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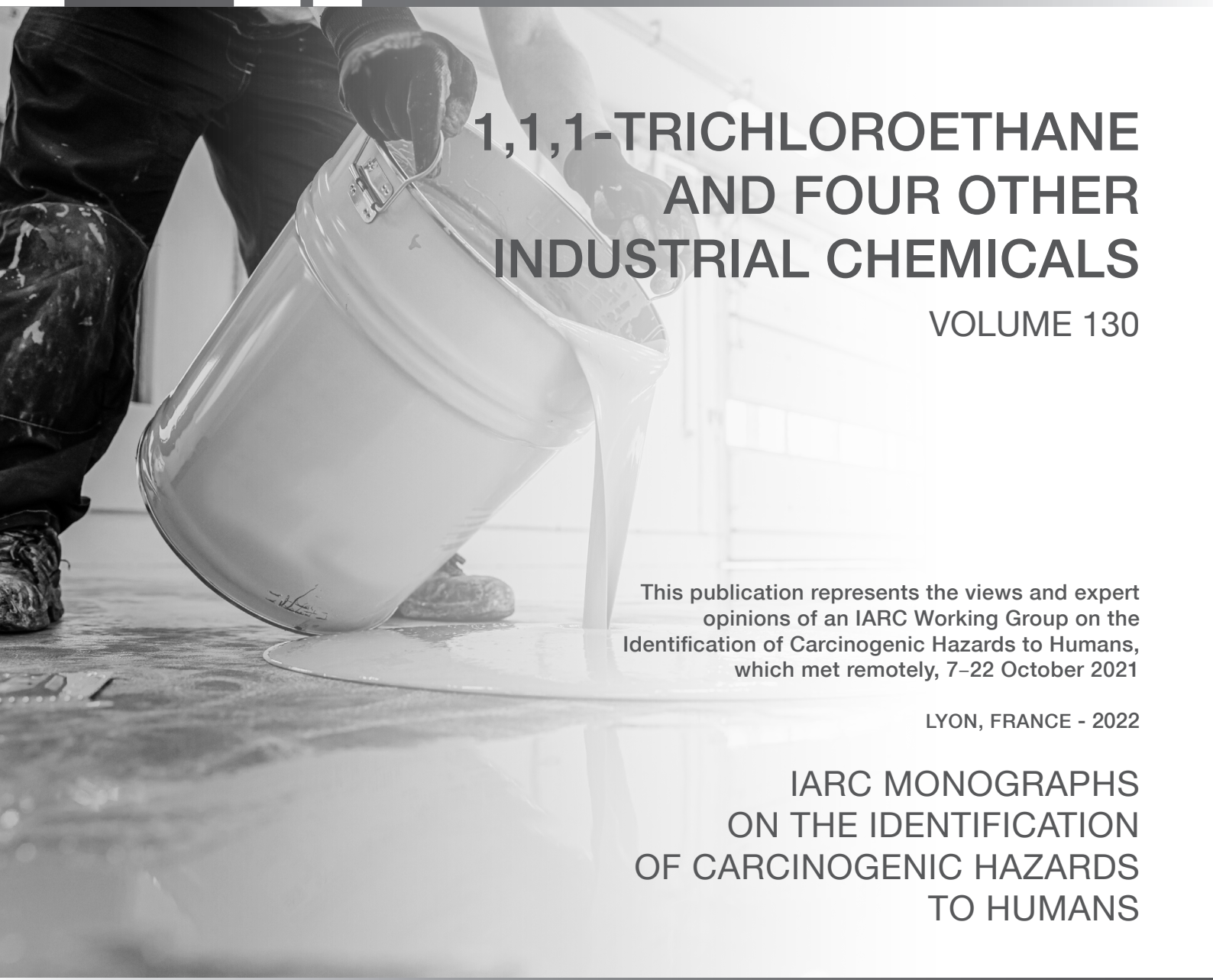

1,1,1-TRICHLOROETHANE AND FOUR OTHER INDUSTRIAL CHEMICALS

VOLUME 130

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IARC MONOGRAPHS ON THE IDENTIFICATION OF CARCINOGENIC HAZARDS TO HUMANS

International Agency for Research on Cancer

World Health Organization

DIPHENYLAMINE

1. Exposure Characterization

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 122-39-4

EC/List No.: 204-539-4

Chem. Abstr. Serv. name: piphenylamine

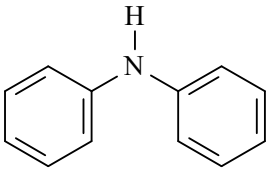
IUPAC systematic name: *N*-phenylaniline

Synonyms: *N*-phenylaniline, diphenylazane, *N*-phenylbenzenamine, anilinobenzene, (phenylamino) benzene, *N*,*N*-diphenylamine, and other depositor-supplied synonyms and acronyms (NCBI, 2021).

1.1.2 Structural and molecular information

Relative molecular mass: 169.22 (NCBI, 2021)

Chemical structure:



Molecular formula: $C_{12}H_{11}N$

1.1.3 Chemical and physical properties

Description: colourless, tan, amber, or brown crystalline solid, with a pleasant, floral odour; sinks in water (NCBI, 2021); forms lamellar crystals (IFA, 2021)

Melting point: 53 °C (IFA, 2021), 54–55 °C (NCBI, 2021)

Boiling point: 302 °C (IFA, 2021)

Density: 1.16 g/cm^3 at 20 °C (IFA, 2021)

Relative vapour density: 5.82 (air = 1) (NCBI, 2021)

Flash point: 153 °C (IFA, 2021)

Auto-ignition temperature: 630 °C (IFA, 2021), 634–635 °C (NCBI, 2021)

Vapour pressure: 6.70×10^{-4} hPa at 25 °C (NCBI, 2021)

Viscosity: 262 cP at 20 °C (NCBI, 2021)

Solubility: practically insoluble in water (50 mg/L at 25 °C) (IFA, 2021); soluble in oxygenated and aromatic solvents, i.e. very soluble in ethanol, propyl alcohol, acetone, benzene, carbon tetrachloride, pyridine, and ethyl acetate; soluble in ether, glacial acetic acid; slightly soluble in chloroform (NCBI, 2021)

Octanol/water partition coefficient (P): log K_{ow} = 3.50 (IFA, 2021, NCBI, 2021)

Odour threshold: 0.05 mg/L (NCBI, 2021)

Dissociation constants (of the conjugated acid BH^+): pK_a = 0.28 at 24 °C (Sangster, 1989)

Reactivity: risk of explosion in contact with oxidizing agents; the substance can react dangerously with strong acids and trichloromelamine; when heated to decomposition, the substance emits fumes of nitrogen oxides; dust explosion possible if in powder or dust form and mixed with air (IFA, 2021; NCBI, 2021).

1.1.4 Impurities

Several primary and secondary amines, including the carcinogen 4-aminobiphenyl (*carcinogenic to humans*, IARC Group 1), may be present as impurities in commercial diphenylamine (Babish et al., 1983). For example, 4-aminobiphenyl was quantified at up to 94 ppm in four out of six commercial brands of diphenylamine (Safe et al., 1977). In addition, 2-aminobiphenyl and *ortho*-cyclohexylaniline were quantified at up to 32 and 93 ppm in several of the six brands, respectively. A single brand (of the six) also contained *para*-cyclohexylaniline as an impurity [no concentration provided]. [The Working Group noted that, on the basis of the age of the studies, the impurities noted above do not necessarily reflect those of current commercial batches.]

1.2 Production and use

1.2.1 Production process

Diphenylamine is an aniline dimer made by heating the parent monomer in the presence of aniline hydrochloride or in the presence of phenol with an acid catalyst at high temperatures (NCBI, 2021).

1.2.2 Production volume

According to Drzyzga (2003), the global annual volume of production of diphenylamine in the 1980s was 40 000 tonnes.

