyperplasia of the spleen was significantly higher in treated male rats than in controls (control group, 1/123; lowest dose, 2/129; intermediate dose, 0/128; and highest dose, 31/130), with a significant positive trend [$P < 0.001$, trend test; $P < 0.0001$ for the group at the highest dose compared with controls]. [The presence of stromal hyperplasia lends further support to the evidence that stromal sarcomas of the spleen were induced by aniline hydrochloride. The authors noted that the stromal hyperplasias in the spleen "often appeared similar in cell type and morphology to the stromal sarcomas, but lacked invasion", so stromal hyperplasia of the spleen probably represents a precursor (pre-neoplastic lesion) to the stromal sarcoma.]

The incidence of tumours in treated female rats was not significantly higher than in controls; however, the incidence of stromal hyperplasia of the spleen was significantly higher in treated female rats than in controls (control group, 0/129; lowest dose, 0/129; intermediate dose, 0/130; and highest dose, 9/130), with a significant positive trend [$P < 0.001$, trend test; $P = 0.003$ for the group at the highest dose compared with controls] (US EPA, 1982). [The Working Group noted the principal strengths of the study: the high number of animals at the start and adequate survival, the use of males and females, the adequate durations of exposure and observation, and multiple doses tested. The principal limitations were: no statistics were reported in the study report, and the data in the report were not clearly presented.]

[The Working Group noted that the aromatic amine *ortho*-toluidine, which is *carcinogenic to humans* (IARC Group 1) also causes malignant tumours of the spleen and mesothelioma of the tunica vaginalis of the testis when administered to male Fischer 344 rats (IARC, 2012).]

3.2.2 Oral administration (drinking-water)

In a lifetime study, 50 rats [strain and sex unspecified] (age, 100 days) were given drinking-water containing aniline hydrochloride [purity not reported] to provide an intake of 22 mg/day. Half of the rats lived for more than 425 days, the last rat surviving up to day 750. The total doses administered were between 14 g and 16.5 g/rat. Necropsy was performed, but only the urinary bladder, liver, spleen, and kidneys were examined in all rats. No tumours were observed (Druckrey, 1950). [The Working Group noted the lack of controls and the limited experimental details. The study was considered inadequate for the evaluation.]

3.2.3 Co-carcinogenicity

Litvinov et al. (1982) gave two groups of 50 albino outbred male rats [strain and age unspecified] (body weight, 150–200 g) drinking-water containing NDMA at a concentration of 10 mg/L (0.5 mg/kg bw per day) alone or with aniline [purity not reported] at a concentration of 0.5 mg/L. A third group of 50 untreated control rats was given drinking-water only; no group treated with aniline alone was available. The experiment lasted 19 months. Necropsy was performed on all rats, and the liver, lung, kidney, and spleen, and all gross lesions were examined histologically. The effective number of animals was the number of surviving rats. The incidence of liver tumours [mainly hepatocellular tumours, NOS] was significantly higher in rats receiving NDMA plus aniline than in rats receiving NDMA alone [34/44 versus 15/42; $P < 0.0002$]. There were no liver tumours in 38 untreated control rats. There was no significant increase in the incidence of kidney or lung tumours in rats receiving NDMA plus aniline compared with rats receiving NDMA alone. No kidney or lung tumours occurred in 38 untreated control rats. [The Working Group noted the lack

of body-weight data and absence of an aniline-only treated group.]

3.3 Hamster

Subcutaneous injection

Two groups of 15 male and 15 female Syrian golden hamsters (age, 8 weeks) were given aniline [purity not reported] at a dose of 1.9 mmol/kg bw in peanut oil by weekly subcutaneous injection for 52 weeks. Two vehicle-control groups of 15 male and 15 female hamsters were injected with peanut oil only (Hecht et al., 1983). After the injections were complete, the hamsters were observed until moribund. The experiment was terminated after 87 weeks. Mean survival times were shorter in treated groups of males and females than in controls: treated males, 67.7 weeks; treated females, 62.1 weeks; male controls, 75.5 weeks; and female controls, 68.7 weeks. There was a decrease in body weight in treated males and females compared with males and females in the vehicle-control groups. Upon necropsy, gross lesions and representative samples of all major organs were processed for microscopic evaluation. Aniline at a total dose of 99 mmol/kg bw did not induce any tumours in male and female hamsters. No tumours were observed in the controls. [The Working Group noted the limited data on survival and body weight, and the incomplete histopathological examination. The outcome of the study may reflect the low dose used, the unconventional route of administration, or a possible species-specific difference.]

3.4 Rabbit

3.4.1 Subcutaneous injection

A group of 12 male rabbits [age not reported; weight, approximately 2 kg] received daily subcutaneous injections of 1 mL of 1% aniline [purity not reported] (10 mg) in water (60 mg/week) for up to 216 days. The rabbits did not develop any urinary bladder tumours (Yamazaki & Sato, 1937). [The Working Group noted the lack of vehicle-control group, and the unconventional route of administration. The study was considered inadequate for the evaluation.]

3.4.2 Bladder instillation

In a lifetime study, a group of 30 male rabbits [age not reported; weight, approximately 2 kg] was given 10 mL of 1% aniline [purity not reported] (0.1 g) in water by daily instillation (0.6 g/week) into the urinary bladder. An additional group of 4 male rabbits received 10 mL of 5% aniline (0.5 g) in water by daily instillation (3.0 g/week). A further group of 3 male rabbits received 10 mL of 10% aniline (1.0 g) in water by daily instillation (6.0 g/week). A group of 20 controls received water only or olive oil only by daily instillation. In the group receiving 1% aniline for up to 362 days, 12/30 rabbits developed urinary bladder papillomas after 13–307 days. The rabbits receiving 5% or 10% aniline died within 4–15 days from urinary bladder necrosis and did not develop any urinary bladder tumours. No urinary bladder tumours were observed in the controls (Yamazaki & Sato, 1937). [The Working Group noted the high quality of gross descriptions and microscopic examinations, and the unconventional route of administration. The Working Group also noted that 12/30 treated rabbits developed urinary bladder papillomas, and there were no tumours in 20 controls that had been instilled with water only or olive oil only. However, the number of rabbits instilled with water only in the control group was not reported, and thus this study was considered limited by the Working Group because of the incomplete reporting regarding vehicle controls.]

3.5 Synthesis

In one independent study in male and female Fischer 344 rats treated by oral administration (in feed), aniline hydrochloride caused a significant increase, with a significant positive trend, in the incidence of fibrosarcoma or sarcoma NOS (combined) of the spleen; haemangiosarcoma of the spleen; fibrosarcoma or sarcoma NOS (combined) of multiple organs other than spleen within the body cavities; fibrosarcoma or sarcoma NOS (combined) of the spleen or of multiple organs other than spleen within the body cavities (combined); and haemangiosarcoma of the spleen or of multiple organs other than spleen within the body cavities (combined) in male rats. Aniline hydrochloride also caused a significant positive trend in the incidence of benign or malignant (combined) pheochromocytoma of the adrenal gland in male rats. Aniline hydrochloride caused a significant positive trend in the incidence of fibrosarcoma or sarcoma NOS (combined) of the spleen or of multiple organs other than spleen within the body cavities (combined), and a significant increase in the incidence of endometrial stromal polyps of the uterus in female rats (NCI, 1978).

In another independent study in male and female Fischer 344 rats treated by oral administration (in feed), aniline hydrochloride caused a significant increase, with a significant positive trend, in the incidence of stromal sarcoma of the spleen, haemangiosarcoma of the spleen, and mesothelioma of the tunica vaginalis of the testis in male rats. There was no significant increase in the incidence of tumours in treated female rats (US EPA, 1982).

In one co-carcinogenicity study in female CBA × C57/Bl6 mice treated by oral administration (in drinking-water), in which NDEA was administered in the presence or absence of aniline, there was a significant increase in the incidence of liver tumours, haemangioma of the liver, and lung adenoma in mice treated with NDEA plus aniline, compared with mice treated with NDEA alone (Litvinov et al., 1986). In another co-carcinogenicity study in male albino outbred rats treated by oral administration (in drinking-water), in which NDMA was administered in the presence or absence of aniline, there was a significant increase in the incidence of liver tumours in mice treated with NDMA plus aniline, compared with mice treated with NDMA alone (Litvinov et al., 1982).

In one study in male rabbits treated by urinary bladder instillation, aniline induced papillomas of the urinary bladder in 12 out of the 30 treated animals; however, this study was considered limited by the incomplete reporting regarding vehicle controls (Yamazaki & Sato, 1937).

In one oral administration (in feed) study in male and female B6C3F$_1$ mice (NCI, 1978), one oral administration (in feed) study in male Wistar rats (Hagiwara et al., 1980), one intraperitoneal injection study in male B6C3F$_1$ mice (Delclos et al., 1984), one subcutaneous injection study in male and female Syrian golden hamsters (Hecht et al., 1983), and one co-carcinogenicity study in female CBA × C57/Bl6 mice (Litvinov et al., 1984), aniline or aniline hydrochloride did not induce any tumours or did not cause a significant increase in the incidence of tumours.

4. Mechanistic Evidence

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Exposed humans

(a) Absorption

Aniline is absorbed via dermal, inhalation, or oral routes. Depending on the task performed and the aniline content of the product handled, reports from controlled studies indicate that the dermal route may contribute significantly

to total uptake (Piotrowski, 1957; Dutkiewicz & Piotrowski, 1961; Baranowska-Dutkeiwicz, 1982; Korinth et al., 2007). A study of occupationally exposed workers for whom urinary aniline and haemoglobin adducts were used as biomarkers of exposure demonstrated that skin lesions (such as erythema and scaling) on the hands facilitated the dermal absorption of aniline (Korinth et al., 2007). The use of barrier creams also increased exposure, a phenomenon known as "penetration enhancement" (Korinth et al., 2008). Shi & Ma (2009) reported a case of fatal acute aniline poisoning by dermal exposure.

Regarding inhalation exposure, Käfferlein et al. (2014) performed a controlled study on 19 non-smoking subjects; they demonstrated that environmental exposure to aniline at 2 ppm [7.6 mg/m^3] for 6 hours (including 1 hour with exercise) resulted in a mean urinary concentration of aniline of 168.0 µg/L (SD, 51.8 µg/L). The corresponding methaemoglobinaemia level was 1.21% (SD, 0.29%) (Table 1.4).

Facile oral absorption of aniline was also demonstrated by a more recent study with isotope-labelled aniline (Modick et al., 2016; described below). Most studies of aniline metabolism in humans have used oral administration.

Studies of methaemoglobinaemia, long-established as the principal clinical manifestation of aniline poisoning, indicate that aniline is absorbed through the skin and by the oral route. As early as 1885, aniline ink (applied to mark diapers) was reported to cause "cyanosis" in infants (Rayner, 1886); reports of similar incidents continued for many years afterwards (Ramsay & Harvey, 1959). Methaemoglobinaemia was also reported after accidental (usually, dermal) (Phillips et al., 1990; Lee et al., 2013; Shatila et al., 2017) or deliberate (criminal) (Iwersen-Bergmann & Schmoldt, 2000) acute oral aniline poisonings and after oral administration of aniline (up to 65 mg) to human subjects (Jenkins et al., 1972).

[The Working Group noted that aniline is a small lipophilic molecule that is expected to be readily absorbed by all routes of exposure, based on its chemical properties.]

(b) Distribution, metabolism, and excretion

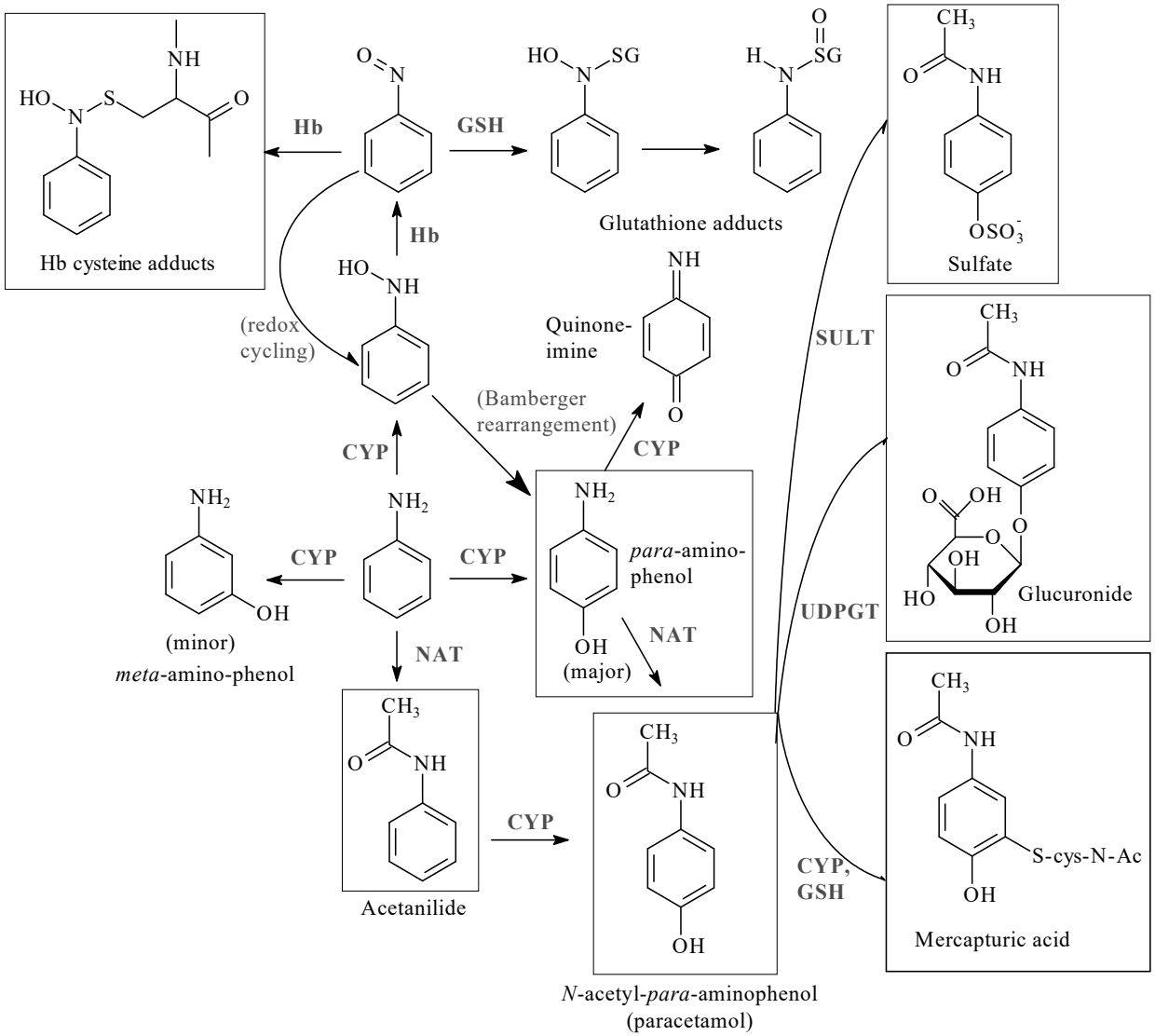
Identified human urinary metabolites and haemoglobin adducts are shown in boxes in Fig. 4.1. See Table 1.4 for a summary of levels of haemoglobin adducts reported in workers occupationally exposed to aniline (Riffelmann et al., 1995; Ward et al., 1996; Thier et al., 2001; Beyerbach et al., 2006; Korinth et al., 2007).

Two metabolic steps, namely cytochrome P450 (CYP)-dependent hydroxylation to *para*-aminophenol [4-aminophenol] and *N*-acetyltransferase (NAT)-dependent N-acetylation (in either sequence), convert aniline into *N*-acetyl-*para*-aminophenol [the chemical name for paracetamol, also known as acetaminophen or *para*-acetyl-aminophenol], which is a widely used analgesic drug. After acute oral exposure of an adult female to aniline, the parent compound and the metabolites acetanilide and *N*-acetyl-*para*-aminophenol were detected in the plasma. The same compounds, as well as *N*-acetyl-*para*-aminophenol conjugates (released by glucuronidase/arylsulfatase treatment), were detected in the urine (GC-MS analysis). Traces of *para*-aminophenol were detected in the urine, but *ortho*-aminophenol was not detected (Iwersen-Bergmann & Schmoldt, 2000).

N-Acetyl-*para*-aminophenol was detected in the urine samples of 21 subjects (not occupationally exposed to aniline) (Modick et al., 2013). This metabolite was detected in all urine samples tested, including samples from the general population, from individuals exposed to aniline in an occupational setting, and from paracetamol (*N*-acetyl-*para*-aminophenol) users (Dierkes et al., 2014).