In the European Union (EU) market in 1992–1993, the approximate total volume of production of diphenylamine was 10 000 tonnes (i.e. ~9000 tonnes of production and ~1000 tonnes of imports) (European Commission, 2008). According to the website of the European Chemicals Agency (ECHA) in 2021, diphenylamine is currently manufactured and/or imported in Europe at a volume of ≥ 10 to < 100 tonnes per year (ECHA, 2021). Many companies no longer produce diphenylamine; only four large companies manufacture diphenylamine in North America, Asia, and Europe (Industry Research, 2020). In 2008, diphenylamine was only produced by two companies in the EU and was mostly processed as a chemical intermediate (approximately 97.5%) (European Commission, 2008). In 2020, worldwide sales of diphenylamine reached 1.02 million tonnes (Chemanalyst, 2021). Asia and Pacific regions represented the largest market share (55.2%) in 2018 owing to industrial development and automobile manufacturing (Industry Research, 2020).

During 2000–2019, the annual use [or production and use] of diphenylamine in the Nordic countries (Denmark, Finland, Norway, Sweden) varied between 11 and 1759 tonnes (mean, 172 tonnes; median, 26 tonnes) (SPIN, 2021). [The values were calculated by the Working Group.]

In Chile, sales of diphenylamine for agricultural use reached 2496 kg and/or 2496 L in 2012 (Servicio Agrícola y Ganadero, 2012). According to this report, the sale was made only in the Maule Region (Servicio Agrícola y Ganadero, 2012), the leading apple-producing region of Chile (ODEPA, 2013).

1.2.3 Uses

(a) Main uses

Diphenylamine is predominantly used in lubricants and greases, hydraulic fluids, metal-working fluids, dyes, and textile treatment products including leather and fur (ECHA, 2021). Diphenylamine is also used as an intermediate and, considering the information reported by industries in the EU, the most common uses were in the production of: antioxidants widely used in the rubber industry and for lubricants; antiozonants used in the rubber industry; and phenothiazine used as stabilizer for plastics and for the preparation of several dyestuffs (Drzyzga, 2003; European Commission, 2008). In 2016, the market share for diphenylamine-derived lubricant and rubber antioxidants combined was 66% (Industry Research, 2020).

(b) Minor uses

Minor uses for diphenylamine include its function as a stabilizer for single- or multi-base propellants, nitrocellulose-containing gunpowder, pharmaceuticals, and perfume oils (content, 0.1%) (Drzyzga, 1999, cited in European Commission, 2008; NCBI, 2021). Depending on its current or pending registration status, diphenylamine can be also used as a scald-suppression agent on fruits in storage in certain geographical regions, including the Americas (Johnson et al., 1997; Muñoz-Quezada et al., 2014), but has not been approved for this or similar uses since 2012 in the EU (European Commission, 2012; Dias et al., 2020).

(c) Former uses

In the EU, diphenylamine was used until 2003 as a colouring agent in low-taxed fuels and heating oils to distinguish them from other fuels (European Commission, 2008). This use was voided in 2001 for gas oils and kerosene (Commission Decision 2001/574/EC; European Commission, 2001). In the past, diphenylamine was also reportedly used in veterinary medicine as an additive in anti-screw worm mixtures and as an active ingredient in biocidal products (Drzyzga, 1999, cited in European Commission, 2008). However, more recent information indicates that diphenylamine is no longer used in veterinary products in the EU and United Kingdom (European Commission, 2008). Also in the EU, commercial use as a stabilizer for carbon tetrachloride is now no longer of importance because the production and use of carbon tetrachloride have been strongly regulated since 1994 (Council Regulation (EC) 3093/94) (European Commission, 2008).

1.3 Detection and quantification

1.3.1 Air

Diphenylamine in the air can be collected on a fibreglass filter (OSHA, 1989). The filter is then extracted with methyl alcohol, and diphenylamine is detected by high-performance liquid chromatography with an ultraviolet detector.

Various solid sorbents (Amberlite XAD-2, Amberlite XAD-4, Supelpak 2, Florisil, and the sorbent bound with octadecyl silica, C-18) have been shown to efficiently retain diphenylamine from the air under different sampling conditions (Gagoulia et al., 2011). Diphenylamine was recovered using low volumes of ethyl acetate or acetone and detected with gas chromatography.

Solid-phase microextraction (SPME) has been used for analyses of diphenylamine in storage environments (Song et al., 2014). Samples were taken from the air in various rooms using an SPME fibre and a portable pump with a flow rate of 1 L/minute for 30 minutes. Detection and identification of diphenylamine were performed using gas chromatography with mass spectroscopy (GC-MS).

1.3.2 Water

Diphenylamine can be extracted from water using methylene chloride, with > 90% recovery by continuous extraction techniques (US EPA, 2000). Detection analysis is performed by gas chromatography-atomic fluorescence.

Seventeen components of three diphenylamine derivatives can be analysed by Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) and gas chromatography-tandem mass spectrometry, and quantified by flame ionization detection (Zhang et al., 2020).

1.3.3 Soil, sediment, and consumer products

Several methods and techniques are used to evaluate levels of diphenylamine.

(a) Chromatography with nitrogen phosphorous

Diphenylamine is extracted with acetone, and the extraction is followed by liquid-liquid partitioning. Subsequent detection can be performed by gas chromatography with nitrogen-phosphorous detection (GC-NPD) (Garrido et al., 1998).

(b) Gas chromatography-mass spectrometry

Residues are extracted with acetonitrile and transferred to acetone. Then GC-MS is applied in the selective-ion monitoring mode (Yu et al., 1997). Residues can also be extracted with dichloromethane. The GC-MS is applied on the residue dissolved in acetone (Robatscher et al., 2012).

(c) Liquid chromatography with electrochemical detection

Residues are extracted with dichloromethane, dissolved in methanol, filtered, and then injected into the chromatograph (Olek, 1988).

(d) Ultraviolet and visible spectrophotometric methods

The diphenylamine residue is dissolved in methanol, filtered, then injected into the chromatograph, using gradient reversed-phase liquid chromatography with ultraviolet-visible absorption and atmospheric pressure chemical ionization detection (LC-UV-vis-APCI-MS) (Rudell et al., 2005).

(e) Fluorimetric methods

To extract the diphenylamine residue, a mobile phase consisting of methanol/water and fluorescence detection is used, followed by reversed-phase high-performance liquid chromatographic (RP-HPLC) method (Saad et al., 2004).

Another study evaluated the potential of combining normal, synchronous, and derived fluorimetry with multivariate methods for the quantitative analysis of diphenylamine in fruit samples to validate a rapid, specific, and sensitive method to determine diphenylamine in food products (Farokhcheh & Alizadeh, 2013).

In whole milk (FAO, 2004), diphenylamine is extracted with acetonitrile divided with hexane to remove fat. The extract is evaporated, re-dissolved in hexane, and analysed by GC with mass-selective detection (GC-MSD). The method for animal tissues is similar, except that after evaporation, the residue is re-dissolved in a small volume of acetonitrile, diluted with water, and partitioned in hexane. The hexane solution is then analysed by GC-MSD (FAO, 2004).

1.3.4 Human biomarkers

Diphenylamine is transformed into hydroxylated metabolites and is rapidly excreted; therefore, it does not bioaccumulate (Alexander et al., 1965; European Commission, 2008).

There are no validated biomarkers of diphenylamine exposure in humans. [The Working Group noted the metabolites 4-hydroxydiphenylamine

and 4,4′-dihydroxydiphenylamine, described in Section 4.1, as possible targets for biomarker development.]

1.4 Occurrence and exposure

1.4.1 Occurrence in the environment, food, and consumer products

Table 1.1 presents a summary of the studies that evaluated the occurrence of diphenylamine in the environment, and in food commodities and consumer products.

Diphenylamine released as a result of use of weapons and ammunition in military bases causes contamination of soil and water (Drzyzga, 2003). A series of studies found high concentrations of diphenylamine and its nitrate derivatives in groundwater at these military bases.

In a study conducted in the province of Jaén, Spain (Robles-Molina et al., 2014), 83 surface-water samples were collected over 20 months in 3 rivers, 5 reservoirs, and 11 wetlands in order to monitor a group of 373 organic pollutants, including diphenylamine, belonging to different compound categories. Diphenylamine was found in 72.7% of the river samples (average concentration, 148.9 ng/L; C_{max}, 220.4 ng/L), in 20% of the studied wetlands (average, 178.9 ng/L; C_{max}, 195.5 ng/L), and in all reservoirs studied.

Diphenylamine is one of the most prevalent compounds found in air and sediment samples from the USA (Chicago) and in electronic-waste ("e-waste") and residential-dust samples from the USA and Canada (Wu et al., 2020).

The same study (Wu et al., 2020) evaluated phenolic and amino antioxidants and ultraviolet filters. The concentrations of 47 such compounds and their transformation products were measured in 20 samples of atmospheric particles collected in the USA (Chicago), 21 e-waste dust samples from Canada, 32 samples of residential dust from Canada and the USA, and 10 sediment samples collected from the Chicago Sanitary and Ship Canal, USA. Diphenylamine was one of the most prevalent compounds of those measured. Total concentrations of diphenylamine were significantly higher in the e-waste dust than in the Canadian residential dust. In addition, diphenylamine was the predominant amino antioxidant found in the US residential dust, but comprised only 4.4% of amino antioxidants in Canadian residential dust, suggesting regional variations in diphenylamine use. The sediment samples showed relatively high levels of other substances measured.

In China, Liu et al. (2019) evaluated the presence of two types of secondary aromatic amines in dust samples from rubber surfaces of outdoor playgrounds and from residential homes, and found diphenylamine in all the playground dust samples at concentrations of 2.33–32.6 ng/g, with a geometric mean of 8.02 ng/g. In indoor dust from residential homes, diphenylamine concentrations ranged from 8.71 to 129 ng/g, with a geometric mean of 25.5 ng/g.

The US EPA (1998) has assessed the dietary risk posed by diphenylamine. The anticipated residue concentration (ARC) for the overall population of the USA represents 2.27% of the reference dose (RfD). Non-nursing infants aged < 1 year had an ARC of 20.8% of the RfD, which was considered an acceptable dietary exposure risk.

Robatscher et al. (2012) evaluated the potential of fruit storage facilities to contaminate apples that had not been treated with diphenylamine. Diphenylamine (in quantities up to 917 g) was found on the walls of a storage room and was associated with cross-contamination of the untreated apples stored within, even years after the last diphenylamine treatment. Of 689 apple samples, 481 samples contained diphenylamine at concentrations ranging from 0.41 to 2 mg/kg, which exceeds the current EU maximum residue limit (MRL) of 0.05 mg/kg (Reg. (EU) 2018/1515) (European Commission, 2018).

Table 1.1 Concentrations of diphenylamine in the environment, and in food and consumer products

Occurrence context	Monitoring method	Analytical technique	No. of samples tested (*n*)	Concentration of diphenylamine			Reference
				Detection frequency (%)	Median (IQR)	Other measure	
During 20 months (April 2009 to November 2010), 83 surface-water samples from 19 sampling sites were collected in the province of Jaén, Spain	Representative water samples from 3 rivers, 5 reservoirs, and 11 wetlands were collected in amber glass bottles with Teflon caps (1 L)	LC-TOFMS for the analysis of 340 compounds, and GC-MS/MS for the analysis of 63 organic contaminants (30 of these compounds were also analysed by LC-TOFMS)	Guadalquivir river and tributary rivers (Guadalimar and Jandulilla river), *n* = 11	72.7%	NR	Average, 148.9 ng/L (C_{max}, 220.4 ng/L)	Robles-Molina et al. (2014)
			10 wetlands, *n* = 11	20%	NR	Average, 178.9 ng/L (C_{max}, 195.5 ng/L)	
			Giribaile reservoirs, *n* = 9	66.7%		Average, 113.0 ng/L (C_{max}, 170.2 ng/L)	
			Quiebrajano reservoirs, *n* = 5	40%	NR	Average, 57.8 ng/L (C_{max}, 64.2 ng/L)	
			Rublar reservoirs, *n* = 10	30%	NR	Average, 128.7 ng/L (C_{max}, 136.6 ng/L)	
			La Fernandina reservoirs, *n* = 11	45.5%	NR	Average, 141.1 ng/L (C_{max}, 203.2 ng/L)	
			Guadalen reservoirs, *n* = 11	36.4%	NR	Average, 125.7 ng/L (C_{max}, 181.2 ng/L)	

Table 1.1 (continued)

Occurrence context	Monitoring method	Analytical technique	No. of samples tested (*n*)	Concentration of diphenylamine			Reference
				Detection frequency (%)	Median (IQR)	Other measure	
E-waste dismantling facility, Ontario, Canada, 2016; houses in Ontario, Canada, 2015; houses in Indiana, USA, in 2013; Chicago Sanitary and Ship Canal, USA, 2013; atmospheric particles in Chicago, USA, September 2018 to April 2019	E-waste dust samples were collected from the floor, work benches, and sorting bins	In each case, half of the sample extract was diluted with hexane and half with methanol. Half of the samples were then analysed by electron impact GC-MS; the other half were analysed by positive or negative ion LC-MS/MS	E-waste dust, *n* = 21	100%	199 mg/g (81.8–439 mg/g)	NR	Wu et al. (2020)
	Residential floor dust samples were collected using a small vacuum cleaner fitted with a precleaned polyester sock inserted at the end of the hose attachment		Residential floor (Ontario), *n* = 20	25%	5.73 ng/g (< LOD to 10.6 ng/g)	NR	
			Residential floor (Indiana), *n* = 20	100%	13.4 ng/g (5.70–53.6 ng/g)	NR	
	Superficial sediment samples were collected from Chicago Sanitary and Ship Canal		Superficial sediment samples, *n* = 10	80%	7.70 ng/g (< LOD to 505 ng/g)	NR	
	Atmospheric particles were collected on quartz fibre filters using a high-volume air sampler (815 m^3 of air was sampled for 24 h every 12 days)		Samples of atmospheric particles, *n* = 20	85%	0.85 pg/m^3 (< LOD to 3.08 pg/m^3)	NR	

Table 1.1 (continued)

Occurrence context	Monitoring method	Analytical technique	No. of samples tested (*n*)	Concentration of diphenylamine			Reference
				Detection frequency (%)	Median (IQR)	Other measure	
Dust samples collected from outdoor rubber playgrounds and residential houses in March 2016, in Beijing, China	Using a wool paint brush, each dust sample was swept onto aluminium foil from the rubber ground, sealed in polyethylene zip bag	UHPLC interfaced with an API 5500 triple-quadrupole mass spectrometer	Dust from outdoor rubber playgrounds, *n* = 30	100%	NR	Geometric mean, 8.02 ng/g (range, 2.33–32.6 ng/g)	Liu et al. (2019)
	In the living room, 0.5 g of indoor dust was collected from the surfaces of upholstery, electronic fans, furniture, and windowsills (sampling procedure similar to above)		Dust from indoor residential houses, *n* = 30	100%	NR	Geometric mean, 25.5 ng/g (range, 8.71–129 ng/g)	

Table 1.1 (continued)

Occurrence context	Monitoring method	Analytical technique	No. of samples tested (*n*)	Concentration of diphenylamine			Reference
				Detection frequency (%)	Median (IQR)	Other measure	
DPA presence in fruit storage facilities	DPA residues in commercially stored apples	GC analysis was performed on an Agilent 6890 Series GC system equipped with an HP 5973 mass selective detector	Apple samples, *n* = 689	85% (587 samples with some level of DPA)	NR	106 samples containing residues at 0.01–0.40 mg/kg 481 samples containing residues at 0.41–2.00 mg/kg 102 samples were < LOD Untreated apples stored for several months in eight different storage rooms that had been used previously for DPA treatment, 0.01–0.07 mg/kg	Robatscher et al. (2012)
	Activated carbon was removed from CO_2 scrubbers		Activated carbon, 2 g	0	NR	< LOD	

Table 1.1 (continued)