Modick et al. (2016) dosed four healthy male subjects (two NAT2 "fast" and two NAT2 "slow" acetylators) orally with 5 mg of isotope-labelled

Fig. 4.1 Major pathways in the metabolism of aniline



CYP, cytochrome P450; GSH, glutathione; Hb, haemoglobin; NAT, *N*-acetyl transferase; SULT, sulfotransferase; UDPGT, uridine diphosphoglucuronyltransferase.
Human urinary metabolites and haemoglobin adducts are shown in boxes.
Created by the Working Group.

aniline and urine samples were collected over 2 days. After enzymatic hydrolysis, *N*-acetyl-*para*-aminophenol was the predominant urinary metabolite of aniline (55.7–68.9% of the oral dose). The mercapturic acid conjugate of *N*-acetyl-*para*-aminophenol represented 2.5–6.1% of the administered dose. Acetanilide and free aniline were found in small amounts, 0.14–0.36%. Combined, these metabolites (urine, 48 hours post-dose) accounted for 62.4–72.1% of the oral aniline dose. The elimination half-lives were: *N*-acetyl-*para*-aminophenol, 3.4–4.3 hours; mer-

capturic acid conjugate, 4.1–5.5 hours; acetanilide, 1.3–1.6 hours; aniline, 0.6–1.2 hours.

The metabolism of aniline in humans in vivo to give protein (haemoglobin and serum albumin) adducts is discussed in Section 4.2.1(a).

4.1.2 Human cells in vitro

(a) CYP-dependent metabolism of aniline

Recombinant human CYP2E1 catalyses the hydroxylation of aniline to *para*-aminophenol [4-aminophenol] (Dai et al., 1993; Yamazaki et al., 1996) and CYP2E1 is thought to be the major CYP enzyme carrying out aniline hydroxylation. [The Working Group noted that, although not demonstrated directly with human CYP2E1, on the basis of rodent metabolism studies it is likely that the other isomers, 2-aminophenol [*ortho*-aminophenol] and 3-aminophenol [*meta*-aminophenol], are also formed, albeit in much smaller amounts.]

Hartman and colleagues studied the metabolism of aniline to *para*-aminophenol [4-aminophenol] catalysed by pooled human liver microsomes. The contributions of specific hepatic CYP isoforms were assessed by examining the effects of enzyme-specific inhibitors. The major contributors to aniline 4-hydroxylation were found to be CYP2E1, CYP2A6, and CYP2C9 (Hartman et al., 2014). Both endoplasmic reticulum-localized and mitochondrion-localized forms of CYP2E1 contributed (Hartman et al., 2015). [The Working Group noted that the N-hydroxylation of aniline is presumably catalysed by CYP in the human liver.]

No data were available to the Working Group on CYP-dependent N-oxidation of aniline. CYP-dependent N-oxidation of the aniline derivative, 2,6-dimethylaniline, has been studied in vitro with human liver microsomes and recombinant human CYPs (Gan et al., 2001; Skipper et al., 2010). Like aniline, 2,6-dimethylaniline can undergo *para*-hydroxylation to the *para*-aminophenol [4-aminophenol], or N-hydroxylation to the hydroxylamine. It was observed that, at micromolar concentrations, only *para*-hydroxylation was detectable, but at nanomolar concentrations, N-hydroxylation was a major pathway. Using CYP-specific inhibitors and inhibitory mouse monoclonal antibodies, CYP2A6 was identified as the major form responsible for N-hydroxylation. Also, recombinant human cytochrome CYP2E1 and human liver microsomes catalysed the NADPH-independent rearrangement of *N*-(2,6-dimethylphenyl) hydroxylamine to 4-amino-3,5-dimethylphenol (the "Bamberger rearrangement") (Gan et al., 2001). [The Working Group noted the relevance of these findings on 2,6-dimethylaniline to aniline metabolism.]

(b) N-acetylation of aniline

N-Acetylation of aniline has been demonstrated in vitro with recombinant forms of both human acetyl coenzyme A (CoA):aromatic amine *N*-acetyltransferase enzymes, NAT1 and NAT2 (Liu et al., 2007). [The Working Group noted that it is likely that aniline and all its metabolites with free arylamine NH_2 groups undergo acetylation, at least to some extent, in humans.]

4.1.3 Experimental systems

Aniline is rapidly absorbed by experimental animals after oral administration, application to the skin, or inhalation (Carpenter et al., 1949; Roudabush et al., 1965; Kiese, 1966). After intravenous administration to male Fischer 344 rats, [^{14}C]-labelled aniline was distributed throughout the body, as observed by whole-body autoradiography (Irons et al., 1980). Aniline was shown to readily pass the placental barrier in pregnant Sprague-Dawley rats (Maickel & Snodgrass, 1973).

Plasma clearance of aniline administered subcutaneously to male albino rats was increased after pretreatment with phenobarbital or benzo[*a*]

pyrene (Wiśniewska-Knypl & Jabłońska, 1975). When [^{14}C]-labelled aniline was administered to dairy cattle, residue was detected in the edible tissues and in the milk (Eisele et al., 1985).

After oral administration of aniline to dogs and acid hydrolysis of conjugates, *para*-aminophenol [4-aminophenol] was identified in the urine (Schmiedeberg, 1877). Smith & Williams (1949) reported that about 28% of aniline, orally administered to rabbits, was excreted as sulfate conjugates of *ortho*-aminophenol [2-aminophenol], *para*-aminophenol [4-aminophenol], and 4-aminoresorcinol (the product of both *ortho*- and *para*-hydroxylation of aniline). About 70% of the administered dose was excreted as glucuronides, including *para*-acetamido- and *para*-aminophenyl glucuronides. Acetylated products were also found.

All species tested (rabbit, rat, mouse, guinea-pig, gerbil, hamster, cat, dog, pig, sheep) hydroxylate aniline to *ortho*- and *para*-aminophenol, which are excreted in the urine as conjugates (Williams, 1959; Parke, 1960; Kao et al., 1978). The ratio of the isomers differs among species. *meta*-Aminophenol was also detected, in trace quantities, in the urine of dogs and rabbits (Parke, 1960).

Small amounts of free aniline, phenylsulfamic acid, and aniline *N*-glucuronide are found in the urine of some species after administration of aniline (Boyland et al., 1957; Parke, 1960). The mercapturic acids of *ortho*- and *para*-aminophenol and *N*-acetyl-*para*-aminophenol are excreted in rats. *N*-acetyl-*para*-aminophenol and its mercapturic acid are excreted in rabbits (Boyland et al., 1963). Acetanilide was found in the urine of rabbits, but not in the urine of dogs (Williams, 1959); the absence of acetylated metabolites in the dog is consistent with the absence of aromatic amine *N*-acetyltransferase genes and enzymes in that species (Trepanier et al., 1997).

Phenylhydroxylamine (*N*-hydroxyaniline) has not been detected in the urine of experimental animals given aniline; however, phenylhydroxylamine and nitrosobenzene are found in the blood (see Section 4.1.4). The N-hydroxylation of aniline by hepatic microsomal preparations from pre-treated rats and mice has been observed in vitro (McCarthy et al., 1985).

In male Sprague-Dawley rats given [^{14}C]-labelled aniline by gavage and killed after 24 hours, dose-dependent binding and accumulation of radiolabel were seen in erythrocytes and in the spleen (Khan et al., 1995).

4.1.4 Methaemoglobinaemia

Methaemoglobinaemia has been demonstrated in humans and in various species of experimental animal, including rats, and is attributed to phenylhydroxylamine formation (Kiese & Taeger, 1976; Harrison & Jollow, 1987). N-Hydroxylation of aniline to give phenylhydroxylamine has been demonstrated after intravenous administration of aniline to dogs (Kiese, 1959), intraperitoneal administration of aniline to rats (Harrison & Jollow, 1987), in liver microsomal preparations of rat and mouse (McCarthy et al., 1985), and rabbit (Burstyn et al., 1991), and in the isolated perfused rat liver, when the perfusion fluid contained human erythrocytes, to trap the metabolites by binding to haemoglobin (Eyer et al., 1980).

The methaemoglobin-inducing species are the oxidized (hydroxylamine and nitroso) metabolites rather than aniline itself. Phenylhydroxylamine is a potent inducer of methaemoglobin (Kiese & Taeger, 1976).

[The Working Group noted that, although the conversion of haemoglobin (Hb) to methaemoglobin is an oxidation, it can be induced by reducing agents (such as nitrite and phenylhydroxylamine), which "unleash" the oxidizing power of bound O_2, via "co-oxidation" processes such as:

$$Hb^{2+}\,O_2 + X_{red} \rightarrow Hb^{3+} + HOOH + X_{ox}$$

The detailed chemical mechanisms of methaemoglobin formation are very complex and are still not fully understood (Gladwin et al., 2009). There are several reasons for this, most or all of which apply in the case of aniline:

(i) Haemoglobin is a macromolecule with four subunits/four iron atoms, and these subunits interact strongly.

(ii) There are many distinct steps in the pertinent chemical processes.

(iii) Haemoglobin has catalytic (peroxidase) activity, in addition to carrying oxygen (Vlasova, 2018).

(iv) There are competing reactions, such as free-radical reactions with the protein's cysteine residues (Maples et al., 1990). These reactions generate covalent addition products (adducts) which can serve as biomarkers of exposure (Pathak et al., 2016).

(v) Redox-cycling can occur, so that the agents inducing methaemoglobinaemia may act as catalysts, with one molecule of toxicant causing multiple haemoglobin oxidations (Vásquez-Vivar & Augusto, 1994).

(vi) The agents inducing methaemoglobinaemia may be metabolites of the toxicant rather than the parent compound.]

4.2 Evidence relevant to key characteristics of carcinogens

This section summarizes the evidence for the key characteristics of carcinogens (Smith et al., 2016) including whether aniline (and aniline hydrochloride) is electrophilic or can be metabolically activated to an electrophile; is genotoxic; induces oxidative stress; or alters cell proliferation, cell death, or nutrient supply. For the evaluation of other key characteristics of carcinogens, data were not available or considered insufficient.

4.2.1 Is electrophilic or can be metabolically activated to an electrophile

This section covers covalent binding to proteins and to DNA.

(a) Protein adducts

See Fig. 4.2.

(i) Haemoglobin adducts in humans

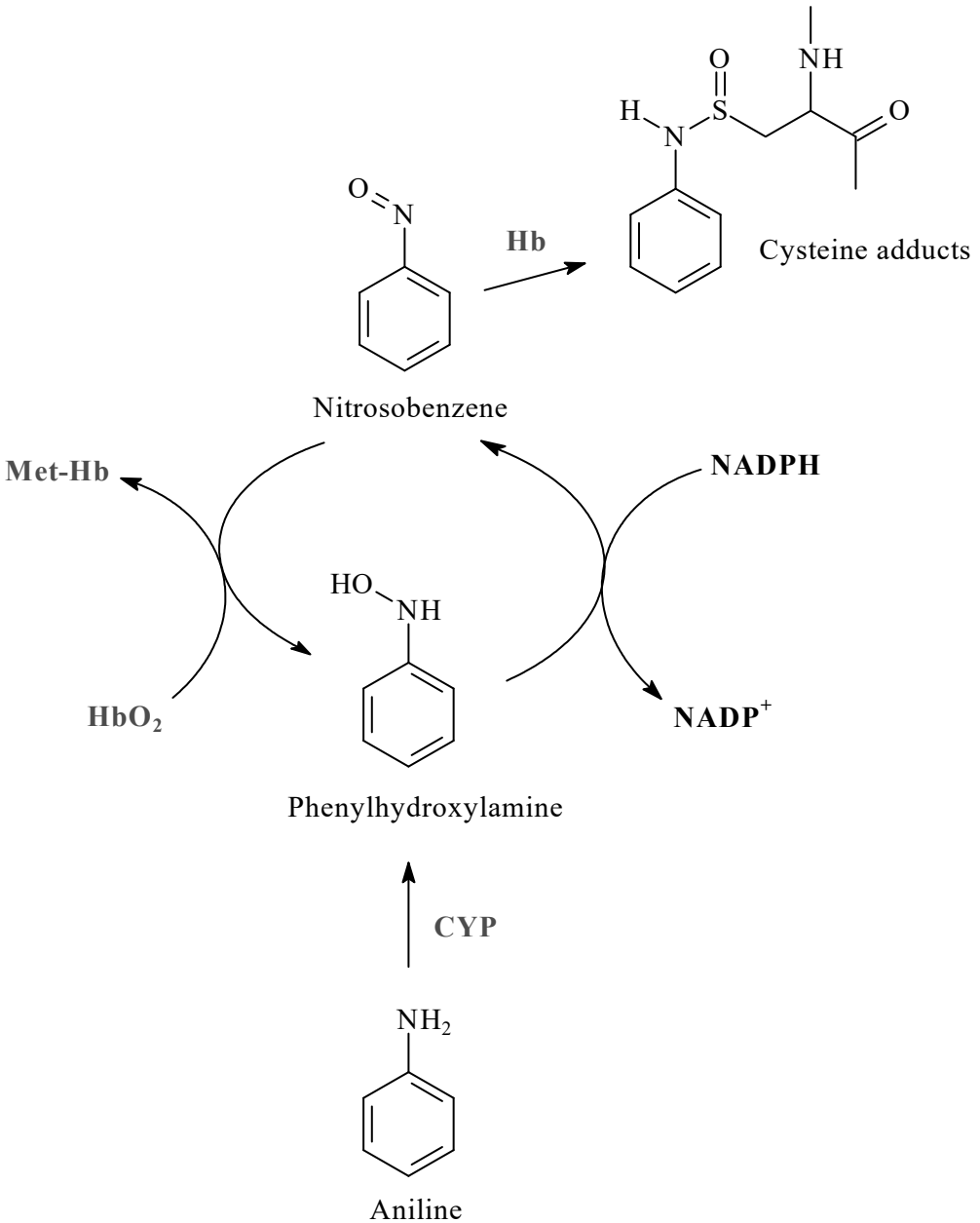
In exposed humans, haemoglobin adducts are commonly used as biomarkers of exposure to aniline. An analytical technique has been applied in which blood haemoglobin is purified and hydrolysed in acid or alkali; bound aniline released by hydrolysis is detected by GC-MS. The bound aniline was used as a biomarker of exposure to aniline, for example, in a study in chemical-plant workers engaged in synthesis and processing of aniline and 4-chloroaniline [*para*-chloroaniline] (Riffelmann et al., 1995), and in a study of workers engaged in the manufacture of rubber additives (Ward et al., 1996) (see also Section 1.4.2). Adducts are also formed with serum albumin, as demonstrated in studies of exposed male workers in a nitrobenzene-reduction plant (Thier et al., 2001; see also Section 1.4.2).

(ii) Protein adducts in experimental systems

Neumann and colleagues (Albrecht & Neumann, 1985; Birner & Neumann, 1988) gave aniline orally to female Wistar rats and showed that aniline bound to haemoglobin, using an analytical technique similar to that used in humans (in which blood haemoglobin was purified and hydrolysed in acid or alkali, and the aniline released by hydrolysis was detected by GC-MS); see also a study in male Fischer 344 rats (Zwirner-Baier et al., 2003).

Adducts with serum albumin were shown to be formed in a study in which beagle dogs were exposed to aniline vapour (Pauluhn, 2002).

Fig. 4.2 Formation of aniline–haemoglobin cysteine adducts



CYP, cytochrome P450; Hb, haemoglobin; NADP, nicotinamide adenine dinucleotide phosphate; NADPH, nicotinamide adenine dinucleotide phosphate, reduced form

Aniline is oxidized to phenylhydroxylamine (*N*-hydroxyaniline) in a cytochrome P450-catalysed reaction. Phenylhydroxylamine is co-oxidized by oxyhaemoglobin (HbO_2) in a complex process that yields nitrosobenzene and methaemoglobin (Met-Hb); the illustration is not to be read as displaying the reaction stoichiometry. Nitrosobenzene can react with cysteine–SH groups on the haemoglobin protein to form covalent adducts. The cysteine adducts can undergo further S-oxidation (not shown).

Roberts & Warwick (1966) reported the binding of [^{3}H]-labelled aniline to protein (as well as to DNA and ribosomal RNA, rRNA) in the liver, spleen, and kidney of male albino rats (Chester Beatty stock), after intraperitoneal administration. In Fischer 344 rats and C57BL/6 × C3H F_1 mice given [^{14}C]-labelled aniline intraperitoneally, protein and RNA of the kidney, large intestine, and spleen were the major macromolecular targets, with low but significant binding to DNA (McCarthy et al., 1985).

In a study of a rat microsomal preparation incubated with [^{14}C]-labelled aniline and an NADPH-generating system, covalent binding to microsomal protein was observed, but adducts were not characterized. In experiments in which the rats were pretreated with either benzene or phenobarbital for CYP induction, the formation of both water-soluble metabolites and protein-bound material was increased (Gut et al., 1996).