Occurrence context	Monitoring method	Analytical technique	No. of samples tested (*n*)	Concentration of diphenylamine			Reference
				Detection frequency (%)	Median (IQR)	Other measure	
DPA presence in fruit storage facilities (cont.)	DPA extraction from storage cell wall paint		12 storage rooms (a sample of each of approximately 2 cm × 2 cm cell wall paint)	75% (8 samples with some level of DPA; 4 rooms < LOD)	NR	DPA amounts exceeding 1000 mg/m^2 in wall paint from storage rooms that had been nebulized with DPA for 3 years Walls of storage rooms in which drenched apples had been stored were contaminated with DPA at 150–300 mg/m^2 Storage room that had never been used for storage of DPA-treated apples yielded DPA residues of 21.0 mg/m^2	Robatscher et al. (2012 (cont.)
	Silica cartridges were installed on the air outlet of a pump and placed into a contaminated storage cell		Three consecutive silica cartridges	NR	NR	DPA measured in the air of storage rooms ranged from 0.9 to 7.3 µg/m^3 and showed strong temperature dependence, with the highest values measured at 20 °C and the lowest at 1 °C	
Grey partridge (*Perdix perdrix*) eggs, collected on 12 intensively cultivated areas of farmland in France, 2010–2011	Eggs from hatched, destroyed, and deserted clutches of radio-tagged grey partridge females; intact failed eggs were opened in the laboratory to examine their contents, including developing embryos	GC-MS/MS and LC-MS/MS screening and measuring about 500 compounds	139 eggs of 52 clutches	NA	NA	Fate: hatching, dead embryo, stage 11 days, 0.01 mg/kg Fate: hatching, infertile, < 0.01 mg/kg Fate: failure, dead embryo, stage 20 days, 0.019 mg/kg	Bro et al. (2016)

Table 1.1 (continued)

Occurrence context	Monitoring method	Analytical technique	No. of samples tested (*n*)	Concentration of diphenylamine			Reference
				Detection frequency (%)	Median (IQR)	Other measure	
Baby food from local markets, Spain, 2012	Baby food samples were purchased from different local markets	LC-MS and LC-MS/MS mode experiments, obtaining a reduction of these effects when working in LC-MS/MS	Fruit-based baby food, *n* = 25	NA	NA	< LOD (full scan, 5.0 μg/kg; LC-MS/MS, 3.0 μg/kg)	Gilbert-López et al. (2012)
Meals of urban and rural schools, Maule Region, Chile, 2010–2011	Presence of pesticide residues (including DPA) in apples	GC-MS	190 school children; 14 schools considered, DPA residues found in 9	Summer, 72% of children consumed fruit treated with DPA Autumn, 50% of children consumed fruit treated with DPA	NR	Summer (mg/kg apple): School (S): S2 = 0.26; S3 = 0.23; S4 = 0.77; S5 = 0.02; S6 = 0.01; S7 = 0.65; S8 = 3.89; S9 = 1.11; S10 = 2.01 Autumn (mg/kg apple): School: S1 = 0.12; S3 = 0.10; S4 = 0.53; S5 = 0.01; S9 = 0.02; S12 = 0.68; S14 = 0.45	Muñoz-Quezada et al. (2014)
Meals prepared and supplied by company cafeterias and by schools, hospitals, and rest homes; samples collected February–December 2005, Italy	Presence of pesticide residues (including DPA) in meals	MS	50 complete meals	[12%]	[1.726 μg]	Quantity of DPA in fruit: Range: 0.0484–132.5 μg per fruit (*n* = 6)	Lorenzin (2007)

DPA, diphenylamine; e-waste, electronic waste; GC, gas chromatography; GC-MS, gas chromatography with mass spectrometry; GC-MS/MS, gas chromatography triple – quadrupole mass spectrometry; HPLC, high-performance liquid chromatography; IQR, interquartile range; LC-MS, liquid chromatography with mass spectrometry; LC-MS/MS, liquid chromatography with tandem mass spectrometry; LC-TOFMS, liquid chromatography electrospray time-of flight mass spectrometry; LOD, limit of detection; MS, mass spectrometry; NA, not applicable; NR, not reported; UHPLC, ultrahigh-performance liquid chromatography.

In a study by Bro et al. (2016) in France, analysis was carried out on a total of 139 eggs from 52 grey partridge clutches collected from 12 intensively cultivated areas of farmland. A total of 15 different compounds, including diphenylamine, were detected in 24 clutches. Diphenylamine concentrations ranged between < 0.01 and 0.019 mg/kg.

In a study by Gilbert-López et al. (2012) in Spain, liquid chromatography-electrospray ionization-ion trap tandem mass spectrometry was used to quantify multiple residues of 10 fungicides, including diphenylamine, in fruit-based baby foods. The limit of detection was 3.0 µg/kg for diphenylamine. None of the analysed samples exceeded the EU standard for infant feeding (EU No. 578/2012) (European Commission, 2018).

In a study by Muñoz-Quezada et al. (2014) in Talca Province, Chile, pesticide concentrations were measured in samples collected from school meals in 14 urban and rural schools in summer and autumn. Families were surveyed about their children's vegetable consumption in school and at home, the use of pesticides in the home, and other sociodemographic variables. Vegetables contained the highest pesticide concentration at both time points, both in urban and rural schools. In summer, diphenylamine residues were found in samples from nine schools. During the 4 days before sample collection in the summer, 72% (n = 136) of the schoolchildren had consumed fruits (apples) that had been treated with diphenylamine, and 65% had consumed fruit (oranges and apples) that had been treated with more than one type of pesticide. In autumn, 70.3% (n = 128) of the children consumed a vegetable or a fruit that had been treated with diphenylamine. The risk of consuming vegetables containing diphenylamine was 2.9 times higher in urban schoolchildren than in rural schoolchildren.

The Italian Ready-Meal Residue Project, promoted by the Pesticides Working Group of Italian environmental agencies (Lorenzin, 2007), evaluated the number of pesticides in pre-prepared meals (first course, side dish, fruit, bread, and wine). In 2005, 50 complete meals were analysed. The results showed residues of pesticides in 39 meals, with an average number of 2.4 pesticides, and a maximum of 10, in each meal. Diphenylamine was one of the most common pesticides found in fruit (6 meals).

1.4.2 Occupational exposure

The most relevant routes of occupational exposure to diphenylamine are respiratory (inhalation) and dermal (skin contact) (European Commission, 2008).

In a study by Gagoulia et al. (2011), a simple method was developed to determine and monitor diphenylamine in the indoor air of two apple-storage plants from September 2006 to March 2007 in Greece. Diphenylamine was detected in indoor air at concentrations ranging between 1.6 and 580 µg/m^3 (Table 1.2). When evaluating the presence of diphenylamine in the air during a typical working day after diphenylamine application in both apple-storage plants, the highest concentrations of diphenylamine residues (483.5 µg/m^3 and 580 µg/m^3) were recorded in the afternoon. Lower concentrations of diphenylamine (3.7–16.8 µg/m^3) were detected in the air from other areas of the building, such as office areas and the sorting line, probably because of the greater distance between these areas and the diphenylamine application area. Indoor air concentrations of diphenylamine 3–4 months after diphenylamine application ranged from 1.6 to 6.9 µg/m^3; these levels were attributed to the desorption of diphenylamine from the building walls.

In a review of occupational exposure by inhalation (European Commission, 2008), data were considered from a study carried out in two rubber-antioxidant factories in 1989–1999 (Table 1.2). In one of these factories, during the bagging of diphenylamine flakes, values of up to 161.2 mg/m^3 were reported, with a measurement

Table 1.2 Occupational exposure to diphenylamine in workplace air

Exposure context	Monitoring method	Analytical technique	No. of samples tested	Concentration of diphenylamine		Reference
				Median	Other measure	
Workers' exposure in two apple-storage buildings located in two different agricultural areas in Greece, 2006–2007	Air sampling and analytical methodology were applied in the field to measure DPA levels in the air	GC-NPD analysis GC-MS analysis	2 apple storage plants; 33 air samples	NR	Range, 1.6–580 μg/m^3	Gagoulia et al. (2011)
Workers' exposure in two rubber-antioxidant factories, 1989–1999	Air monitoring (measurement duration, ≤ 420 minutes)	NR	122	0.92 mg/m^3	Range, 0.1–162 mg/m^3 90th percentile, 0.3 and 1.05 mg/m^3 95th percentile, 1.65 mg/m^3	European Commission (2008)
Workers' exposure in rubber-manufacturing industry, 1990s	Air samples taken over a 3 h period in two stable positions near the mixing and personal air samples were taken over 2 h period during a normal work day from five workers involved in different operations (mixing, weighing, calendering, compounding and extruding)	GC-MS	7	NR	DPA detected in the stationary air samples collected near the mixing and calendering areas	Fracasso et al. (1999)

DPA, diphenylamine; GC-MS, gas chromatography with mass spectrometry; GC-NPD, gas chromatography-nitrogen phosphorus detector; NR, not reported.

duration of up to 420 minutes, and a 95th percentile for the collective measurement of 1.65 mg/m^3. Concentrations during the "bagging of diphenylamine-chips" activity reached 0.4 mg/m^3 (duration, 60 minutes), with a 90th percentile of 0.3 mg/m^3. Since some exposure information was missing, the ECHA risk analysis used the Estimation and Assessment of Substance Exposure (EASE) model (August 1997) to evaluate the effects of various production parameters and diphenylamine physical states (liquids or flakes). Exposure levels were found to be similar to the measured levels across a variety of modelled parameters tested and for both physical states (European Commission, 2008).

Owing to the lack of data for dermal exposure, estimations of skin exposure were also performed using the EASE model. In this case, the input parameters used in the EASE model were non-dispersive use, direct and intermittent handling, an exposed area of 210 cm^2, and the use of suitable gloves with a protection efficiency of 90%. These input parameters led to exposure levels of 2.1–21 mg/person per day, which was considered to represent the reasonable worst case. Using the same model, the dermal exposure assessment was also carried out for a worker who did not wear personal protective equipment and was exposed to diphenylamine-containing lubricants. The estimated exposure levels (42–126 mg/person per day) were calculated for a 1% diphenylamine formulation over an exposed skin area of 840 cm^2. For this occupation, exposure by inhalation was considered negligible unless diphenylamine was in aerosol form (European Commission, 2008).

Mixers, loaders, and applicators of pesticides may also be exposed to diphenylamine during and after regular use in agricultural and other settings. The pesticide handlers may be exposed to diphenylamine used as a drench on apples after harvest (US EPA, 1998). A study developed in a rubber manufacturing industry located in Italy (Fracasso et al., 1999) detected diphenylamine through GC-MS analysis of airborne extracts on the basis of similarity of the mass spectra index to that in the Wiley library system. Diphenylamine was detected in ambient air samples taken over a 3-hour period in two stable positions near the mixing Banbury mixer and calendering areas, probably produced by degradation processes facilitated by the high temperatures (100–200 °C) to which the raw materials (e.g. antioxidants) are subjected in these workplaces (Table 1.2).

1.4.3 Exposure of the general population

According to the European Commission (2008), the route of exposure for consumers is oral intake by eating fruits and vegetable foods that have been preserved with diphenylamine, but dermal exposure from lubricants in consumer products is also possible.

In the Total Diet Study by the Food and Drug Administration, conducted between 1986 and 1991 (Gunderson, 1995), the average daily intake of diphenylamine was determined for eight age groups as follows: 6–11 months, 0.0034 µg/kg body weight (bw) per day; 2 years, 0.0410 µg/kg bw per day; girls aged 14–16 years, 0.0073 µg/kg bw per day; boys aged 14–16 years, 0.0099 µg/kg bw per day; women aged 25–30 years, 0.0074 µg/kg bw per day; men aged 25–30 years, 0.0051 µg/kg bw per day; women aged 60–65 years, 0.0079 µg/kg bw per day; and men aged 60–65 years, 0.0065 µg/kg bw per day.

1.5 Regulations and guidelines

1.5.1 Exposure limits and guidelines

(a) Occupational exposure limits

In the USA, the Occupational Safety and Health Administration (OSHA) and the National Institute for Occupational Safety and Health (NIOSH) both recommend an 8-hour time-weighted average (TWA) limit of 10 mg/m^3 to be applied only in construction and

Table 1.3 Occupational exposure limits for diphenylamine in various countries

Country	8-hour TWA (mg/m³)	Short-term (15 minutes) (mg/m³)	Reference
Australia	10		IFA (2021)
Austria	5	10	IFA (2021)
Belgium	10		IFA (2021)
Canada – province of Ontario	10		IFA (2021)
Canada – province of Quebec	10		IFA (2021)
China	10		IFA (2021)
Denmark	5	10	IFA (2021)
Finland	5	10	IFA (2021)
France	10		IFA (2021)
Germany	5	10	IFA (2021)
Ireland	10	20	IFA (2021)
New Zealand	10		IFA (2021)
Italy	10		European Commission (2008)
Netherlands	0.7		European Commission (2008)
Norway	5		IFA (2021)
Poland	8		IFA (2021)
Republic of Korea	10		IFA (2021)
Romania	4	6	IFA (2021)
Singapore	10		IFA (2021)
Spain	10		IFA (2021)
Sweden	4	12	IFA (2021)
Switzerland	10		IFA (2021)
United Kingdom	10	20	IFA (2021)
USA – NIOSH	10		IFA (2021)

NIOSH, National Institute for Occupational Safety and Health; TWA, time-weighted average.

maritime settings. The American Conference of Governmental Industrial Hygienists (ACGIH) and the Division of Occupational Safety and Health of California (Cal/OSHA) recommend the same value for the 8-hour TWA, but do not restrict to specific types of occupational settings (OSHA, 2020). The same exposure limit is used by Safe Work Australia (2019) (Table 1.3).

Several European countries have national occupational limits for diphenylamine (also summarized in Table 1.3) (European Commission, 2008).

(b) Environmental exposure limits

In the USA (United States Government, 2014), the tolerances for residues of diphenylamine are as follows: apple wet pomace, 30.0 mg/kg; apple from pre-harvest or post-harvest use, including use of impregnated wraps, 10.0 mg/kg; cattle fat, cattle meat, cattle meat by-products, except liver, goat fat and meat and goat meat by-products, except liver, horse fat and meat and horse meat by-products, except liver, milk, sheep fat and meat, and sheep meat by-products, except liver, 0.01 mg/kg; cattle, goat, horse, and sheep liver, 0.1 mg/kg.

According to the Codex Alimentarius (FAO, 2021), the MRLs that exist for diphenylamine in food are: apple, 10 mg/kg; apple juice, 0.5 mg/kg; cattle kidney and meat, 0.01 mg/kg; cattle liver, 0.05 mg/kg; milk and milk fats, 0.01 mg/kg; and pear, 5 mg/kg. The MRLs for apple and for processed foods accommodate post-harvest treatment of the commodity. Most non-European

countries define MRLs in food on the basis of these Codex Alimentarius indications (FAO, 2021). At the EU level, the value of 0.05 mg/kg is used for all commodities (Reg. (EU) 2018/1515) (European Commission, 2018).

The state drinking-water guideline in the USA is 175 µg/L (US EPA, 1993).

According to the harmonized classification and labelling framework implemented in the EU (Classification, Labelling and Packaging (CLP) Regulation, 1272/2008/EC), diphenylamine has the following classification: acute toxicity category 3; specific target organ toxicity-repeated exposure category 2; aquatic acute 1; aquatic chronic 1. Employers are obliged under the CLP Regulation to minimize worker exposure to diphenylamine and must arrange for medical surveillance of exposed workers (Council Directive 98/24/EC; European Commission, 1998).

1.5.2 Reference values for biological monitoring of exposure

No reference values related to diphenylamine biological monitoring were available to the Working Group.

2. Cancer in Humans

No epidemiological studies were available that directly investigated the relationship between exposure to diphenylamine and cancer risk. Although there was a case–control study on occupational exposures (workers employed in gunpowder production mentioning use of diphenylamine) and bladder cancer risk (Nizamova, 1991), the study was considered by the Working Group to be uninformative and was excluded here since there was no information on the risk of cancer in relation to diphenylamine exposure specifically.

3. Cancer in Experimental Animals

See Table 3.1.