(iii) Aniline metabolites involved in haemoglobin adduct formation

The formation of covalent adducts with haemoglobin is attributed to N-oxidation of aniline via formation of the aniline metabolite phenylhydroxylamine. Phenylhydroxylamine is co-oxidized with haemoglobin in the erythrocyte to form nitrosobenzene. Nitrosobenzene binds to the haem iron centre of haemoglobin even more tightly than does oxygen (O_2) (Eyer & Ascherl, 1987). It is also an electrophilic compound that reacts with the sulfhydryl groups of glutathione or of haemoglobin cysteine residues, forming sulfinamide adducts that can be further oxidized to sulfonamides (Kiese & Taeger, 1976; Eyer et al., 1980; Maples et al., 1990; Sabbioni & Beyerbach, 1995; Möller et al., 2017).

(b) DNA adducts

See Fig. 4.3.

(i) DNA adducts in humans

No data were available to the Working Group.

(ii) DNA adducts in experimental systems

Roberts & Warwick (1966) reported the binding of [^{3}H]-labelled aniline to DNA, as well as to rRNA and to protein, in the liver, spleen, and kidney of rats exposed in vivo. In Fischer 344 rats given [^{14}C]-labelled aniline intraperitoneally, binding of the radiolabel was predominantly to protein and RNA. Binding to DNA was detected in various tissues, with the highest levels detected in the kidney, large intestine, and spleen; however, complete purification of the adducted DNA was not attempted and the adducts were not identified (McCarthy et al., 1985). [The Working Group noted that the level of binding was described as "low but significant".] The covalent binding index values for all tissues were less than 15, in contrast to a covalent binding index of 17 000 for aflatoxin B_1 and 560 for 2-acetylaminofluorene (Lutz, 1979, 1981).

[The Working Group noted that, although aniline itself was not examined, alkyl derivatives (2,6-dimethylaniline, 3,5-dimethylaniline, and 3-ethylaniline) were found to bind to DNA (Skipper et al., 2006). Wildtype C57/BL6 mice were given [^{14}C]-labelled compounds intraperitoneally, and tissue DNA was analysed for labelling by sensitive accelerator mass spectrometry. All the alkylanilines produced detectable labelling of DNA, up to about one modified base per 10^7 bases (for 3,5-dimethylaniline, in the liver) (Skipper et al., 2006); (see also the ^{32}P-post-labelling studies in Section 4.2.2)].

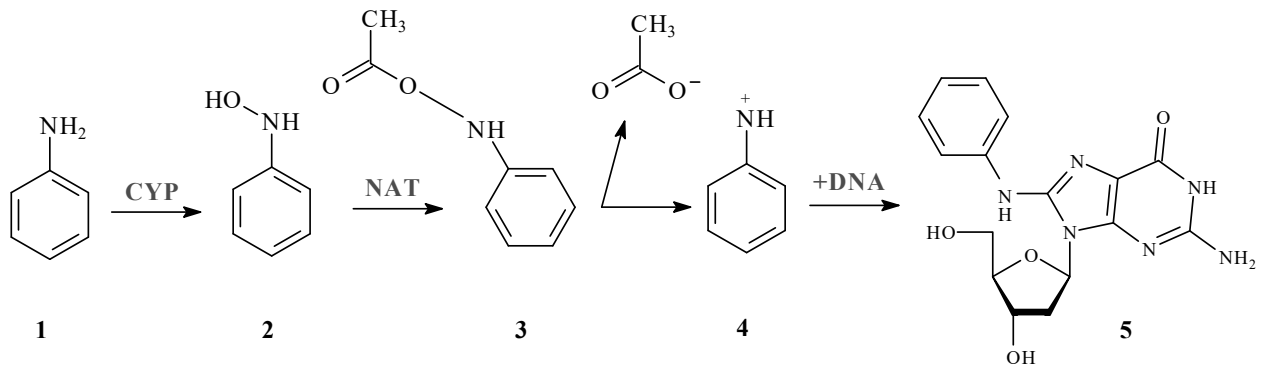
(iii) Aniline metabolites involved in DNA adduction

See Fig. 4.3.

As noted above, no aniline–DNA adducts have been identified in vivo.

N-Acetoxyaniline (the putative metabolic precursor of the phenylnitrenium ion), prepared synthetically, reacts in an acellular system with the DNA nucleoside deoxyguanosine to give an aniline–C8 adduct (Famulok & Boche, 1989). In a subsequent study using a similar chemical

Fig. 4.3 Postulated route to formation of aniline–DNA adducts



CYP, cytochrome P450; NAT, *N*-acetyl transferase.
Aniline–DNA adducts have not been isolated and characterized from cells or tissues, but they have been synthesized chemically by the reactions of *N*-acetoxyaniline (a presumed phenylnitrenium ion precursor) with deoxynucleosides or DNA (Famulok & Boche, 1989; Králík et al., 2015). The scheme shows: the cytochrome P450 (CYP)-catalysed N-oxidation of aniline (1) to phenylhydroxylamine (*N*-hydroxyaniline) (2); O-acetylation (possibly catalysed by *N*-acetyltransferase + acetyl-coenzyme A) to give *N*-acetoxyaniline (3); heterolysis of *N*-acetoxyaniline to give phenylnitrenium ion (4) and a typical DNA adduct (deoxyguanosine C adduct) (5). Králík et al. (2015) isolated adenine adducts at positions C2, C8, *N*7, and N^6, and guanine adducts at positions C8, *N*7, and N^2.
Created by the Working Group.

approach, Králík et al. (2015) separated and identified multiple adducts formed in DNA treated with *N*-acetoxyaniline, characterizing adenine C2, C8, *N*7, and N^6 aniline adducts and guanine C8, *N*7, and N^2 aniline adducts. [The Working Group noted that a similar spectrum of DNA adducts is seen with other aromatic amines such as 4-aminobiphenyl (*para*-phenylaniline), which is classified as *carcinogenic to humans* (IARC Group 1) by the *IARC Monographs* programme (IARC, 2012).]

Adducted aniline is predicted (on the basis of molecular mechanics calculations) to distort the DNA double-helix structure less than do larger aromatic amine ring systems (Shapiro et al., 1998).

As shown in Fig. 4.3, a possible route to the formation of these DNA adducts begins with CYP-catalysed N-hydroxylation of aniline (see Section 4.1), a process that is known to occur, as discussed in Section 4.2.1(a), and that is an obligate step in the pathway leading to haemoglobin adducts. The hydroxylamine metabolite can be further activated, for example, by O-acetylation catalysed by the *N*-acetyltransferase. [The Working Group noted that further activation of the *N*-hydroxy compound by acetylation has not been directly demonstrated in an in vitro system.] The resulting *N*-acetoxyaniline, mentioned above, undergoes spontaneous heterolysis (loss of acetate anion) to give a reactive electrophilic nitrenium ion (Shamovsky et al., 2012). As discussed above, N-hydroxylation of aniline to phenylhydroxylamine has been shown in dogs and rats; in liver microsomal preparations from rat, mouse, and rabbit; and in the isolated perfused rat liver, when the perfusion fluid contained human erythrocytes, to trap the oxidized metabolites by binding to haemoglobin. [The Working Group noted that this pathway of DNA adduct formation parallels an established paradigm for aromatic amines, including 4-aminobiphenyl (*para*-phenylaniline), 2-naphthylamine, and *ortho*-toluidine (*ortho*-methylaniline), which have been classified as *carcinogenic to humans* (IARC Group 1)

Table 4.1 Genetic and related effects of aniline, aniline hydrochloride, and aniline metabolites in human cells in vitro

End-point	Tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Aniline						
DNA strand breaks (comet assay)	Human MCL-5 cells	+	NT	2.44 mM [227 µg/mL]		Martin et al. (1999)
DNA damage (γ-H2AX induction)	Human urothelial cell line 1T1	+	NT	7.5 mM [697.5 µg/mL]		Qi et al. (2020)
Sister-chromatid exchange	Human lymphoblastoid cells, NL3 (cell line)	–	–	0.1 mM [9.3 µg/mL]	Only one concentration studied	Tohda et al. (1983)
Aniline hydrochloride						
Unscheduled DNA synthesis	Primary human hepatocytes	–	–	1 mM [129 µg/mL]	Test for 6 cases	Butterworth et al. (1989)
Sister-chromatid exchange	Human fibroblasts, GM-3468 (cell line)	+	NT	5 or 10 mM [645 or 1290 µg/mL]	Minimal although statistically significant increases [reported as negative in Suppl. 6 of the *IARC Monographs* (IARC (1987)]	Wilmer et al. (1981)
Sister-chromatid exchange	Human lymphocytes	–	NT	1 mM [129 µg/mL]		Wilmer et al. (1984)
Sister-chromatid exchange	Human whole blood	+	NT	1 mM [129 µg/mL]		Wilmer et al. (1984)
Sister-chromatid exchange	Human lymphocytes, peripheral blood (primary culture)	–	–	1 mM [129 µg/mL]		Takehisa & Kanaya (1982)
Aniline metabolites						
Sister-chromatid exchange	Human fibroblasts, GM-3468 (cell line)	–	NT	Acetanilide 10 mM [1351.7 µg/mL]		Wilmer et al. (1981)
Sister-chromatid exchange	Human fibroblasts, GM-3468 (cell line)	+	NT	2-Aminophenol 0.1 mM [10.91 µg/mL]		Wilmer et al. (1981)
Sister-chromatid exchange	Human fibroblasts, GM-3468 (cell line)	–	NT	4-Aminophenol 0.2 mM [21.83 µg/mL]		Wilmer et al. (1981)
Sister-chromatid exchange	Human fibroblasts, GM-3468 (cell line)	–	NT	2-Hydroxyacetanilide 10 mM [1511.63 µg/mL]		Wilmer et al. (1981)

Table 4.1 (continued)

End-point	Tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Sister-chromatid exchange	Human fibroblasts, GM-3468 (cell line)	–	NT	4-Hydroxyacetanilide [paracetamol] 10 mM [1511.65 µg/mL]		Wilmer et al. (1981)
Sister-chromatid exchange	Human fibroblasts, GM-3468 (cell line)	+	NT	Phenylhydroxylamine 0.5 mM [54.56 µg/mL]		Wilmer et al. (1981)

HIC, highest ineffective concentration; LEC, lowest effective concentration; NT, not tested.

[a] +, positive; –, negative.

Table 4.2 Genetic and related effects of aniline and aniline hydrochloride in non-human mammals in vivo

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Aniline							
Covalent DNA binding	Rat, F344, (NR)	Kidney, large intestine, and spleen	+	100 mg/kg bw	Predosed with unlabelled aniline for 7 days and then i.p.; 1×; sampled after 24 h; 50 and 100 mg/kg bw [^{14}C]-labelled aniline	At high dose, CBI of 14.2, 4.3, 3.7 µmol/mol nucleotides	McCarthy et al. (1985)
Covalent DNA binding	Rat, F344 (NR)	Liver and small intestine	–	100 mg/kg bw	Predosed with unlabelled aniline for 7 days and then i.p.; 1×; sampled after 24 h; 50 and 100 mg/kg bw [^{14}C]-labelled aniline	CBI, < 2 µmol/mol nucleotides	McCarthy et al. (1985)
Covalent DNA binding	Mice, B6C3F$_1$ (NR)	Liver, kidney, spleen, small intestine, and large intestine	–	500 mg/kg bw	Predosed with unlabelled aniline for 7 days and then i.p.; 1×; sampled after 24 h; 250 and 500 mg/kg bw [^{14}C]-labelled aniline	CBI, < 2.6 µmol/mol nucleotides	McCarthy et al. (1985)
DNA strand breaks (comet assay)	Rat, Wistar (M)	Liver, bladder, colon, lung, and kidney	+	150 mg/kg bw	Oral, in saline; 1×; sampled after 3 h; single dose		Sekihashi et al. (2002)
DNA strand breaks (comet assay)	Rat, Wistar (M)	Stomach, brain, and bone marrow	–	150 mg/kg bw	Oral, in saline; 1×; sampled after 3 h; single dose		Sekihashi et al. (2002)
DNA strand breaks (comet assay)	Rat, Wistar (M)	Stomach, bladder, kidney, and lung	+	150 mg/kg bw	Oral, in saline; 1×; sampled after 8 h; single dose		Sekihashi et al. (2002)
DNA strand breaks (comet assay)	Rat, Wistar (M)	Liver, colon, brain, and bone marrow	–	150 mg/kg bw	Oral, in saline; 1×; sampled after 8 h; single dose		Sekihashi et al. (2002)
DNA strand breaks (comet assay)	Rat, Wistar (M)	Stomach, kidney, and lung,	+	150 mg/kg bw	Oral, in saline; 1×; sampled after 24 h; single dose		Sekihashi et al. (2002)
DNA strand breaks (comet assay)	Rat, Wistar (M)	Colon, brain, bladder, liver, and bone marrow	–	150 mg/kg bw	Oral, in saline; 1×; sampled after 24 h; single dose		Sekihashi et al. (2002)
DNA strand breaks (alkaline elution assay)	Rat, Sprague-Dawley (M)	Liver	+	210 mg/kg bw	i.p.; 1×; sampled after 4 h; single dose		Parodi et al. (1981)

Table 4.2 (continued)

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
DNA strand breaks (alkaline elution assay)	Rat, Sprague-Dawley (M)	Liver	+	105 mg/kg bw	i.p.; 1×; sampled after 4 h; 53, 105, 210, 420, and 840 mg/kg bw		Parodi et al. (1982)
DNA strand breaks (alkaline elution assay)	Rat, Sprague-Dawley (M)	Liver	+	210 mg/kg bw	i.p.; 1×; sampled after 24 h; 210 and 840 mg/kg bw		Parodi et al. (1982)
DNA strand breaks (alkaline elution assay)	Rat, Sprague-Dawley (M)	Liver	–	210 mg/kg bw	i.p.; 1×; sampled after 48, 72, and 120 h; single dose		Parodi et al. (1982)
DNA strand breaks (alkaline elution assay)	Rat, Sprague-Dawley (M)	Kidney	+	210 mg/kg bw	i.p.; 1×; sampled after 4 h; 105, 210, and 420 mg/kg bw		Parodi et al. (1982)
DNA strand breaks (alkaline elution assay)	Rat, Sprague-Dawley (M)	Spleen	–	210 mg/kg bw	i.p.; 1×; sampled after 4 h; single dose		Parodi et al. (1982)
DNA strand breaks (alkaline elution assay)	Rat, Sprague-Dawley (M)	Liver	+	210 mg/kg bw	i.p.; 1×; sampled after 4 h; single dose		Parodi et al. (1982)
DNA strand breaks (viscometric assay)	Rat, Sprague-Dawley (M)	Liver	–	210 mg/kg bw	i.p.; 1×; sampled after 4, 12, and 24 h; single dose		Brambilla et al. (1985)
DNA strand breaks (comet assay)	Mouse, ddY (M)	Liver, lung, brain, and bone marrow	+	1000 mg/kg bw	Oral, in saline; 1×; sampled after 3 h; single dose		Sasaki et al. (1999)
DNA strand breaks (comet assay)	Mouse, ddY (M)	Stomach, colon, kidney, and bladder	–	1000 mg/kg bw	Oral, in saline; 1×; sampled after 3 h; single dose		Sasaki et al. (1999)
DNA strand breaks (comet assay)	Mouse, ddY (M)	Liver, bladder, lung, brain, and bone marrow	+	1000 mg/kg bw	Oral, in saline; 1×; sampled after 8 h; single dose		Sasaki et al. (1999)
DNA strand breaks (comet assay)	Mouse, ddY (M)	Stomach, colon, and kidney	–	1000 mg/kg bw	Oral, in saline; 1×; sampled after 8 h; single dose		Sasaki et al. (1999)
DNA strand breaks (comet assay)	Mouse, ddY (M)	Liver, lung, brain, and bone marrow	+	100 mg/kg bw	Oral, in saline; 1×; sampled after 3 h; single dose		Sekihashi et al. (2002)

Table 4.2 (continued)

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
DNA strand breaks (comet assay)	Mouse, ddY (M)	Stomach, colon, kidney, and bladder	–	100 mg/kg bw	Oral, in saline; 1×; sampled after 3 h; single dose		Sekihashi et al. (2002)
DNA strand breaks (comet assay)	Mouse, ddY (M)	Liver, bladder, lung, brain, and bone marrow	+	100 mg/kg bw	Oral, in saline; 1×; sampled after 8 h; single dose		Sekihashi et al. (2002)
DNA strand breaks (comet assay)	Mouse, ddY (M)	Stomach, colon, and kidney	–	100 mg/kg bw	Oral, in saline; 1×; sampled after 8 h; single dose		Sekihashi et al. (2002)
DNA strand breaks (comet assay)	Mouse, ddY (M)	Colon, bladder, lung, and brain	+	100 mg/kg bw	Oral, in saline; 1×; sampled after 24 h; single dose		Sekihashi et al. (2002)
DNA strand breaks (comet assay)	Mouse, ddY (M)	Liver, stomach, kidney, and bone marrow	–	100 mg/kg bw	Oral, in saline; 1×; sampled after 24 h; single dose		Sekihashi et al. (2002)
DNA strand breaks (alkaline elution assay)	Mouse, Swiss (M)	Liver	–	300 mg/kg bw	i.p.; 1×; sampled after 4 h; single dose		Cesarone et al. (1982)
DNA strand breaks (alkaline elution assay)	Mouse, Swiss (M)	Kidney	+	300 mg/kg bw	i.p.; 1×; sampled after 4 h; single dose		Cesarone et al. (1982)
DNA strand breaks (alkaline elution assay)	Mouse, Swiss (M)	Liver, kidney, and bone marrow	–	420 mg/kg bw	i.p.; 1×; sampled after 4 h; single dose for liver and kidney assay; 210 and 420 mg/kg bw for bone marrow assay		Parodi et al. (1982)
Gene mutation	Rat, Big Blue F344 (M)	Liver, spleen, and bone marrow	–	100 mg/kg bw	Oral, vehicle NR; gavage daily for 28 days; sample collected at day 31; single dose		Koenig et al. (2018)
Gene mutation	Rat, Sprague-Dawley (M)	Urine (Host-mediated activation using *Salmonella typhimurium* TA98)	+	300 mg/kg bw	Oral, in olive oil; 1×; sampled after 24 h; single dose		Tanaka et al. (1980)
Micronucleus formation	Rat, Big Blue F344 (M)	Peripheral blood	+	100 mg/kg bw	Oral, vehicle NR; gavage daily for 28 days; sampled at day 4 and 29; single dose		Koenig et al. (2018)

Table 4.2 (continued)

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Micronucleus formation	Mouse, SJL Swiss (M, F)	Bone marrow	+	50 mg/kg bw	i.p.; 1×; sampled after 24 h; 5, 50, 100, and 200 mg/kg bw		Sicardi et al. (1991)
Micronucleus formation	Mouse, CBA (M)	Bone marrow	–	300 mg/kg bw	i.p.; 2×; sampled after 6 h; 100, 200, 250, and 300 mg/kg bw		Ashby et al. (1991)
Micronucleus formation	Mouse, CBA (M)	Bone marrow	+	380 mg/kg bw	i.p.; 2×; sampled after 24 h; 237.5 and 380 mg/kg bw		Ashby et al. (1991); Tinwell & Ashby (1991)
Micronucleus formation	Mouse, CBA (M)	Bone marrow	–	380 mg/kg bw	i.p.; 2×; sampled after 48 h; 237.5 and 380 mg/kg bw		Ashby et al. (1991)
Micronucleus formation	Mouse, ICR Swiss (M)	Bone marrow	–	250 mg/kg bw	Oral, in olive oil; 1×; sampled after 24 h; 125 and 250 mg/kg bw;		Harper et al. (1984)
Micronucleus formation	Mouse, $B6C3F_1$ (M)	Bone marrow	+	23 mg/kg bw	Oral, in corn oil; 2×; sampled after 24 h; 12, 23, 47, 120, and 470 mg/kg bw		Ress et al. (2002)
Sister-chromatid exchange	Mouse, Swiss (M)	Bone marrow	+	210 mg/kg bw	i.p.; 1×; sampled after 24 h; 61, 123, 210, and 420 mg/kg bw	Commercial aniline	Parodi et al. (1982)
Sister-chromatid exchange	Mouse, Swiss (M)	Bone marrow	+	210 mg/kg bw	i.p.; 1×; sampled after 24 h; single dose	Distilled (purified) aniline	Parodi et al. (1982)
Sister-chromatid exchange	Mouse, Swiss (M)	Bone marrow	+	210 mg/kg bw	i.p.; 1×; sampled after 24 h; 210 and 420 mg/kg bw		Parodi et al. (1983)
Aniline hydrochloride							
DNA damage (γ-H2AX induction)	Rat, F344/DuCrl-Crlj (M)	Bladder	–	0.6% in the feed	Oral, in feed; for 4 weeks		Toyoda et al. (2019)
Chromosomal aberration	Rat, PVG (M)	Bone marrow	+	500 mg/kg bw	Oral, in water; 1×; sampled after 18 h; 300, 400, and 500 mg/kg bw		Bomhard (2003)
Chromosomal aberration	Rat, PVG (M)	Bone marrow	–	500 mg/kg bw	Oral, in water; 1×; sampled after 30 h; single dose		Bomhard (2003)
Chromosomal aberration	Mouse, CBA (M)	Bone marrow	–	380 mg/kg bw	i.p.; 2×; sampled after 16, 20 or 24 h; 220, 300, and 380 mg/kg bw		Jones and Fox (2003)
Micronucleus formation	Rat, PVG (M)	Bone marrow	+	287 mg/kg bw	Oral, in water; 1×; sampled after 24 h; 215, 287, 400, and 500 mg/kg bw		George et al. (1990)
Micronucleus formation	Rat, PVG (M)	Bone marrow	+	400 mg/kg bw	Oral, in water; 1×; sampled after 48 h; 215, 287, 400, and 500 mg/kg bw		George et al. (1990)

Table 4.2 (continued)

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Micronucleus formation	Rat, PVG (M)	Bone marrow	+	300 mg/kg bw	Oral, in water; 1×; sampled after 24 h; 300, 400, and 500 mg/kg bw		Bomhard (2003)
Micronucleus formation	Rat, PVG (M)	Bone marrow	–	300 mg/kg bw	Oral, in water; 1×; sampled after 48 h; 300, 400, and 500 mg/kg bw		Bomhard. (2003)
Micronucleus formation	Mouse, CHR (M)	Bone marrow	+	1000 mg/kg bw	Oral, in water; 1×; sampled after 24 h; 400, 500, and 1000 mg/kg bw		Westmoreland & Gatehouse (1991)
Micronucleus formation	Mouse, CHR (M)	Bone marrow	–	1000 mg/kg bw	Oral, in water; 1×; sampled after 48 h; 400, 500, and 1000 mg/kg bw		Westmoreland & Gatehouse (1991)
Micronucleus formation	Mouse, CHR (M)	Bone marrow	+	380 mg/kg bw	i.p.; 1×; sampled after 24 h; single dose		Westmoreland & Gatehouse (1991)
Micronucleus formation	Mouse, $B6C3F_1$ (M, F)	Peripheral blood	+	65 mg/kg bw	Oral, in feed; daily for 90 days; 500, 1000, and 2000 mg/kg in feed, equivalent to 65, 130, and 260 mg/kg bw/day		Witt et al. (2000)

bw, body weight; CBI, covalent binding index; F, female; h, hour; γ-H2AX, phosphorylated histone 2AX; HID, highest ineffective dose; i.p., intraperitoneal; LED, lowest effective dose; M, male; NR, not reported.

[a] +, positive; –, negative.

Table 4.3 Genetic and related effects of aniline and aniline hydrochloride in non-human mammals in vitro

End-point	Species, tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Aniline						
DNA strand breaks (comet assay)	Rat, Sprague-Dawley, primary hepatocytes	+	NT	2.5 µg/mL		Wang et al. (2016)
DNA strand breaks (alkaline elution assay)	Mouse, lymphoma, L5178Y	–	(+)	21.5 mM [2000 µg/mL]		Garberg et al. (1988)
DNA strand breaks (alkaline elution assay)	Chinese hamster, lung, V79	–	–	3 mM [279 µg/mL]		Swenberg et al. (1976); Swenberg (1981)
Unscheduled DNA synthesis (DNA repair assay)	Rat, primary hepatocytes	–	NT	1 mM [93 µg/mL]		Williams (1980; 1981)
Unscheduled DNA synthesis (DNA repair assay)	Rat, F344, primary hepatocytes	–	NT	10^{-5} M [0.93 µg/mL]	Single dose only	McQueen et al. (1981)
Unscheduled DNA synthesis (DNA repair assay)	Mice, CD-1, primary hepatocytes	–	NT	10^{-5} M [0.93 µg/mL]	Single dose only	McQueen et al. (1981)
Unscheduled DNA synthesis (DNA repair assay)	Hamster, Syrian golden, primary hepatocytes	–	NT	10^{-5} M [0.93 µg/mL]	Single dose only	McQueen et al. (1981)
Gene mutation, *Tk*$^{+/-}$	Mouse, lymphoma, L5178Y	NT	+	3.7 mM [344 µg/mL]		Amacher et al. (1980)
Gene mutation, *Tk*$^{+/-}$	Mouse, lymphoma, L5178Y	+	NT	2.5 mM [581 µg/mL]		Wangenheim & Bolcsfoldi (1988)
Gene mutation, *Tk*$^{+/-}$	Mouse, lymphoma, L5178Y	NT	+	0.5 mM [46.5 µg/mL]		Wangenheim & Bolcsfoldi (1988)
Gene mutation, *Tk*$^{+/-}$	Mouse, lymphoma, L5178Y	+	+	1 µL/mL [1000 µg/mL]		Myhr & Caspary (1988)
Gene mutation, *Tk*$^{+/-}$	Mouse, lymphoma, L5178Y	+	NT	0.8 µL/mL [800 µg/mL]		Mitchell et al. (1988); Caspary et al. (1988)
Gene mutation, *Tk*$^{+/-}$	Mouse, lymphoma, L5178Y	NT	+	0.41 µL/mL [410 µg/mL]		Mitchell et al. (1988); Caspary et al. (1988)
Gene mutation, *Tk*$^{+/-}$	Mouse, lymphoma, L5178Y	+	NT	1600 µg/mL		McGregor et al. (1991)
Gene mutation, *Tk*$^{+/-}$	Mouse, lymphoma, L5178Y	NT	+	500 µg/mL		McGregor et al. (1991)
Gene mutation, *Hprt*$^{+/-}$	Chinese hamster, lung, V79	–	NT	20 mM [1860 µg/mL]		Fassina et al. (1990)
Gene mutation, *Hprt*$^{+/-}$	Chinese hamster, lung, V79	NT	+	60 mM [5580 µg/mL]		Fassina et al. (1990)

Table 4.3 (continued)

End-point	Species, tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Gene mutation, *Hprt*$^{+/-}$	Chinese hamster, lung, V79 (co-cultured with rat hepatocytes)	–	NT	10 mM [930 µg/mL]		Fassina et al. (1990)
Chromosomal aberrations	Chinese hamster, ovary, CHO	–	+	5 mg/mL		Galloway et al. (1987)
Chromosomal aberrations	Chinese hamster, ovary, CHO	+	NT	444 µg/mL		Chung et al. (1995; 1996)
Micronucleus formation	Syrian hamster, embryo, SHE	–	NT	NR		Fritzenschaf et al. (1993)
Micronucleus formation	Chinese hamster, lung, CHL/IU	–	NT	2 mg/mL		Matsushima et al. (1999)
Micronucleus formation	Chinese hamster, lung, CHL/IU	NT	+	250 µg/mL		Matsushima et al. (1999)
Sister-chromatid exchange	Rat, liver epithelial, RL_4	+	NT	0.5 mM [46.5 µg/mL]		Cunningham & Ringrose (1983)
Sister-chromatid exchange	Chinese hamster, ovary, CHO	+	+	5 mg/mL	Different treatment periods: 2 h (+S9) vs about 26 h (–S9)	Galloway et al. (1987)
Aniline hydrochloride						
Unscheduled DNA synthesis	Rat, primary hepatocytes	–	NT	1 mM [129 µg/mL]		Yoshimi et al. (1988)
Unscheduled DNA synthesis	Rat, primary hepatocytes	–	NT	1 mM [129 µg/mL]		Butterworth et al. (1989)
Sister-chromatid exchange	Chinese hamster, ovary, CHO	+	NT	1 mM [129 µg/mL]		Takehisa et al. (1988)
Sister-chromatid exchange	Chinese hamster, ovary, CHO	NT	–	1 mM [129 µg/mL]	Negative in the presence of rat liver S9 or *Vicia* root S10	Takehisa et al. (1988)
Chromosomal aberrations	Chinese hamster, Don	–	NT	5 mM [645 µg/mL]		Abe & Sasaki (1977)
Chromosomal aberrations	Chinese hamster, lung fibroblasts, CHL	–	NT	1 mg/mL		Ishidate (1983)
Chromosomal aberrations	Chinese hamster, lung fibroblasts, CHL	NT	–	2 mg/mL		Ishidate (1983)

Table 4.3 (continued)

End-point	Species, tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Chromosomal aberrations	Chinese hamster, ovary, CHO	–	NT	4 mM [516 µg/mL]		Kanaya (1996)
Chromosomal aberrations	Chinese hamster, ovary, CHO	NT	+	1 mM [129 µg/mL]	With *Vicia* S10 mix	Kanaya (1996)
Chromosomal aberrations	Chinese hamster, ovary, CHO	–	NT	4 mM [516 µg/mL]	With *Pisum* or *Lactuca* S10 mix	Kanaya (1996)
Sister-chromatid exchange	Chinese hamster, ovary, CHO	–	–	4 mM [516 µg/mL]	With *Vicia*, *Pisum*, or *Lactuca* S10 mix	Kanaya (1996)
Sister-chromatid exchange	Chinese hamster, Don	+	NT	10^{-6} M [0.13 µg/mL]		Abe & Sasaki (1977)
Aniline metabolites (2-AP, 3-AP, 4-AP)						
Chromosomal aberrations	Chinese hamster, ovary, CHO	+	NT	0.1 mM 2-AP		Kanaya (1996)
Chromosomal aberrations	Chinese hamster, ovary, CHO	+	NT	2 mM 3-AP		Kanaya (1996)
Chromosomal aberrations	Chinese hamster, ovary, CHO	+	NT	0.05 mM 4-AP		Kanaya (1996)
Sister-chromatid exchange	Chinese hamster, ovary, CHO	+	NT	0.1 mM 2-AP		Kanaya (1996)
Sister-chromatid exchange	Chinese hamster, ovary, CHO	+	NT	2 mM 3-AP		Kanaya (1996)
Sister-chromatid exchange	Chinese hamster, ovary, CHO	+	NT	0.1 mM 4-AP		Kanaya (1996)

2-AP, 2-aminophenol; 3-AP, 3-aminophenol; 4-AP, 4-aminophenol; h, hour; Hprt, hypoxanthine-guanine phosphoribosyltransferase; HIC, highest ineffective concentration; LEC, lowest effective concentration, NR, not reported; NT, not tested; S9, 9000 × *g* supernatant; S10, 10 000 × *g* supernatant; *Tk*, thymidine kinase.

[a] +, positive; (+), weakly positive; –, negative.