3.1 Mouse

3.1.1 Oral administration (feed)

In a well-conducted study of chronic toxicity and carcinogenicity that complied with Good Laboratory Practice (GLP), groups of 50 male and 50 female Crj:BDF_1 [B6D2F_1/Crlj] mice (age, 6 weeks) were given feed containing diphenylamine (purity, 100.5%) at a concentration of 0, 250, 1000, or 4000 ppm for the control group and the groups at the lowest, intermediate, and highest dose, respectively, for 104 weeks (JBRC, 2011a, b). The survival rate of males at the highest dose was significantly lower than that of the controls, probably due to urinary retention. The highest dose level was considered to exceed the maximum tolerated dose. The survival rate of females at the highest dose was significantly higher than that of controls. At study termination, survival was: 31/50, 29/50, 29/50, and 16/50 in males, and 23/50, 25/50, 25/50, and 35/50 in females, for the control group and the groups at the lowest, intermediate, and highest dose, respectively. The body weights at the highest dose were significantly decreased in males and females compared with their respective controls. All mice underwent complete necropsy. All organs and tissues were sampled for histopathology in all the animals.

In male mice, there was a significant positive trend ($P < 0.05$, Peto test) in the incidence of haemangioma in the liver, haemangioma or haemangiosarcoma (combined) in the liver, and haemangiomas in all organs. The incidence of haemangioma or haemangiosarcoma (combined) was significantly increased ($P < 0.01$, Fisher exact test) both in the spleen and in all organs (spleen, liver, subcutis, bone marrow, and heart) combined in the group at the intermediate dose: for the spleen – control, 1/50 (2%);

Table 3.1 Studies of carcinogenicity with diphenylamine in experimental animals

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, Crj:BDF_1 [$B6D2F_1$/Crlj] (M) 6 wk 104 wk JBRC (2011a, b)	Oral administration (feed) Diphenylamine, 100.5% Feed 0, 250, 1000, 4000 ppm (w/w), 1×/day 50, 50, 50, 50 31, 29, 29, 16	*Liver* Haemangioma 2/50, 2/50, 5/50, 3/50 Haemangiosarcoma 0/50, 1/50, 2/50, 1/50 Haemangioma or haemangiosarcoma (combined) 2/50, 3/50, 7/50, 4/50 *Spleen* Haemangioma 1/50, 0/50, 6/50, 2/50 Haemangiosarcoma 0/50, 0/50, 3/50, 1/50 Haemangioma or haemangiosarcoma (combined) 1/50 (2%), 0/50, 9/50 (18%)*, 3/50 (6%) *All organs (spleen, liver, subcutis, bone marrow, and heart)* Haemangioma 3/50 (6%), 2/50 (4%), 10/50 (20%)*, 6/50 (12%) Haemangiosarcoma 0/50, 1/50, 4/50, 1/50 Haemangioma or haemangiosarcoma (combined) 3/50 (6%), 3/50 (6%), 14/50 (28%)*, 6/50 (12%)	 $P < 0.05$, Peto trend test NS $P < 0.05$ by Peto trend test NS NS *$P < 0.01$, Fisher exact test $P < 0.05$, Peto trend test; *$P < 0.05$, Fisher exact test NS *$P < 0.01$, Fisher exact test	Principal strengths: multiple doses used; duration of exposure and observation was adequate; well-conducted GLP study; adequate number of mice per group Historical controls: haemangioma or haemangiosarcoma (combined) of the spleen: 107/2244 (4.8%); range, 0–14%; haemangioma in all organs: 145/2245 (6.5%); range, 0–18%; haemangioma or haemangiosarcoma (combined) in all organs: 279/2245 (12.4%); range, 0–22%; liver haemangioma: 70/2245 (3.1%); range, 0–14%

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, Crj:BDF_1 [$B6D2F_1$/Crlj] (F) 6 wk 104 wk JBRC (2011a, b)	Oral administration (feed) Diphenylamine, 100.5% Feed 0, 250, 1000, 4000 ppm (w/w), 1×/day 50, 50, 50, 50 23, 25, 25, 35	*Uterus*: histiocytic sarcoma 8/50 (16%), 7/50 (14%), 17/50 (34%)*, 12/50 (24%)	*$P < 0.05$, Fisher exact test	Principal strengths: multiple doses used; the duration of exposure and observation was adequate; well-conducted GLP study; adequate number of mice per group Historical controls: histiocytic sarcoma of the uterus, 464/2245 (20.7%); range, 0–34%
Full carcinogenicity Mouse, NMRI (M) 8 wk 126 wk Holmberg et al. (1983)	Oral administration (gavage) Diphenylamine, ≥ 99% Soybean oil 0, 300 mg/kg bw 1×/wk for 18 mo (78 wk) 30, 125 NR	Total tumours (all types) 22.2%, 22.9%	NS	Principal limitations: only one sex used; only one dose used; unusual dosing regimen Other comments: after 26 wk, 28 animals were killed in the diphenylamine-treated group, and 7 animals in the vehicle control group; after 52 wk, 24 animals were killed in the diphenylamine-treated group, and 7 animals in the vehicle control group In both groups, the most common tumour types were lymphoma and alveolar adenoma: diphenylamine-treated group, lymphoma (8.3%) and alveolar adenoma (16.5%); vehicle control group, lymphoma (11.1%) and alveolar adenoma (11.1%)

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/DuCrlCrlj (M) 6 wk 104 wk JBRC (2011c, d)	Oral administration (feed) Diphenylamine, 100.5% Feed 0, 250, 1000, 4000 ppm (w/w), 1×/day 50, 50, 50, 50 37, 40, 43, 41	*Spleen* Haemangiosarcoma 0/50, 0/50, 0/50, 3/50 (6%) Haemangioma or haemangiosarcoma (combined) 0/50, 1/50 (2%), 0/50, 3/50 (6%) *Subcutis* Fibroma 2/50, 11/50*, 3/50, 2/50 Fibrosarcoma 0/50, 2/50, 0/50, 1/50 Fibroma or fibrosarcoma (combined) 2/50, 13/50*, 3/50, 3/50 Haemangiosarcoma 0/50, 0/50, 0/50, 1/50 *All organs* Haemangioma 0/50, 1/50, 0/50, 1/50 Haemangiosarcoma 0/50, 0/50, 0/50, 4/50 (8%) Haemangioma or haemangiosarcoma (combined) 0/50, 1/50 (2%), 0/50, 5/50 (10%)* *Testis*: interstitial cell tumour 37/50, 40/50, 46/50*, 46/50*	 $P < 0.01$, Peto trend test and Cochran–Armitage test $P < 0.05$, Peto trend test and Cochran–Armitage test *$P < 0.01$, Fisher exact test NS *$P < 0.01$, Fisher exact test NS NS $P < 0.01$, Peto trend test and Cochran–Armitage test *$P < 0.05$, Fisher exact test $P < 0.05$ by Peto trend test and Cochran–Armitage test; *$P = 0.05$, Fisher exact test	Principal strengths: multiple doses used; the duration of exposure and observation was adequate; well-conducted GLP study; adequate number of rats per group Historical controls: haemangiosarcoma in the spleen, 7/2748 (0.3%); range, 0–4%; haemangiosarcoma in all organs, 8/2748 (0.3%); range, 0–4%; haemangioma or haemangiosarcoma (combined) in all organs, 19/2748 (0.7%); range, 0–4%

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/DuCrlCrlj (F) 6 wk 104 wk JBRC (2011c, d)	Oral administration (feed) Diphenylamine, 100.5% Feed 0, 250, 1000, 4000 ppm (w/w), 1×/day 50, 50, 50, 50 40, 43, 45, 43	*Uterus* Adenocarcinoma 1/50 (2%), 0/50, 0/50, 4/50 (8%) Adenoma or adenocarcinoma (combined) 1/50 (2%), 1/50 (2%), 0/50, 4/50 (8%) *Spleen*: mononuclear cell leukaemia 3/50 (6%), 2/50 (4%), 0/50, 5/50 (10%)	$P < 0.01$, Peto trend test and Cochran–Armitage test $P < 0.05$, Peto trend test and Cochran–Armitage test $P < 0.05$ by Peto trend test	Principal strengths: multiple doses used; the duration of exposure and observation was adequate; well-conducted GLP study; adequate number of rats per group Historical controls: adenocarcinoma of the uterus, 15/2544 (0.6%); range, 0–4%; adenoma or adenocarcinoma of the uterus, 22/2544 (0.9%); range, 0–4%; mononuclear cell leukaemia of the spleen, 314/2547 (12.3%); range, 2–26%

bw, body weight; F, female; GLP, Good Laboratory Practice; M, male; mo, month; NR, not reported; NS, not significant; ppm, parts per million; w/w, weight per weight; wk, week.