Table 4.4 Genetic and related effects of aniline and aniline hydrochloride in non-mammalian experimental systems

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Aniline						
Embryo-fetal chicken livers	DNA adducts, nucleotide ^{32}P-post-labelling	–	NA	20 mg/egg (injection)		Kobets et al. (2019)
Embryo-fetal chicken livers	DNA strand breaks, comet assay	–	NA	20 mg/egg (injection)		Kobets et al. (2019)
Drosophila melanogaster	Mutation, sex-linked recessive lethal mutations	–	NA	400 mg/kg (injection)		Yoon et al. (1985)
Drosophila melanogaster	Mutation, sex-linked recessive lethal mutations	–	NA	600 mg/kg (feeding)		Yoon et al. (1985)
Drosophila melanogaster	Interchromosomal mitotic recombination (white/white$^+$) eye mosaic assay	–	NA	600 mg/kg (feeding)		Vogel & Nivard (1993)
Wheat seeds	Micronucleus formation	+	NA	5 mg/L		Tao et al. 2017
Aspergillus nidulans	Reverse mutation	–	NA	200 µg/mL		Prasad (1970)
Saccharomyces cerevisiae D3	Homozygosis, mitotic recombination	–	–	0.5% v/v [10 000 µg/mL]	One concentration only	Simmon (1979)
Saccharomyces cerevisiae, RS112	DEL recombination	+	NA	4 mg/mL		Schiestl (1989); Schiestl et al. (1989)
Saccharomyces cerevisiae, RS112	DEL recombination	+	NA	5 mg/mL		Brennan & Schiestl (1997)
Saccharomyces cerevisiae, HAN	Mutation or small deletion	+	NA	10 µL/mL cell suspension		Schafer et al. (2008)
Saccharomyces cerevisiae, DAN	Recombination	+	NA	10 µL/mL cell suspension		Schafer et al. (2008)
Salmonella typhimurium TA98	DNA adducts, nucleotide ^{32}P-post-labelling	–	–	4 mg/4 mL cell culture		Mori et al. (1996)
Salmonella typhimurium TA1357/pSK1002	DNA damage, *umu* test	–	–	4000 µg/mL		Sakagami et al. (1988)
Bacillus subtilis H17 rec$^+$ and M45 rec$^-$	DNA damage, rec assay	–	NT	3 µg/well	MIC, 3 µg/well in H17 rec$^+$; and 0.2 µg/well in M45 rec$^-$	McCarroll et al. (1981)
Escherichia coli KWP2, WP100	DNA damage, rec assay	NT	–	2000 µg/mL		Mamber et al. (1983)

Table 4.4 (continued)

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Escherichia coli K12	DNA damage, prophage induction	NT	–	2000 µg/plate	Only dose reported	Mamber et al. (1984)
Escherichia coli pol A$^+$/pol A$^-$	DNA damage	+	+	25 µL/plate		Fluck et al. (1976)
Escherichia coli pol A$^+$/pol A$^-$	DNA damage	+	+	25 µL/plate	5 days after distillation	Fluck et al. (1976)
Escherichia coli pol A$^+$/pol A$^-$	DNA damage	–	–	25 µL/plate	Freshly distilled	Fluck et al. (1976)
Vibrio fischeri	DNA damage	+	–	2760 µM	EC_{50}	Osano et al. (2002)
Salmonella typhimurium TA98, TA100	Reverse mutation	NT	–	2500 µg/plate		Parodi et al. (1981)
Salmonella typhimurium TA98, TA100	Reverse mutation	NT	–	1000 µg/plate	Co-incubated with plant cells	Gentile et al. (1987)
Salmonella typhimurium TA98, TA100	Reverse mutation	–	–	10 000 µg/plate	Rat liver S9	Gentile et al. (1987)
Salmonella typhimurium TA100	Reverse mutation	–	+	10 000 µg/plate	Pea apical bud S9; concentration-related increase but significant only at the highest concentration	Gentile et al. (1987)
Salmonella typhimurium TA98, TA100	Reverse mutation	–	–	1000 µg/plate	Rat liver S9	Rashid et al. (1987)
Salmonella typhimurium TA98, TA100	Reverse mutation	–	–	698 µg/plate	Rat S9	Nohmi et al. (1984)
Salmonella typhimurium TA98, TA100	Reverse mutation	–	–	0.05 µmol/100 µL per plate	Positive after nitrite treatment	Kato et al. (1991)
Salmonella typhimurium TA98, TA100	Reverse mutation	–	–	5000 µg/plate		Assmann et al. (1997)
Salmonella typhimurium TA98, TA100	Reverse mutation	–	–	3000 µg/plate	Rat liver S9	Chung et al. (1995)
Salmonella typhimurium TA98, TA100	Reverse mutation	NT	–	5000 µg/plate	Ethanol-induced rat liver S9	Burke et al. (1994)
Salmonella typhimurium TA98, TA98NR, TA100, TA100NR	Reverse mutation	–	–	3000 µg/plate	Rat liver S9	Chung et al. (1996)

Table 4.4 (continued)

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Salmonella typhimurium TA98, TA100, TA97	Reverse mutation	–	–	2000 µg/plate		Brams et al. (1987)
Salmonella typhimurium TA98, TA100, TA1535, TA1537	Reverse mutation	NT	–	2500 µg/plate		Ashby et al. (1981)
Salmonella typhimurium TA98, TA100, TA1535, TA1537	Reverse mutation	–	–	3333 µg/plate		Haworth et al. (1983)
Salmonella typhimurium TA98, TA100, TA102, TA1535, TA1537	Reverse mutation	NT	–	5000 µg/plate	Pyrazole-induced rat liver S9	Burke et al. (1994)
Salmonella typhimurium TA102	Reverse mutation	–	–	5000 µg/plate	Rat liver S9	Jung et al. (1992)
Salmonella typhimurium TA98, TA100, TA1538	Reverse mutation	–	–	5000 µg/plate	Rat liver S9	Chung et al. (1981)
Salmonella typhimurium TA98	Reverse mutation	–	NT	500 µg/plate	Concentrations, 0.8, 4, 20, 100, and 500 µg/plate	Ashby et al. (1983)
Salmonella typhimurium TA98	Reverse mutation	NT	–	200 µg/plate		Nagao et al. (1977)
Salmonella typhimurium TA98	Reverse mutation	NT	–	1000 µg/plate		Ho et al. (1981)
Salmonella typhimurium TA98	Reverse mutation	–	–	10 000 µg/plate	Pea apical bud S9	Gentile et al. (1987)
Salmonella typhimurium TA1538	Reverse mutation	–	–	100 µg/plate	Rat liver S9	Garner & Nutman (1977)
Escherichia coli (WP2 *uvra*)	Reverse mutation	–	NT	2 mM [186 µg/mL]		Pai et al. (1985)
Aniline hydrochloride						
Drosophila melanogaster	Mutation, sex-linked recessive lethal mutations	–	NA	10% solution for feeding	Increases in nondisjunction observed	Muñoz & Barnett (1998)
Drosophila melanogaster	Mutation, sex-linked recessive lethal mutations	–	NA	5% (0.4 µL) for injection		Muñoz & Barnett (1998)
Vicia faba	Chromosomal aberrations	–	NA	1 mM [129 µg/mL]	At 1 mM, positive response seen at 20 h recovery time but not longer	Kanaya (1990)

Table 4.4 (continued)

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Vicia faba	Sister-chromatid exchange	–	NA	4 mM [4386 µg/mL]	Positive responses from metabolites 2-, 3-, and 4-aminophenol	Kanaya (1990)
Salmonella typhimurium TA100	Reverse mutation	NT	–	2000 µg/plate		Imamura et al. (1983)
Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	Reverse mutation	–	–	5000 µg/plate	S9 from rat liver, rat spleen, hamster liver and hamster spleen	Shahin (1989)
Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	Reverse mutation	–	–	10 000 µg/plate	S9 from livers of rat, mouse or hamster	Dunkel et al. (1985)
Escherichia coli (WP2 *uvrA*)	Reverse mutation	–	–	10 000 µg/plate	S9 from livers of rat, mouse or hamster	Dunkel et al. (1985)
Escherichia coli (WP2 *uvrA*), IC188, IC203	Reverse mutation	–	NT	1000 µg/plate	Single dose tested only	Martínez et al. (2000)

DEL, deletion; EC_{50}, half maximal effective concentration; h, hour; HIC, highest ineffective concentration; LEC, lowest effective concentration; MIC, minimal inhibitory concentration; NA, not applicable; NT, not tested; TA98NR, TA98 nitroreductase-deficient mutant strain; TA100NR, TA100 nitroreductase-deficient mutant strain.

[a] +, positive; –, negative.

by the *IARC Monographs* programme (IARC, 2010, 2012).]

4.2.2 Is genotoxic

Table 4.1, Table 4.2, Table 4.3, and Table 4.4 summarize the available studies of the genetic and related effects of aniline and its hydrochloride.

(a) Humans

(i) Exposed humans

No data in exposed humans were available to the Working Group.

(ii) Human cells in vitro

See Table 4.1.

DNA damage

Significantly increased DNA damage measured by comet assay was seen in metabolically competent human MCL-5 cells treated with aniline at a concentration of 2.44 mM and higher. DNA repair inhibitors (hydroxyurea and cytosine arabinoside) markedly enhanced the effects (Martin et al., 1999). A significant induction of γ-H2AX (a biomarker of DNA damage) was observed in the human urothelial 1T1 cell line exposed to aniline at a concentration of 7.5 mM and the response was concentration-related (Qi et al., 2020). However, a test for unscheduled DNA synthesis in primary human hepatocytes was reported to give negative results with aniline hydrochloride at concentrations up to 1 mM (Butterworth et al., 1989).

Chromosomal damage

At a low concentration, exposure of human lymphoblast NL3 cells to aniline (0.1 mM) did not result in induction of sister-chromatid exchange in the presence of absence of endogenous metabolic activation (Tohda et al., 1983). Wilmer et al. (1981) reported marginal but significant increases in the frequency of sister-chromatid exchange in human fibroblasts exposed to aniline hydrochloride at higher concentrations (5 mM). Further study confirmed that aniline hydrochloride at a lower concentration (1 mM) also induced a significant concentration-related increase in the frequency of sister-chromatid exchange in human whole blood cell cultures, but not in mononuclear leukocytes (Wilmer et al., 1984). Furthermore, Takehisa & Kanaya (1982) showed that aniline hydrochloride at a concentration of 1 mM was unable to induce an increase in sister-chromatid exchange in human peripheral blood lymphocytes in the presence or absence of endogenous metabolic activation. [The Working Group noted that cell type and concentration of aniline were the two main factors that influenced the outcomes of the exposure.] Some hydroxylated metabolites of aniline, e.g. 2-aminophenol and phenylhydroxylamine, were more potent than aniline in sister-chromatid exchange induction; others (acetanilide, 4-aminophenol, and 2- and 4-hydroxyacetanilide) gave negative results (Wilmer et al., 1981).

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.2.

Several studies investigated the genotoxic effects of exposure to aniline or aniline hydrochloride in experimental animals in vivo. The end-points include DNA binding, DNA damage, gene mutation, chromosomal aberration, micronucleus formation, and sister-chromatid exchange.

DNA damage

Significantly increased values for the covalent binding index were reported in the kidney, spleen and large intestine of Fischer 344 rats after intraperitoneal injection of [^{14}C]-labelled aniline at a dose of 100 mg/kg bw (McCarthy et al., 1985).

No increases in phosphorylated histone H2AX (γ-H2AX) formation (a biomarker of DNA damage) were observed in the bladder urothelium of Fischer 344/DuCrl-Crlj rats given feed

containing 0.6% aniline hydrochloride (Toyoda et al. 2019).

DNA strand breaks measured by comet assay were investigated by several techniques in various tissues of rats and mice after oral or intraperitoneal exposure to aniline or aniline hydrochloride. Significant increases in the frequency of single-strand breaks were observed in the liver, bladder, lung, colon, and kidney; but not in the stomach, brain, or bone marrow in Wistar rats after a single oral dose of aniline of 150 mg/kg bw at the 3-hour sampling time. At two other sampling times (8 hours or 24 hours after treatment), the pattern of organs testing positive for DNA damage was slightly different. For example, at the 24-hour time point, DNA damage was seen only in stomach, kidney, and lung (Sekihashi et al., 2002). [The Working Group noted that spleen was not investigated in these studies with the comet assay in rats.]

In a preliminary study in which aniline was administered by intraperitoneal injection, DNA fragmentation (measured by alkaline elution assay) was observed in liver samples from male Sprague-Dawley rats 4 hours and 24 hours after a single dose of aniline at 210 mg/kg bw (half of the median lethal dose, LD_{50}) (Parodi et al., 1981). In a more in-depth study, male Sprague-Dawley rats were given a single intraperitoneal injection of aniline at a dose ranging from 53 to 840 mg/kg bw, with sampling at 4 hours after treatment, or at a dose of 210 mg/kg bw with sampling times at 4–120 hours after treatment. In the 4-hour dose–response experiment, DNA fragmentation (alkaline elution assay) was significantly increased in the liver (at 105 mg/kg bw and above) and in the kidneys (at 210 and 420 mg/kg bw), but not in the spleen (at up to 210 mg/kg bw). In the time-course experiment, no DNA fragmentation was observed in the liver samples collected at 48 hours or after (Parodi et al., 1982). [The Working Group noted that sampling time seems to be a critical factor for observation of DNA damage.] Moreover, a purified aniline sample after distillation was found to behave very similarly to the commercial grade, at the test dose of 210 mg/kg bw (Parodi et al., 1982). Brambilla et al. (1985) reported no DNA damage (by viscometric assay) in liver samples (24 hours after treatment) in male Sprague-Dawley rats that received a single intraperitoneal injection of aniline at 210 mg/kg bw.

In male ddY mice given a single oral dose of aniline at 1000 mg/kg bw, significantly increased DNA damage, as measured by the comet assay, was observed in liver, lung, brain, and bone marrow, but not in stomach, colon, kidney, or bladder at the sampling time of 3 hours after treatment. At a sampling time of 8 hours after treatment, DNA damage also occurred in the bladder (Sasaki et al., 1999). Following a similar protocol, Sekihashi et al. (2002) obtained similar DNA damage results in varied organs in male ddY mice given much lower single oral doses (100 mg/kg bw) at three sampling times, 3, 8, and 24 hours after treatment. [The Working Group noted that the patterns of the positive results for DNA damage slightly varied at the different sampling time-points (see Table 4.2), and that the spleen was not investigated in these studies with the comet assay in mice.]

Significant induction of single-strand breaks as measured by alkaline elution assay was observed in male Swiss CD1 mouse kidney 4 hours after a single intraperitoneal injection of aniline (300 mg/kg bw), whereas a negative finding was obtained in liver (Cesarone et al., 1982). No induction of DNA fragmentation as measured by alkaline elution assay was seen in the liver, kidney, or bone marrow of male Swiss mice after a single intraperitoneal injection of aniline at a dose of up to 420 mg/kg bw (Parodi et al., 1982).

Gene mutation

One study on gene mutation in vivo in Big Blue rats and one host-mediated assay were available. No significant increases in mutant

frequency at the *cII* gene were observed in the liver, spleen, or bone marrow after a daily exposure at 100 mg/kg bw by gavage for 28 consecutive days (Koenig et al., 2018). Aniline was mutagenic in a host-mediated assay for mutation in *Salmonella*. Urine samples were collected for 24 hours from rats given aniline orally at a dose of 300 mg/kg bw. Ether extracts of the urine samples were tested for mutagenicity in *Salmonella typhimurium* TA98 and TA100. A clear concentration-dependent increase in gene mutation frequency was obtained with TA98 in the presence of endogenous metabolic activation (Tanaka et al., 1980).

Chromosomal aberration

There are few studies in vivo on chromosomal aberration with aniline or aniline hydrochloride. A slight but significant increase in the frequency of chromosomal aberration was observed in the bone marrow of male PVG rats given aniline hydrochloride as a single oral dose at 500 mg/kg bw (the highest dose tested) with a sampling time of 18 hours after treatment; however, no increased effect on chromosomal aberration was seen at the sampling time of 30 hours (Bomhard, 2003). Similarly, no clastogenic effect was observed in the bone marrow of male CBA mice treated with up to two intraperitoneal injections of aniline hydrochloride (380 mg/kg bw) and with sampling times of 16, 20, and 24 hours after the second treatment (Jones & Fox, 2003).

Micronucleus formation

Several studies have investigated micronucleus induction by aniline or aniline hydrochloride in rats. Significant increases in the frequency of micronucleus formation were seen in peripheral blood of Big Blue F344 rats treated with aniline at dose of 100 mg/kg bw by gavage daily for 4 days or 28 days (Koenig et al., 2018). A dose-related increase in the frequency of micronucleus formation was observed in the bone marrow of male PVG rats that received aniline hydrochloride as a single oral dose at 0, 215, 287, 400, or 500 mg/kg bw (as aniline base) (George et al., 1990). The lowest effective dose was 287 mg/kg bw when the bone marrow was collected at 24 hours after exposure. In a similar study carried out by Bomhard (2003), a small but statistically significant and dose-related induction of micronuclei was observed 24 hours but not 48 hours after a single oral dose of aniline hydrochloride at 0, 300, 400, or 500 mg/kg bw in male PVG rats.

Micronucleus induction by aniline or aniline hydrochloride has also been investigated in mice dosed via feed, gavage, or intraperitoneal injection.

In a 90-day study, an increased frequency of micronucleus formation was observed in peripheral blood in male and female B6C3F$_1$ mice given feed containing aniline hydrochloride at a concentration of 500, 1000, or 2000 mg/kg (equivalent to 65, 130, and 260 mg/kg bw per day) for 90 days (Witt et al., 2000).

Harper et al. (1984) reported a negative result for micronucleus induction in male ICR mice given a single oral dose of aniline at up to 250 mg/kg bw. [The Working Group noted that aniline enhanced the effect of benzene in the micronucleus test in a dose-related manner.] In a study on micronucleus formation in male B6C3F$_1$ mice (treated with aniline at 12, 23, 47, 120, or 470 mg/kg bw for 24 hours, orally), a significant induction of micronucleus was only observed at doses of 23 and 470 mg/kg bw. No dose–response relationship was seen (Ress et al., 2002). Aniline hydrochloride significantly induced micronucleus formation in the bone marrow of male CRH mice only at the highest dose tested (1000 mg/kg bw) at the 24-hour but not the 48-hour time point (Westmoreland & Gatehouse, 1991).

A dose-related increase in the frequency of micronucleated polychromatic erythrocytes was observed in bone marrow cells of Swiss mice given a single intraperitoneal injection of

purified aniline (5, 50, 100, or 200 mg/kg bw for 24 hours) (Sicardi et al., 1991). After intraperitoneal injections of aniline (up to 300 mg/kg bw), male CBA mice failed to show a significant increase in micronucleus frequency 6 hours after treatment (Ashby et al., 1991). A significant increase (about 7-fold over the control) was seen at a dose of 380 mg/kg bw 24 hours after treatment (Ashby et al., 1991; Tinwell & Ashby, 1991); the effect was not significant at 48 hours (Ashby et al., 1991). Westmoreland & Gatehouse (1991) confirmed the positive response for micronucleus induction in bone marrow of male CHR mice 24 hours after administration of aniline hydrochloride as a single intraperitoneal dose at 380 mg/kg bw.

[The Working Group noted that single oral dose studies seemed to be less sensitive than intraperitoneal injection studies.]

Sister-chromatid exchange

Only two in vivo studies were available for the induction of sister-chromatid exchange, both in Swiss mice given aniline intraperitoneally. A clear dose-dependent induction of sister-chromatid exchange in bone marrow cells of male Swiss mice was reported 24 hours after a single injection of aniline at a dose of 61–420 mg/kg bw. The lowest effective dose was 210 mg/kg bw, and raw chemical and purified aniline showed similar activities (Parodi et al., 1982). In addition, a significant dose-related induction of sister-chromatid exchange in male Swiss mice was seen after a single intraperitoneal injection of 210 or 420 mg/kg bw with sampling time of 24 hours after treatment (Parodi et al., 1983).

Sperm head abnormalities

In (CBA × BALB/c)F_1 male mice that received five daily intraperitoneal injections of aniline hydrochloride at doses ranging from 17 to 200 mg/kg bw, no increase in the frequency of sperm head abnormalities was observed for 5 weeks after the last dose (Topham, 1980).

(ii) Non-human mammalian cells in vitro

See Table 4.3.

Several studies investigated the genotoxic effects of exposure to aniline or aniline hydrochloride in non-human mammalian cells in vitro. The end-points included DNA damage, gene mutation, chromosomal aberration, sister-chromatid exchange, and unscheduled DNA synthesis.

DNA damage

Aniline (1.25–10 µg/mL for 24 hours) caused a concentration-related increase in the frequency of DNA strand breaks (comet assay) in cultured primary hepatocytes isolated from Sprague-Dawley rats. Addition of *N*-acetyl-L-cysteine reduced the effects (Wang et al., 2016).

No DNA damage (measured by alkaline elution assay) was seen in mouse lymphoma L5178Y cells in the absence of endogenous metabolic activation, whereas positive results were seen in the presence of endogenous metabolic activation, at the higher dose (Garberg et al., 1988).

Exposure of Chinese hamster lung fibroblasts (V79) to aniline (up to 3 mM for 4 hours) with or without endogenous metabolic activation did not affect the rate of elution of DNA (Swenberg et al., 1976; Swenberg, 1981).

Unscheduled DNA synthesis tests with aniline or aniline hydrochloride in primary rat or mouse hepatocytes all gave negative results (Williams, 1980, 1981; McQueen et al., 1981; Yoshimi et al., 1988; Butterworth et al., 1989).

Gene mutation

Aniline or aniline hydrochloride gave positive results for gene mutation in the mouse lymphoma L5178Y *Tk*$^{+/-}$ assay consistently in several independent studies, even though most positive results occurred at high concentrations. In the presence of endogenous metabolic activation, the mutation frequency in the *Tk*$^{+/-}$ mutation assay increased in a concentration-related manner up

to 4.98 mM and doubled at a concentration of 3.7 mM (Amacher et al., 1980). Wangenheim & Bolcsfoldi (1988) reported a concentration-related increase in aniline-induced mutation frequency in the $Tk^{+/-}$ mutation assay in both the presence (aniline, 0.5–5 mM) and absence (aniline, 2.5–15 mM) of endogenous metabolic activation. The lowest effective dose was 0.5 mM and the presence of endogenous metabolic activation enhanced the sensitivity of aniline-induced mutation. Similar results for aniline concentration-related increases in frequency of gene mutation were also obtained in several other studies (Caspary et al., 1988; Mitchell et al., 1988; Myhr & Caspary, 1988; McGregor et al., 1991). [The Working Group noted that aniline or aniline hydrochloride has the potential to cause mutations at the thymidine kinase (*Tk*) locus in mouse lymphoma L5178Y cells. The $Tk^{+/-}$ mutation assay can detect a wide range of genetic events, including point mutations, deletions, chromosomal rearrangements, mitotic recombination, and nondisjunction. Generally, the induction of small colonies of mutants is associated with chemicals that induce gross chromosomal aberrations, whereas the induction of large colonies of mutants is associated with chemicals that induce point mutations; however, in the available studies, the colony sizes were not reported.]

In addition, Fassina et al. (1990) reported a marginal but significant increase in mutation frequency in the *Hprt* mutation assay in Chinese hamster V79 cells exposed to aniline at up to 60 mM in the presence of endogenous metabolic activation from rat S9; however, no induction of mutation was seen with aniline in the absence of S9 in the cell culture or when co-cultured with rat hepatocytes. [The Working Group noted that aniline caused mutations at the *Hprt* locus only at a high concentration in the presence of endogenous metabolic activation.]

Chromosomal aberrations

Clastogenic activity with aniline or aniline hydrochloride has also been studied in mammalian cells. The studies from Galloway et al. (1987) showed negative results in Chinese hamster ovary cells exposed to aniline in the absence of endogenous metabolic activation, whereas a significantly increased frequency of chromosomal aberrations was seen at the highest test concentration of aniline (5 mg/mL) in the presence of endogenous metabolic activation. [The Working Group noted that the test gave weakly positive results at the highest dose; and that a dose–response relationship with endogenous metabolic activation was observed.] Aniline induced a concentration-related increase in the frequency of chromosomal aberrations in Chinese hamster embryo cells in the absence of endogenous metabolic activation. The lowest effective concentration was 444 µg/mL and the main aberrations were dicentric chromosomes and breaks (Chung et al., 1995, 1996).

Early studies showed that aniline hydrochloride was not clastogenic in Chinese hamster Don cells at concentrations of up to 5 mM [0.645 mg/mL] (Abe & Sasaki, 1977) or in Chinese hamster lung cells at up to 1 mg/mL in the absence of endogenous metabolic activation (Ishidate & Odashima, 1977; Ishidate, 1983). Moreover, Kanaya (1996) reported that *Vicia faba* extract could activate aniline to induce chromosomal damage in Chinese hamster ovary cells, whereas extracts from *Pisum sativum* and *Lactuca sativa* did not. Furthermore, aniline metabolites *ortho*-, *meta*-, and *para*-aminophenol [2-, 3-, and 4-aminophenol] induced an increased frequency of chromosomal aberrations in Chinese hamster ovary cells in a concentration-related manner (Kanaya 1996).

Micronucleus formation

Fritzenschaf et al. (1993) reported that aniline [dose not reported] did not induce micronucleus formation in Syrian hamster embryo cells in the

absence of endogenous metabolic activation, whereas Matsushima et al. (1999) reported a significant increase in the frequency of micronucleated cells in the Chinese hamster lung cell line exposed to aniline at a concentration of 125–2000 µg/mL with endogenous metabolic activation. No induction of micronuclei was seen in the absence of endogenous metabolic activation in the test system (Matsushima et al., 1999).

Sister-chromatid exchange

A concentration-related increase in sister-chromatid exchange frequency was seen in RL4 rat liver epithelial cells treated with aniline (0.1, 0.2, 0.5, 1 mM for 24 hours) in the absence of endogenous metabolic activation. The doubling effect occurred at concentration of 0.5 mM (Cunningham & Ringrose, 1983). Galloway et al. (1987) reported a slight increase in the frequency of sister-chromatid exchange in Chinese hamster ovary W-B1 cells treated with aniline (50–500 µg/mL for 26 hours) without endogenous metabolic activation; and at concentrations of 4 mg/mL and 5 mg/mL in an experiment with incubation for 2 hours with endogenous metabolic activation.

Takehisa et al. (1988) also reported that aniline hydrochloride (0.01, 0.1, 1 mM, for 24 hours) alone could induce sister-chromatid exchange in Chinese hamster ovary cells, although the response was weak; however, the presence of endogenous metabolic activation with *Vicia* root S10 or rat liver S9 did not induce an increase in sister-chromatid exchange frequency above that of the controls. An early study showed that aniline hydrochloride caused a concentration-related effect on sister-chromatid exchange induction in Chinese hamster Don cells (Abe & Sasaki, 1977). Kanaya (1996) reported that none of the plant extracts tested (*Vicia*, *Pisum*, or *Lactuca* S10 mix) could activate aniline to induce sister-chromatid exchange in Chinese hamster ovary cells; however, the aniline metabolites *ortho*-, *meta*-, and *para*-aminophenol [2-, 3-, and 4-aminophenol] increased the frequency of sister-chromatid exchange in Chinese hamster ovary cells in a concentration-related manner.

(iii) Non-mammalian experimental systems in vivo and in vitro

See Table 4.4.

Aniline did not induce DNA adduct formation (measured by nucleotide ^{32}P-post-labelling) or cause DNA strand breaks when assessed in embryo-fetal chicken livers (Kobets et al., 2019).

No DNA adducts were found (by ^{32}P-post-labelling) in *S. typhimurium* TA98 after treatment with aniline alone at a concentration of 1 mg/mL with or without endogenous metabolic activation; however, DNA adducts were detected in the presence of norharman and endogenous metabolic activation (Mori et al., 1996).

Yoon et al. (1985) reported that aniline was not mutagenic in the test for sex-linked recessive lethal mutations in *Drosophila melanogaster* exposed to aniline at 400 mg/kg by injection or at 600 mg/kg via feed. Moreover, in an eye mosaic (white/white$^+$) assay, aniline induced a modest increase in the frequency of interchromosomal mitotic recombination after treatment of larvae at 2 mM (Vogel & Nivard, 1993). Muñoz & Barnett (1998) showed that aniline hydrochloride was not mutagenic in the test for sex-linked recessive lethal mutations in *Drosophila* after intra-abdominal injection (0.4 µL) or feeding (10% solution); however, significant increases in the frequency of nondisjunction were observed in *Drosophila* in the feeding study.

In a plant study, aniline increased the frequency of micronucleus formation in wheat (*Triticum aestivum*) root tip cells, in a dose-dependent manner at concentrations as low as 5 mg/L in the culture solution (Tao et al., 2017). Exposure of *Vicia faba* seeds to aniline hydrochloride resulted in significant increases in the frequency of chromosomal aberrations but not sister-chromatid exchange in the root cells (Kanaya, 1990).

Previous studies indicated that aniline did not cause mutation in *Aspergillus nidulans* (Prasad, 1970) and gave negative results for recombinogenic activity in an assay in vitro with *Saccharomyces cerevisiae* D3 (Simmon, 1979). It was subsequently reported that aniline (1, 2, 4, or 7 mg/mL, for 17 hours) induced intrachromosomal recombination in yeast *S. cerevisiae* RS112 at a higher concentration (7.9-fold at 7 mg/mL) (Schiestl, 1989; Schiestl et al., 1989). Furthermore, Brennan & Schiestl (1997) reported that aniline (5, 10, or 12 mg/mL, for 17 hours) and its metabolites 2- and 4-aminophenol induced intrachromosomal (DEL) recombination in *S. cerevisiae* strain RS112. Schafer et al. (2008) showed that aniline was mutagenic and a recombinogen in the eukaryotic organisms *S. cerevisiae* strains HAN and DAN.

Sakagami et al. (1988) reported that aniline did not caused DNA damage in *S. typhimurium* as measured by the SOS/*umu* genotoxicity assay at concentrations of up to 4000 µg/mL with or without metabolic activation. Aniline did not cause DNA damage in *Escherichia coli* KWP2, WP100, or K12 (Mamber et al., 1983, 1984) or in the rec assay with *Bacillus subtilis* strains H17 rec^+ and M45 rec^- (McCarroll et al., 1981). Stock aniline or aniline that was not freshly distilled (5 days after distillation) was reported to cause DNA damage in the *E. coli* pol A^+/pol A^- assay with or without metabolic activation; but the freshly distilled aniline gave negative results (Fluck et al., 1976). Moreover, aniline induced DNA damage in the Mutatox genotoxicity test with *Vibrio fischeri* (dark variant) without endogenous metabolic activation; but was not genotoxic in the presence of endogenous metabolic activation (Osano et al., 2002).

Negative results were obtained in most of the *S. typhimurium* assays with aniline or aniline hydrochloride in tester strains TA98, TA100, TA1535, TA1537, TA1358, TA98NR, or TA100NR in the presence or absence of metabolic activation from varied types of S9 (from rat, mouse, hamster, pig, or plant) (Garner & Nutman, 1977; Nagao et al., 1977; Ashby et al., 1981, 1983; Chung et al., 1981, 1995, 1996; Ho et al., 1981; Parodi et al., 1981; Haworth et al., 1983; Imamura et al., 1983; Nohmi et al., 1984; Dunkel et al., 1985; Brams et al., 1987; Gentile et al., 1987; Rashid et al., 1987; Shahin, 1989; Kato et al., 1991; Jung et al., 1992; Burke et al., 1994; Assmann et al., 1997). Only one positive result was seen in strain TA100 at the highest test concentration, 10 000 µg/plate, in the presence of metabolic activation from pea apical bud S9 (Gentile et al., 1987). Notably, Shahin (1989) re-evaluated the mutagenicity of aniline hydrochloride in TA98, TA100, TA1535, TA1537, and TA1538 with metabolic activation from S9 prepared from rat liver or spleen, or hamster liver or spleen, and the results confirmed that aniline was not mutagenic in *S. typhimurium* at concentrations up to 5 mg/plate.

Similarly, aniline (Pai et al., 1985) and aniline hydrochloride (Dunkel et al., 1984, 1985; Martínez et al., 2000) were also not mutagenic in forward mutation assays in *E. coli* with or without metabolic activation from S9 from various animal livers.

4.2.3 Induces oxidative stress

Table 4.5, Table 4.6, and Table 4.7 summarize the available studies of effects related to oxidative stress after exposure to aniline and aniline hydrochloride.

(a) Humans

No data were available to the Working Group on oxidative stress related to aniline or aniline hydrochloride in exposed humans.

In human cells in vitro, Horinouchi et al. (2015) reported that aniline did not cause membrane damage or free radical generation (measured by immuno-spin trapping using in-cell Western experiments and confocal microscopy) in HepG2 cells at a concentration of 100 µM (the only concentration tested).

Table 4.5 Oxidative damage to DNA in experimental animals

End-point/biomarker	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Reference
Aniline hydrochloride						
8-OHdG	Rat, Sprague-Dawley (M)	Spleen	+	1 mmol/kg bw per day [129 mg/kg bw]	Gavage, 7 days	Wu et al. (2005)
8-OHdG, Ogg1 mRNA, protein levels and enzyme activity	Rat, Sprague-Dawley (M)	Spleen	+	0.5 mmol/kg bw per day [64.8 mg/kg bw]	Drinking-water, 30 days	Ma et al. (2008)
Base excision repair enzymes Neil1/2, Nth1, and Ape-1 mRNA, protein levels and enzyme activity	Rat, Sprague-Dawley (M)	Spleen	+	0.5 mmol/kg bw per day [64.8 mg/kg bw]	Drinking-water, 30 days	Ma et al. (2011, 2013)

Ape-1, apurinic/apyrimidinic endonuclease 1; bw, body weight; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; HID, highest ineffective dose; LED, lowest effective dose; M, male; Neil1/2, nei-like DNA glycosylase; Nth1, endonuclease III homologue 1; Ogg1, 8-oxoguanine glycosylase 1.
[a] +, positive.

(b) Experimental systems

(i) Oxidative damage to DNA

See Table 4.5.

Aniline treatment of male Sprague-Dawley rats resulted in a significant increase of 200% in splenic iron content and an 83% increase in 8-hydroxy-2′-deoxyguanosine (8-OHdG) in spleen compared with controls (Wu et al., 2005). 8-Oxoguanine glycosylase (Ogg1) is a specific DNA glycosylase enzyme and plays an important role in the removal of 8-OHdG adducts during base excision repair (BER). Subchronic exposure of male Sprague-Dawley rats to aniline for 30 days resulted in increases of 2.8-fold in 8-OHdG levels, 2-fold in *Ogg1* messenger RNA (mRNA) expression, and 1.3-fold in Ogg1 BER activity (Ma et al., 2008). [The Working Group noted that the results suggest that aniline-induced oxidative stress is associated with increased oxidative damage to DNA.]