lowest dose, 0/50; intermediate dose, 9/50 (18%); and highest dose, 3/50 (6%); and for all organs combined – control, 3/50 (6%); lowest dose, 3/50 (6%); intermediate dose, 14/50 (28%); and highest dose, 6/50 (12%). The incidence of haemangioma or haemangiosarcoma (combined) at the intermediate dose both in the spleen and in all organs combined exceeded the upper bound of the range observed in historical controls from this laboratory – spleen, 107/2244 (4.8%); range, 0–14%; and all organs combined, 279/2245 (12.4%); range, 0–22%. The incidence of haemangioma in all organs combined was significantly increased at the intermediate dose: control, 3/50 (6%); lowest dose, 2/50 (4%); intermediate dose, 10/50 (20%); and highest dose, 6/50 (12%); $P < 0.05$, Fisher exact test. The incidence of haemangioma in all organs combined at the intermediate dose exceeded the upper bound of the range observed in historical controls – 145/2245 (6.5%); range, 0–18%.

In female mice, there was a significant increase in the incidence of histiocytic sarcoma of the uterus at the intermediate dose: control, 8/50 (16%); lowest dose, 7/50 (14%); intermediate dose, 17/50 (34%); and highest dose, 12/50 (24%); $P < 0.05$, Fisher exact test. The incidence of histiocytic sarcoma of the uterus at the intermediate dose was at the upper bound of the range observed in historical controls from this laboratory – 464/2245 (20.7%); range, 10–34%.

In all treated groups of male and female mice, diphenylamine caused methaemoglobinaemia, anaemia, increased haematopoiesis of the bone marrow, splenic enlargement, haematopoiesis, and hemosiderosis (JBRC, 2011a, b). [The Working Group noted that this was a well-conducted study that complied with GLP, the number of animals per group was adequate, the study used both sexes and multiple dose groups, and the duration of exposure and observation was adequate.]

3.1.2 Oral administration (gavage)

A group of 125 male NMRI mice (age, 8 weeks) was treated with diphenylamine (purity, ≥ 99%) at a dose of 300 mg/kg bw in soybean oil by gavage once per week for 18 months (78 weeks). A control group of 30 male NMRI mice was given the vehicle only (soybean oil, 10 mL per kg bw) using the same protocol (Holmberg et al., 1983). Groups of mice were killed at 26 weeks (7 controls and 28 diphenylamine-treated mice) and 52 weeks (7 controls and 24 diphenylamine-treated mice). The remaining mice were observed up to experimental week 126. Diphenylamine decreased the mean body weight but not the survival of the treated animals compared with vehicle controls. Histopathological examination was performed on main organs and tissues.

There were no changes in the frequency of any type of tumour in treated animals compared with vehicle controls. [The Working Group noted that only one sex and dose were used, and that the dosing regimen was unusual.]

3.2 Rat

3.2.1 Oral administration (feed)

In a well-conducted chronic toxicity and carcinogenicity study that complied with GLP, groups of 50 male and 50 female F344/DuCrlCrlj rats (age, 6 weeks) were given feed containing diphenylamine (purity, 100.5%) at a dose of 0, 250, 1000, or 4000 ppm for the control group and the groups at the lowest, intermediate, and highest dose, respectively, for 104 weeks (JBRC, 2011c, d). Survival analysis showed no differences between the treated groups and their respective control groups. At study termination, survival was: 37/50, 40/50, 43/50, and 41/50 in males, and 40/50, 43/50, 45/50, and 43/50 in females, for the control group and the groups at the lowest, intermediate, and highest dose, respectively. At termination of treatment, the body weights of males

at the highest dose and females at the intermediate and highest dose were significantly lower than those of their respective controls. Food consumption was decreased in males at the highest dose for most of the duration of the study. Food consumption was also decreased in females at the intermediate and highest dose for most (weeks 0–78) of the duration of the study. All rats underwent complete necropsy. All organs and tissues were sampled for histopathology in all the animals.

In male rats, there was a significant positive trend in the incidence of haemangiosarcoma in the spleen ($P < 0.01$, Peto and Cochran–Armitage tests), of haemangiosarcoma in all organs (spleen and subcutis) combined ($P < 0.01$, Peto and Cochran–Armitage tests), and of haemangioma or haemangiosarcoma (combined) in the spleen ($P < 0.05$, Peto and Cochran–Armitage tests). The incidence of haemangioma or haemangiosarcoma (combined) in all organs combined was significantly increased at the highest dose: control, 0/50; lowest dose, 1/50 (2%); intermediate dose, 0/50; and highest dose, 5/50 (10%); $P < 0.05$, Fisher exact test. The incidence of haemangiosarcoma in the spleen (6%), haemangiosarcoma in all organs combined (8%), and of haemangioma or haemangiosarcoma (combined) in all organs combined (10%), all at the highest dose, exceeded the overall incidence and upper bound of the range for these tumours observed in historical controls from this laboratory – incidence, 7/2748 (0.3%), 8/2748 (0.3%), and 19/2748 (0.7%), respectively; all ranges: 0–4%. The incidence of subcutis fibroma was 2/50, 11/50, 3/50, and 2/50; the incidence of subcutis fibrosarcoma was 0/50, 2/50, 0/50, and 1/50; and the incidence of fibroma or fibrosarcoma (combined) of the subcutis was 2/50, 13/50, 3/50, and 3/50 in the control groups and in the groups at the lowest, intermediate, and highest dose, respectively. The incidence of fibroma of the subcutis and of fibroma or fibrosarcoma (combined) of the subcutis was significantly increased at the lowest dose compared with controls ($P < 0.01$, Fisher exact test). There was a significant positive trend in the incidence of interstitial cell tumour of the testis – control, 37/50; lowest dose, 40/50; intermediate dose, 46/50; highest dose, 46/50; $P < 0.05$, Peto and Cochran–Armitage tests – with a significant increase ($P < 0.05$, Fisher exact test) at the intermediate and highest dose.

In female rats, there was a significant positive trend in the incidence of adenocarcinoma of the uterus – control, 1/50 (2%); lowest dose, 0/50; intermediate dose, 0/50; and highest dose, 4/50 (8%); $P < 0.01$, Peto and Cochran–Armitage tests – and of adenoma or adenocarcinoma (combined) of the uterus –control, 1/50 (2%); lowest dose, 1/50 (2%); intermediate dose, 0/50; and highest dose, 4/50 (8%); $P < 0.05$, Peto and Cochran–Armitage tests. The incidence of adenocarcinoma of the uterus at the highest dose and adenoma or adenocarcinoma (combined) of the uterus at the highest dose exceeded the upper bound of the range observed in historical controls from this laboratory – incidence of adenocarcinoma of the uterus, 15/2544 (0.6%); range, 0–4%; and incidence of adenoma or adenocarcinoma (combined) of the uterus, 22/2544 (0.9%); range, 0–4%). A significant positive trend in the incidence of mononuclear cell leukaemia of the spleen ($P < 0.05$, Peto test) was also observed. The incidence of mononuclear cell leukaemia of the spleen in all dose groups – control, 3/50 (6%); lowest dose, 2/50 (4%), intermediate dose, 0/50; and highest dose, 5/50 (10%) – did not exceed the upper bound of the range (2–26%) observed in historical controls from this laboratory.

In treated males (at the intermediate and highest dose) and females (at all doses), diphenylamine caused methaemoglobinaemia. Anaemia occurred in males (at the highest dose) and females (at the intermediate and highest dose). Splenic enlargement, increased haematopoiesis, and haemosiderosis were observed in the spleen of treated male rats. Splenic enlargement, capsular hyperplasia, angiectasis, and fibrosis were

observed in the spleen of treated female rats (JBRC, 2011c, d). [The Working Group noted that this was a well-conducted GLP study that used an adequate number of animals per group, males and females, and multiple dose groups, and with the duration of exposure and observation was adequate.]