Like Ogg1, nei-like DNA glycosylase (Neil1/2) is also a BER enzyme; distinct from Ogg1, it is able to excise oxidized base lesions from regions of single-stranded DNA. Ma et al. (2011) reported that male Sprague-Dawley rats exposed to aniline had increased levels of Neil1/2 activity, mRNA and protein, and Neil immunoreactivity. [The Working Group noted that NEIL1/2 may play a unique role in maintaining the functional integrity of mammalian genomes. This study confirmed that aniline-induced oxidative stress and related DNA damage could also be removed by another BER enzyme, Neil1/2, in addition to Ogg1.] Ma et al. (2013) further reported that aniline exposure led to increased levels of mRNA, protein, protein-associated BER activity, and immunoreactivity for the BER enzymes endonuclease III homologue 1 (Nth1) [nth like glycosylase 1, Nthl1] and apurinic/apyrimidinic endonuclease 1 (Ape1) [apurinic/apyrimidinic endodeoxyribonuclease 1, Apex1]. [The Working Group noted that these data consistently revealed that aniline exposure caused reactive oxygen species (ROS)-mediated DNA damage in rats, as demonstrated by the increased splenic level of 8-OHdG (Wu et al., 2005) and increased expression and activity of BER proteins Ogg1 (Ma et al., 2008), Neil1/2, Nth1, and Ape1 (Ma et al., 2011).]

(ii) Other oxidative stress markers

See Table 4.6.

In studies in experimental animals, a single acute exposure to aniline vapour induced superoxide dismutase (SOD) isozymes in rabbit lung (Kakkar & Viswanathan, 1987) and increased

Table 4.6 Oxidative stress in experimental animals

End-point/ biomarker	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Aniline							
SOD activity	Rabbit, albino (M)	Lung	+	15 302 ppm [58 300 mg/m^3]	Chamber exposure, 30 min, 15 302 ppm aniline vapours		Kakkar & Viswanathan. (1987)
SOD activity and MDA content	Rat, Wistar (M)	Brain	+	15 302 ppm [58 300 mg/m^3]	Chamber exposure, 10 min, 15 302 ppm aniline vapours		Kakkar et al. (1992)
Aniline hydrochloride							
MDA content	Rat, Sprague-Dawley (M)	Spleen	+	0.5 mmol/kg bw per day	Gavage; 0.25, 0.5, 1, 2 mmol/kg bw per day for 4 days		Khan et al. (1997)
MDA, protein carbonyl content and splenic iron	Rat, Sprague-Dawley (M)	Spleen	+	1 mmol/kg bw per day	Gavage; 4, 7 days		Khan et al. (1997)
MDA, protein carbonyl content, and splenic iron	Rat, Sprague-Dawley (M)	Spleen	+	65 mg/kg bw per day	Drinking-water; 30, 60, 90 days	Negative results for protein carbonyl content at day 30; stronger response in longer exposure	Khan et al. (1999a)
MDA protein adducts	Rat, Sprague-Dawley (M)	Spleen	+	1 mmol/kg bw per day [129.5 mg/kg bw per day]	Gavage; 7 days		Khan et al. (2003a)
MDA protein adducts	Rat, Sprague-Dawley (M)	Spleen	+	65 mg/kg bw per day	Drinking-water; 30 days		Khan et al. (2003b)
MDA, GSH, NO content	Rat, Wistar (M)	Spleen and liver	+	100 ppm [381 mg/m^3]	Drinking-water; 30 days, ± *Dioscorea alata L* extract		Khan et al. (2014)
MDA, GSH, NO content	Rat, Wistar (M)	Spleen	+	100 ppm [381 mg/m^3]	Drinking-water; 28 days, ± protocatechuic acid or ascorbic acid		Khairnar et al. (2016)
Free iron level, total iron or ferritin level	Rat, Sprague-Dawley (M)	Spleen	+	1 mmol/kg bw per day	Gavage; 1, 4, 7 days	Significant increased free iron content at day 7 Significant increased total iron or ferritin level at day 4 and 7	Wang et al. (2010)
Formation of nitrated protein, iNOS mRNA and protein expression	Rat, Sprague-Dawley (M)	Spleen	+	0.5 mmol/kg bw per day	Drinking-water; 30 days		Fan et al. (2011)

Table 4.6 (continued)

End-point/ biomarker	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Phenylhydroxylamine, an oxidized metabolite of aniline							
MDA, protein carbonyl content and splenic iron	Rat, Sprague-Dawley (M)	Spleen	+	0.025 mmol/kg bw per day	Gavage; 0.025, 0.05, 0.1, 0.2 mmol/kg bw per day for 4 days		Khan et al. (1998)
Nitrosobenzene, an N-oxidized metabolite of aniline							
MDA content and MDA-protein adducts	Rat, Sprague-Dawley (M)	Spleen	+	0.025 mmol/kg bw per day	Gavage; 0.025, 0.05, 0.1, and 0.2 mmol/kg bw per day for 4 days		Khan et al. (2000)
Protein carbonyl content and splenic iron	Rat, Sprague-Dawley (M)	Spleen	+	0.1 mmol/kg bw per day	Gavage; 0.025, 0.05, 0.1, and 0.2 mmol/kg bw per day for 4 days		Khan et al. (2000)

bw, body weight; GSH, glutathione; HID, highest ineffective dose; iNOS, inducible nitric oxide synthase; LED, lowest effective dose; M, male; MDA, malondialdehyde; min, minute; NO, nitric oxide; ppm, parts per million; SOD, superoxide dismutase.

[a] +, positive.

Table 4.7 Gene expression responses to aniline and aniline hydrochloride related to the key characteristics of carcinogens

End-point/ biomarker	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Aniline hydrochloride							
TGF-β1 mRNA and protein	Rat, Sprague-Dawley (M)	Spleen	+	1 mmol/kg bw per day	Gavage; 7 days		Khan et al. (2003b)
IL1α, IL6, and TNFα mRNA and protein; NF-κB binding activity	Rat, Sprague-Dawley (M)	Spleen	+	0.5 mmol/kg bw per day	Drinking-water; 30 days		Wang et al. (2005)
IL1α, IL6, TNFα mRNA and protein; NF-κB and AP-1 binding activity	Rat, Sprague-Dawley (M)	Spleen	+	1 mmol/kg bw per day	Gavage; 7 days		Wang et al. (2008)
HO-1 mRNA and protein	Rat, Sprague-Dawley (M)	Spleen	(+)	1 mmol/kg bw per day	Gavage; 1, 4, 7 days	Purity, NR	Wang et al. (2010)
Cyclins and cyclin-dependent kinases	Rat, Sprague-Dawley (M)	Spleen	+	0.5 mmol/kg bw per day	Drinking-water; 30 days		Wang et al. (2011)
Cell cycle regulatory proteins	Rat, Sprague-Dawley (M)	Spleen	+	0.5 mmol/kg bw per day	Drinking-water; 30 days	Significant increase in the expression of cyclins, Cdk1 and aberrant regulation of miRNAs	Wang et al. (2015)
Cell cycle regulatory proteins	Rat, Sprague-Dawley (M)	Spleen	+	1 mmol/kg bw per day	Gavage; 7 days		Wang et al. (2017)
Aniline							
Gene expression of *Gst D2* and *Gst D5*	*Drosophila melanogaster*	NA	+	NR; < 2.0 µL/tube	On filter paper; 1.2, 1.4, 1.6, 1.8, and 2.0 µL/tube		Chan et al. (2015)
Gene expression of *Gst D6*	*Drosophila melanogaster*	NA	+	2.0 µL/tube	On filter paper; 1.2, 1.4, 1.6, 1.8, and 2.0 µL/tube		Chan et al. (2015)

AP-1, activator protein-1; bw, body weight; Cdk1, cyclin-dependent kinase 1; *Gst D*, glutathione *S*-transferase delta; HO-1, haem oxygenase-1; HID, highest ineffective dose; IL, interleukin; LED, lowest effective dose; M, male; miRNA, microRNA; NA, not available; NR, not reported; TGF-β1, tumour growth factor beta 1; TNFα, tumour necrosis factor alpha, NF-κB, nuclear factor-kappa B.

[a] +, positive; (+), weakly positive.

lipid peroxidation (measured by malondialdehyde formation) and SOD activity in rat brain (Kakkar et al., 1992). Using male Sprague-Dawley rats as an experimental model, Khan and colleagues investigated the early biological effects of aniline-induced short-term or subchronic splenic toxicity after oral exposure. Dose-related increases in splenic iron concentration, malondialdehyde, and protein carbonyl content were reported in the spleen (Khan et al., 1997; Wang et al., 2010). Further studies demonstrated that the aniline metabolites phenylhydroxylamine and nitrosobenzene also produced these biological effects, but at much lower concentrations than did aniline (Khan et al., 1998, 2000). Khan et al. (2014) showed that an ethanol extract of *Dioscorea alata L.* (an antioxidant) significantly reduced aniline-induced effects on iron deposition, lipid peroxidation, reduced glutathione, and nitric oxide levels in the spleen in Wistar rats. Moreover, subchronic supplementation with protocatechuic acid (a natural phenolic compound) or with the combination of protocatechuic acid and ascorbic acid significantly ameliorated the effect on glutathione depletion by aniline, and reduced aniline-induced iron deposition, lipid peroxidation, and nitric oxide generation in the spleen of Wistar rats (Khairnar et al., 2016). [The Working Group noted that the protective effects of the antioxidants support the tenet that aniline-induced splenic effects occur through oxidative stress.]

Using proteomic approaches, Fan et al. (2011) showed that aniline exposure resulted in significantly increased formation of nitrated proteins in the spleen of rats. Furthermore, aniline exposure also led to significantly increased expression of inducible nitric oxide synthase mRNA and protein in the spleen. [The Working Group noted that aniline exposure induced nitrosative stress by generating reactive nitrogen species, which contributed to the increase in nitrated proteins.]

Early studies in vitro suggested that aniline depleted the cellular glutathione pool in liver microsomes (Aikawa et al., 1978). In a study in cultured primary rat hepatocytes in vitro, Wang et al. (2016) demonstrated that aniline exposure significantly increased the levels of ROS and malondialdehyde; and significantly decreased the levels of glutathione, catalase, SOD activity, and mitochondrial membrane potential, and caused DNA damage. The addition of *N*-acetyl-l-cysteine, an ROS scavenger, significantly reduced the adverse effects.

(iii) Gene expression responses related to the key characteristics of carcinogens

See Table 4.7.

Acute exposure to aniline induces methaemoglobinaemia, haemolytic anaemia, and haemolysis. The damaged erythrocytes may be scavenged by the spleen and the resulting splenotoxicity may be associated with the release of iron, oxidative and nitrosative stress, and induction of oxidative stress-related gene expression. In male Sprague-Dawley rats, short-term (7 days) exposure to aniline led to upregulation of transforming growth factor-beta 1 (TGF-β1 gene) (Khan et al., 2003b, 2006); activation of transcription factor activator protein-1 (AP-1) and mitogen-activated protein kinases (Khan et al., 2006). The short-term exposure also activated both redox-sensitive transcription factors, nuclear factors NF-κB and AP-1; upregulated fibrogenic cytokines (interleukins IL1 and IL6, and tumour necrosis factor alpha, TNFα) (Wang et al., 2008); and significantly increased levels of free iron, total iron, or ferritin, haem oxygenase mRNA and protein levels in rat spleen (Wang et al., 2010). Subsequently, 30-day studies in male rats confirmed that aniline exposure caused the increased expression of IL1α, IL6, and TNFα at both mRNA and protein levels; and activation of NF-κB (Wang et al., 2005). Such exposure also enhanced expression of cyclins (D1, D2, D3, E) and cyclin-dependent kinases (CDKs), and overexpression of cell proliferation marker proteins nuclear Ki67 and mini-chromosome

maintenance MCM2 protein (Wang et al., 2011). Moreover, subchronic exposure of rats to aniline resulted in a significant increase in the expression of cyclins and CDK1, and aberrant regulation of microRNAs (miRNAs), which led to accelerated G2/M transition of the splenocytes, and potentially to a tumorigenic response (Wang et al., 2015, 2017). Thus, oxidative stress leads to transcriptional upregulation of fibrogenic/inflammatory factors (cytokines IL1, IL6, and TNFα) through the activation of NF-κB, AP-1, and other redox-sensitive transcription factors. In addition, Chan et al. (2015) reported that aniline exposure increased glutathione transferase delta gene expression in *Drosophila melanogaster* in a dose-related manner (see also Section 4.2.5).

4.2.4 Alters cell proliferation, cell death, or nutrient supply

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

Male Wistar rats given feed containing aniline (0.03–0.12%) for 80 weeks did not present hyperplasia in the urinary bladder (Hagiwara et al., 1980). Male and female Fischer 344 rats given feed containing aniline hydrochloride at 0.3% or 0.6% for 103 weeks showed fibrosis and papillary hyperplasia of the spleen, as well as endometrial stromal polyps (NCI, 1978). Stromal hyperplasia and fibrosis of the splenic red pulp, which may represent a precursor lesion of sarcoma, was also observed in male Fischer 344 rats treated with aniline at 100 mg/kg bw and, to a lesser degree, in female rats (US EPA, 1982).

Male Sprague-Dawley rats receiving drinking-water containing aniline hydrochloride at a dose of 65 mg/kg bw per day for 1, 2, or 3 months presented splenomegaly accompanied by morphological changes, including marked red pulp expansion due to prominently dilated splenic sinusoids and fibroblasts, vascular congestion, and splenic hyperplasia. Mitotic activity was not prominent (Khan et al., 1999).

In male Sprague-Dawley rats receiving drinking-water containing aniline hydrochloride (0.5 mmol/kg bw per day for 30 days) (dose that caused upregulation of pro-fibrogenic cytokines) resulted in increased spleen weight and increased splenocyte population (Wang et al., 2011). These findings were confirmed by a significant increase in the expression of splenic proteins that are considered markers of cell proliferation: proliferating cell nuclear antigen (PCNA), nuclear Ki67 protein and minichromosome maintenance 2 (MCM2) protein (Wang et al., 2011). The associated molecular mechanisms were: (i) the increased protein expression of cell cycle regulators, such as cyclins (A, B1, D1, D2, D3, and E), cyclin-dependent kinases (CDK1, CDK2, CDK4, and CDK6), and phosphorylated retinoblastoma protein (pRb-p) (Wang et al., 2011, 2015); and (ii) downregulation of CDK inhibitors p21 and p27 at protein and mRNA levels (Wang et al., 2015). In experimental conditions that precede fibrogenic responses in rats (given drinking-water containing aniline at 1 mmol/kg bw per day, for 7 days), activation of mitogen-activated protein kinases (MAPKs) (Wang et al., 2008) and increased protein expression of cyclins (A, B1, D3, and E) and CDKs (CDK1, CDK2, CDK4, and CDK6) (Wang et al., 2017) were observed.

4.2.5 Evidence relevant to other key characteristics of carcinogens

Experimental evidence related to other key characteristics of carcinogens is described below, including whether aniline or aniline hydrochloride: alters DNA repair; induces epigenetic alterations; induces chronic inflammation; is immunosuppressive; modulates receptor-mediated effects; and causes immortalization.

As noted above (see Section 4.2.3(b), rats given drinking-water containing aniline hydrochloride at a dose of 0.5 mmol/kg bw per day

for 30 days presented increased expression and activity of BER enzymes in the spleen, including Ogg1, Neil1/2, Nth1, and Ape1 (Ma et al., 2008, 2011, 2013).

Decreased expression of miRNAs that may regulate the expression of cyclins and increased expression of miRNAs that may regulate the expression of CDKs were also observed in rats exposed to aniline hydrochloride (Wang et al., 2015, 2017).

In male Fischer rats given feed containing aniline hydrochloride (100 mg/kg bw per day) for 4 weeks there was induction of focal perisplenitis (Mellert et al., 2004). In Sprague-Dawley rats given aniline hydrochloride at a dose that elicits a fibrogenic response (in drinking-water, 0.5 mmol/kg bw per day, for 30 days) there was overexpression (both at mRNA and protein levels) of three cytokines (interleukin IL1α, IL6, and TNFα) through the activation of NF-κB (Wang et al., 2005). Administration of aniline under experimental conditions that precede fibrogenic responses in rats (in drinking-water, 1 mmol/kg bw per day, for 7 days) resulted in activation of NF-κB and AP-1, phosphorylation of IκB kinase (IKK) and upregulation of pro-inflammatory and pro-fibrogenic cytokines in the spleen (Wang et al., 2008). Aniline hydrochloride (0.1–10 μM) inhibited α/β interferon induction in mouse embryo fibroblast cell cultures (Sonnenfeld, 1983). There was a decrease in interferon induction when 3.25 mg of aniline hydrochloride were injected intraperitoneally into mice 24 hours after interferon induction (Sonnenfeld & Hudgens, 1983). [The Working Group noted that the effects of aniline hydrochloride on interferon inhibition were more modest than the effects of 4-aminobiphenyl.]

Increased levels of progesterone, 17α-hydroxyprogesterone, and testosterone were reported in human adrenocortical carcinoma cell lines exposed for 48 hours with different concentration of aniline (0.0001–1000 μM) (Holm et al., 2015). Repeated daily subcutaneous administration of aniline to rats caused significant adrenal enlargement and increase in lipid accumulation in corpora lutea and adrenal cortex (Kovacs et al., 1970, 1971; Hatakeyama et al., 1971; Horvath et al., 1971). [The Working Group noted that these studies are in general descriptive.] Plasma corticosterone levels were decreased 24 hours after a single subcutaneous dose of aniline (30 mg) (Toth et al., 1971).

Aniline induced cell transformation in rodent cells in some studies. Aniline at doses of 485, 544, and 908 μg/mL gave positive results in the Syrian hamster embryo cell transformation assay (Plöttner et al., 2013). Aniline at 0.8 μg/mL induced cell transformation in mouse BALB/C3T3 cells (Dunkel et al., 1981). The results were negative in Fischer rat embryo cells with aniline at 0.001 μg/mL (Price & Mishra, 1980) and in Syrian hamster embryo cells at 5 μg/mL (Dunkel et al., 1981), and at up to 10 μg/mL (Pienta, 1980), and in Syrian hamster kidney cells (BHK21) at up to 250 μg/mL (Purchase et al., 1978). [The Working Group noted that Fischer rat embryo cells are not metabolically competent.]

4.3 Data relevant to comparisons across agents and end-points

The analysis of the in vitro bioactivity of the agents reviewed in the present volume was informed by data from high-throughput screening assays generated by the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the government of the USA (Thomas et al., 2018).

Aniline and aniline hydrochloride were among thousands of chemicals tested across the large assay battery of the Tox21 and ToxCast research programmes as of 26 April 2020. Detailed information about the chemicals tested, assays used, and associated procedures for data analysis is publicly available (US EPA, 2021). [The Working Group noted that the metabolic

capacity of the cell-based assays is variable, and generally limited, as acknowledged in Kavlock et al. (2012).]

Among the 235 assays in which aniline was tested, it was found to be inactive in all assays related to key characteristics of carcinogens (US EPA, 2019a). Among the 432 assays in which aniline hydrochloride was tested (at concentrations up to 100 µM), it was found to be active in 9 assays (US EPA, 2019b).

An effect on upregulation of nuclear receptor subfamily 1, group H, member 4 (NR1H4) was reported in the human kidney cell line, HEK293T, at a half-maximal activity concentration (AC_{50}) of 39.2 µM. Thyroid peroxidase inhibition was observed in a rat thyroid gland cell line at an AC_{50} of 0.592 µM and in a porcine thyroid gland cell line at an AC_{50} of 15.3 µM.

Borderline activity was reported for several assays. These included the activation of the receptors estrogen receptor 1 (ESR1), estrogen receptor 2 (ESR2), retinoic acid receptor α (RARA), androgen receptor (AR), and peroxisome proliferator activated receptor α (PPARα) in different cell lines and increase in NaCl co-transporter (NCCT) assay in *E. coli*. [The Working Group considered the relevance of these end-points to the key characteristics of carcinogens to be unclear.]

4.4 Other relevant evidence

4.4.1 Humans

No data were available to the Working Group, other than on methaemoglobinaemia (see Section 4.1).

4.4.2 Experimental systems

A histopathological review of rat spleens from a bioassay with aniline hydrochloride from the National Cancer Institute (NCI, 1978) reported an increased incidence of splenotoxic changes including fatty metamorphosis, fibrosis, capsule hyperplasia, and haemorrhage in male and female Fischer 344 rats treated with aniline hydrochloride (0.6% in feed, for 103 weeks) (Weinberger et al., 1985). In another bioassay, fatty metamorphosis of splenic red pulp and haematological alterations were observed in male and female Fischer 344 rats given feed containing aniline hydrochloride, as was an increase in the spleen weight, associated with vascular congestion (US EPA, 1982).

In several short-term studies in rats, a correlation was observed between haematopoietic toxicity of aniline (leading to methaemoglobinaemia and iron deposition in spleen) and splenotoxicity (Khan et al., 1997, 1999; Mellert et al., 2004; see also Section 4.2.3(b)).

Aniline hydrochloride induced an increase in spleen weight, associated with vascular congestion, when fed to male Fischer rats for 1 or 4 weeks at a dietary dose of 30 or 100 mg/kg bw (actual intake, ≥ 17 and ≥ 57 mg/kg bw, respectively), and induced hypercellularity of the bone marrow, predominantly of the erythropoietic cell line, at 100 mg/kg bw after 4 weeks. Other observations included regenerative toxic haemolytic anaemia (lower erythrocyte, reduced haemoglobin, and reduced haematocrit values) and alteration in erythrocyte and leukocyte parameters such as: polychromasia, hyperchromasia, normoblasts, higher leukocyte values, anisocytosis, and higher polymorphonuclear neutrophil values. In addition, increased transferrin concentration and total iron binding capacity and haemosiderin deposition in Kupffer cells of the liver were observed (Mellert et al., 2004).

5. Summary of Data Reported

5.1 Exposure characterization

Aniline, the parent compound of aniline hydrochloride, is a basic compound and will undergo acid–base reactions. Aniline and its hydrochloride salt will achieve a pH-dependent acid–base equilibrium in the body.

Aniline is primarily produced by catalytic reduction of nitrobenzene. Aniline hydrochloride is prepared by reacting aniline vapour and hydrogen chloride gas.

Almost no information was found on occurrence, use, and exposure to aniline hydrochloride.

Aniline is a High Production Volume chemical. In the chemical industry, aniline is used for the synthesis of many compounds, including isocyanates, dyes and pigments, antioxidants and accelerators in rubber processing, pharmaceuticals, varnishes, perfumes, photographic chemicals, herbicides, and fungicides. Northeastern Asia was the largest producer of aniline during 2013–2018, accounting for more than half of the world's production of aniline, followed by western Europe and the USA.

The general population may be exposed to aniline from the release of industrial effluents in the environment to air, water, land, or groundwater. Aniline has been detected in drinking-water in several well-conducted studies in North America and Europe. On the basis of limited recent data, food does not appear to be a significant source of aniline at present. Aniline is used as an intermediate for the production of pharmaceuticals and in many consumer products, such as fabrics, textiles and apparel, leather, paper, plastic, and colourants, including tattoo ink. Cigarette smoking is one of the main contributors of aniline in non-occupational environments. Most measurements showed that the levels of aniline in sidestream tobacco smoke were considerably higher than those in mainstream tobacco smoke.

The main scenarios for occupational exposure to aniline are during production and distribution of aniline, production of other chemicals and products for which aniline is used as a chemical intermediate, and by handling and using products containing residual aniline. Production of 4,4-methylene diphenyl diisocyanate (mainly used in the production of rigid polyurethane) accounts for more than 90% of aniline use, followed by production of rubber-processing chemicals, and to a minor extent, dyes prepared from aniline derivatives. In many occupational settings, exposure to aniline co-occurs with exposure to other chemicals that are known bladder carcinogens. Despite the widespread use of aniline, exposure data is scarce for all industries and scenarios where there is a significant potential for aniline exposure. Time-weighted average occupational exposure limits for aniline have been established in several countries.

Although inhalation and skin exposures to aniline occur, only limited biomonitoring data for aniline have been reported for either occupational settings (rubber-chemical manufacturing workers and aniline-production workers) or the general population (mostly analysed separately for smokers and non-smokers).

5.2 Cancer in humans

The body of research available related to cancers in humans was sparse and was limited to four cohort studies in aromatic amine dye- and rubber-chemical manufacturing plants, four population-based case–control studies, and several case reports and case series of bladder cancer in occupational settings. All cohort studies evaluated bladder cancer outcomes, but only two were considered to be potentially informative for the evaluation. Both studies were of good quality, but for one it was not possible to separate any aniline-specific effect from the effect of other co-exposures such as *ortho*-toluidine. The other cohort study evaluated the association

between aniline exposure and bladder cancer while controlling for concurrent exposures; however, the small sample size and strong correlations between the exposures resulted in statistically unstable estimates of the effect for aniline and confounding from co-exposures to other agents with *sufficient* and *limited* evidence of bladder carcinogenicity in humans (*ortho*-toluidine and 2-mercaptobenzothiazole, respectively) could not be ruled out. One case–control study on bladder cancer did not provide information on case or control ascertainment, aniline exposure, or occupational co-exposures. Among the case reports and case series, confounding from co-exposures to occupational bladder carcinogens and/or tobacco smoking could not be ruled out. Overall, the available studies do not permit a conclusion to be drawn about the presence of a causal association between aniline exposure and bladder cancer.

There was no convincing or consistent evidence reported for any other cancer in humans.

5.3 Cancer in experimental animals

Aniline hydrochloride caused an increase in the incidence of malignant neoplasms in two independent studies in one species.

In one independent study in male and female Fischer 344 rats, aniline hydrochloride administered orally (in feed) caused an increase in the incidence of fibrosarcoma or sarcoma (not otherwise specified, NOS) (combined) of the spleen; haemangiosarcoma of the spleen; fibrosarcoma or sarcoma NOS (combined) of multiple organs other than spleen within the body cavities; fibrosarcoma or sarcoma NOS (combined) of the spleen or of multiple organs other than spleen within the body cavities (combined); and haemangiosarcoma of the spleen or of multiple organs other than spleen within the body cavities (combined) in male rats. A positive trend in the incidence of benign or malignant (combined) pheochromocytoma of the adrenal gland was also observed in male rats receiving aniline hydrochloride. A positive trend in the incidence of fibrosarcoma or sarcoma NOS (combined) of the spleen or of multiple organs other than spleen within the body cavities (combined) was observed in female rats receiving aniline hydrochloride.

In another independent study in male Fischer 344 rats, aniline hydrochloride administered orally (in feed) caused an increase in the incidence of stromal sarcoma of the spleen, haemangiosarcoma of the spleen, and mesothelioma of the tunica vaginalis of the testis.

5.4 Mechanistic evidence

Regarding the absorption, distribution, metabolism, and excretion of aniline, data are available from studies in humans, and from experimental systems. In humans, aniline is readily absorbed by the dermal, oral and inhalation routes. Studies of aniline-induced methaemoglobinaemia, dating back to the 1800s, indicate skin absorption. Facile oral absorption of aniline was demonstrated in a recent thorough study of aniline metabolism in human subjects. Cytochrome P450 (CYP)-dependent hydroxylation to *para*-aminophenol [4-aminophenol] followed by N-acetylation (or the reverse sequence) converts aniline into *N*-acetyl-*para*-aminophenol (the chemical name for the analgesic drug paracetamol, also known as acetaminophen). *N*-Acetyl-*para*-aminophenol, acetanilide, and the parent compound were detected in the plasma and urine. More than half of an orally administered dose of aniline is excreted in the urine as *N*-acetyl-*para*-aminophenol and its conjugates (glucuronide, sulfate, mercapturic acid). The elimination half-lives of these urinary metabolites range from less than 1 hour to a few hours. *N*-Acetyl-*para*-aminophenol is found in urine samples from the general population, from individuals exposed to aniline in an occupational setting, and from paracetamol users. Multiple

studies of aniline metabolism in experimental animals, including rat, mouse, rabbit, guinea-pig, gerbil, hamster, cat, dog, pig, and sheep, are consistent with the evidence in humans.

There is consistent and coherent evidence in experimental systems that aniline exhibits multiple key characteristics of carcinogens. Aniline is metabolically activated to electrophiles. In exposed humans, aniline forms haemoglobin adducts, which are commonly used as biomarkers of aniline exposure. No data on DNA adducts in humans were available. In experimental systems, binding of aniline to DNA has been observed, including in the liver, spleen, and kidney of rats treated with aniline. Although not directly demonstrated at every step, there is a plausible pathway for formation of aniline–DNA adducts that parallels an established paradigm for aromatic amines. This bioactivation pathway begins with CYP-catalysed N-hydroxylation to phenylhydroxylamine; further activation by O-acetylation; and spontaneous heterolysis of the *N*-acetoxy metabolite to give a DNA-reactive electrophilic nitrenium ion. The presence of this pathway is supported by evidence showing N-hydroxylation of aniline to phenylhydroxylamine in dogs and rats; in liver microsomal preparations from rat, mouse, and rabbit; and in the isolated perfused rat liver, when the perfusion fluid contained human erythrocytes, to trap the oxidized metabolites by binding to haemoglobin. The *N*-acetoxy derivative of aniline has been prepared synthetically; it reacts with DNA to give a guanine C8 adduct as well as guanine *N*7 and N^2, and adenine C2, C8, *N*7, and N^6 adducts.

Aniline is also genotoxic. No data were available in exposed humans, and only one study, with positive results for sister-chromatid exchange, was conducted in human primary cells. DNA damage was seen in human cell lines in vitro, and in both rats and mice. Aniline was consistently clastogenic, with dose-dependent increases in the frequency of micronucleus formation in orally exposed rats and mice, and the induction of chromosomal aberrations, micronucleus formation, and sister-chromatid exchange in mammalian cells. Aniline was mutagenic in the mouse lymphoma L5178Y cell $Tk^{+/-}$ assay and in the Chinese hamster V79 *Hprt* assay. In experiments with bacteria, aniline neither formed DNA adducts nor caused DNA damage, and it did not cause gene mutations in standard *Salmonella typhimurium* or *Escherichia coli* assays.

In addition, aniline induces oxidative stress. Aniline induces 8-hydroxy-2′-deoxyguanosine and upregulates DNA base-excision repair proteins in rats. In rodent studies in vivo and in vitro, aniline exposure variously increased reactive oxygen and reactive nitrogen species, depleted glutathione, and increased malondialdehyde and protein carbonyl contents.

Aniline alters cell proliferation, cell death, or nutrient supply. Hyperplasia of the spleen was seen in male and female Fischer 344 rats after chronic exposure and in male Sprague-Dawley rats after short-term exposure. After short-term exposure, aniline increased markers of cell proliferation, including proliferating cell nuclear antigen and nuclear Ki67 protein in rats.

Aniline or its hydrochloride form was mostly without effects in the assay battery of the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes.

Overall, the evidence is consistent and coherent that aniline belongs, on the basis of mechanistic considerations, to a class of aromatic amines. Members of this class, including 4-aminobiphenyl (*para*-phenylaniline), 2-naphthylamine, and *ortho*-toluidine (*ortho*-methylaniline) have been classified as *carcinogenic to humans* (IARC Group 1) by the *IARC Monographs* programme. Aniline is structurally similar to these aromatic amines. It also bears similarity with respect to the mechanism of bioactivation to electrophiles, its genotoxicity, and the target organs of carcinogenicity in chronic animal bioassays. For instance, both aniline and *ortho*-toluidine cause malignant

tumours of the spleen and mesothelioma of the tunica vaginalis of the testis when administered orally to male Fischer 344 rats. Therefore, these mechanistic considerations go beyond chemical structural similarity to encompass biological and biochemical similarities relevant to common key characteristics of carcinogens.

6. Evaluation and Rationale

6.1 Cancer in humans

There is *inadequate evidence* in humans regarding the carcinogenicity of aniline and aniline hydrochloride.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of aniline hydrochloride.

6.3 Mechanistic evidence

There is *strong evidence* that aniline belongs, based on mechanistic considerations, to a class of aromatic amines for which several members have been classified as carcinogenic to humans. There is also *strong evidence* that aniline exhibits key characteristics of carcinogens in experimental systems.

6.4 Overall evaluation

Aniline and aniline hydrochloride are *probably carcinogenic to humans (Group 2A).*

6.5 Rationale

The *Group 2A* evaluation is based on *strong* mechanistic evidence that aniline, on the basis of mechanistic considerations, belongs to a class of aromatic amines for which several members have been classified as carcinogenic to humans. Aniline is concordant with other agents in this class with respect to its bioactivation mechanism to electrophiles, genotoxicity, and target organs of carcinogenicity in chronic animal bioassays.

There was also *sufficient evidence* of carcinogenicity in experimental animals, on the basis of increased incidence of malignant neoplasms in two independent studies in one species. In addition, there is *strong evidence* that aniline exhibits key characteristics of carcinogens in experimental systems. Aniline is metabolically activated to electrophiles, it is genotoxic, it induces oxidative stress, and it alters cell proliferation, cell death, or nutrient supply.

The evidence for cancer in humans was *inadequate* because the effects of aniline in workers could not be distinguished from those of co-exposures to other occupational bladder carcinogens in the two available high-quality cohort studies.

Aniline hydrochloride exists in equilibrium with aniline; therefore, the classification of carcinogenic hazard applies to both aniline and its hydrochloride form.

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