In another study, groups of 20 male and 20 female weanling Slonaker-Addis strain rats were given feed containing diphenylamine (purity, ≥ 99.9%) at a concentration of 0 (control), 0.001%, 0.01%, 0.1%, 0.5%, or 1.0% for 2 years (Thomas et al., 1967a). All rats surviving for at least 640 days (including those that survived until study termination at 734 days) were given a complete postmortem examination. The incidence of tumours of any type was not affected by diphenylamine treatment. [The Working Group noted that this study was inadequate for the evaluation of the carcinogenicity of diphenylamine in experimental animals due to the small number of animals and lack of details regarding the postmortem examination.]

3.2.2 Oral administration (gavage)

Twenty female Sprague-Dawley rats (age, 50–55 days) were given a single dose of diphenylamine [purity unspecified] of 300 mg per rat (in sesame oil) by gavage. Complete necropsy was performed 6 months after diphenylamine administration. A group of 89 female Sprague-Dawley rats were given sesame oil only and served as controls. No increased incidence of tumours of any type was reported (Griswold et al., 1966). [The Working Group noted that this study was inadequate for the evaluation of the carcinogenicity of diphenylamine in experimental animals due to the limited duration of observation, small number of animals, and the administration of a single dose.]

3.3 Dog

Oral administration (feed)

Four groups of two male and two female beagle dogs (age, 8 months) were given feed containing diphenylamine (purity, ≥ 99.9%) at a concentration of 0, 0.01%, 0.1%, or 1.0% for the control group and the groups at the lowest, intermediate, and highest dose, respectively, for 2 years. No neoplasms were reported in any treatment group (Thomas et al., 1967b). [The Working Group noted that this study was inadequate for the evaluation of the carcinogenicity of diphenylamine in experimental animals due to the small number of animals, lack of details regarding the postmortem evaluation, and limited duration of observation.]

3.4 Evidence synthesis for cancer in experimental animals

The carcinogenicity of diphenylamine has been assessed in one well-conducted GLP study in male and female Crj:BDF$_1$ mice (JBRC, 2011a, b) and in one well-conducted GLP study in male and female F344/DuCrlCrlj rats (JBRC, 2011c, d) treated by oral administration (in the feed); in two additional studies in male and female Slonaker-Addis strain rats (Thomas et al., 1967a) and male and female beagle dogs (Thomas et al., 1967b) treated by oral administration (in the feed); in one study in female Sprague-Dawley rats treated by oral administration (gavage) (Griswold et al., 1966), and in one study in male NMRI mice treated by oral administration (gavage) (Holmberg et al., 1983).

In the well-conducted GLP study in male and female Crj:BDF$_1$ mice treated by oral administration (JBRC, 2011a, b), there was a significant positive trend in the incidence of haemangioma in the liver, haemangioma or haemangiosarcoma (combined) in the liver, and haemangioma in all organs combined in male mice. The incidence

of haemangioma or haemangiosarcoma (combined) was significantly increased both in the spleen and in all organs combined in male mice at the intermediate dose. The incidence of haemangioma in all organs combined was significantly increased in male mice at the intermediate dose. In female mice at the intermediate dose, oral administration of diphenylamine caused a significant increase in the incidence of histiocytic sarcoma of the uterus (JBRC, 2011a, b).

In a well-conducted GLP study in male and female F344/DuCrlCrlj rats treated by oral administration (JBRC, 2011c, d), there was a significant positive trend in the incidence of haemangiosarcoma in the spleen, haemangiosarcoma in all organs combined, and haemangioma or haemangiosarcoma (combined) in the spleen of male rats. The incidence of haemangioma or haemangiosarcoma (combined) in all organs combined was significantly increased in male rats at the highest dose. The incidence of fibroma and of fibroma or fibrosarcoma (combined) of the subcutis was significantly increased in male rats at the lowest dose. There was a significant positive trend in the incidence of interstitial cell tumours of the testis, with a significant increase in the incidence in male rats at the intermediate dose and highest dose. In female rats, there was a significant positive trend in the incidence of adenocarcinoma of the uterus and of adenoma or adenocarcinoma (combined) of the uterus. A significant positive trend in the incidence of mononuclear cell leukaemia of the spleen was also observed in female rats (JBRC, 2011c, d).

There was no significant increase in the incidence of tumours in the study in male NMRI mice treated by oral administration (Holmberg et al., 1983).

Both studies in male and female weanling Slonaker-Addis strain rats (Thomas et al., 1967a) and in male and female beagle dogs (Thomas et al., 1967b) treated by oral administration, and the one study in female Sprague-Dawley rats treated by oral administration (Griswold et al., 1966), were judged to be inadequate for the evaluation of the carcinogenicity of diphenylamine in experimental animals.

4. Mechanistic Evidence

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

(a) Exposed humans

Only one study on the absorption, distribution, metabolism, and excretion of diphenylamine in humans was available. Diphenylamine was found to be metabolized to 4-hydroxydiphenylamine and 4,4′-dihydroxydiphenylamine after analysis of the urine of two human subjects for 24 hours after administration of a single oral dose of 100 mg of diphenylamine (Alexander et al., 1965). As well as the two identified metabolites, diphenylamine was also shown to be excreted in its unmetabolized form into the urine. No 2-hydroxydiphenylamine was found in the urine. The findings in the urine samples collected 24 hours after oral dosing suggested that diphenylamine is absorbed in humans via the gastrointestinal tract; however, the extent and rate of absorption is unclear. Data on other routes of absorption in humans were not available.

Piechocki et al. (2018) reported the accidental exposure of a 23-year-old patient to diphenylamine in the workplace, resulting in methaemoglobinaemia. [The Working Group noted that this study was not informative because the patient was co-exposed to 1,4-diaminobenzene and the precise amount and duration of exposure were not reported.]

(b) Human cells in vitro

Most metabolites of diphenylamine undergo conjugation. Fig 4.1 illustrates the proposed metabolic pathways for diphenylamine. Green et al. (1998) reported on the direct N-glucuronidation of diphenylamine by human UDP-glucuronosyltransferase 1A3 (UGT1A3) transiently expressed in human embryonic kidney cells (HEK293). However, the rate of glucuronide formation by UGT1A3 was low compared with that by UGT1A4 (Green et al., 1998). [The Working Group noted that this suggests that this enzyme is not a major contributor to the metabolic clearance of diphenylamine in vivo.] Similarly, the metabolism of diphenylamine was shown to be catalysed by human UGT1A8 transfected-HEK293 cells with low glucuronidation rates (Cheng et al., 1998).

As a part of the Toxicity Forecaster/Toxicity Testing in the 21st Century (ToxCast/Tox21) analysis (Section 4.2.4), the intrinsic hepatic clearance rate in vitro for diphenylamine was measured to be 64.57 µL/minute per 10^6 hepatocytes from a human donor pool. The in vitro and computationally derived estimates of pharmacokinetic parameters therefore included half-life and volume of distribution values of 7.35 hours and 0.62 L/kg, respectively (US EPA, 2021).

4.1.2 Experimental systems

(a) Absorption and distribution

Diphenylamine was found to be well absorbed in male and female Sprague-Dawley rats; 68–89% of an oral dose of [^{14}C]-labelled diphenylamine of 5 mg/kg bw was recovered in the urine after 168 hours (WHO, 1998). Adequate absorption was observed across experimental systems. About 85–91% of the daily dose was recovered in the urine of two lactating Toggenburg goats given [^{14}C]-labelled diphenylamine at 50 mg/kg bw per day by oral administration for 7 days. In goats, diphenylamine was reported to distribute both as parent and as metabolites to the liver, kidney, leg muscle, loin muscle, back fat, omental fat, and milk (WHO, 1998). No appreciable tissue accumulation of diphenylamine was noted in male and female rats tested over a wide dose range (5 and 750 mg/kg bw) on the basis of percentage of radiolabelled dose in the carcass and tissues (WHO, 1998).

(b) Metabolism

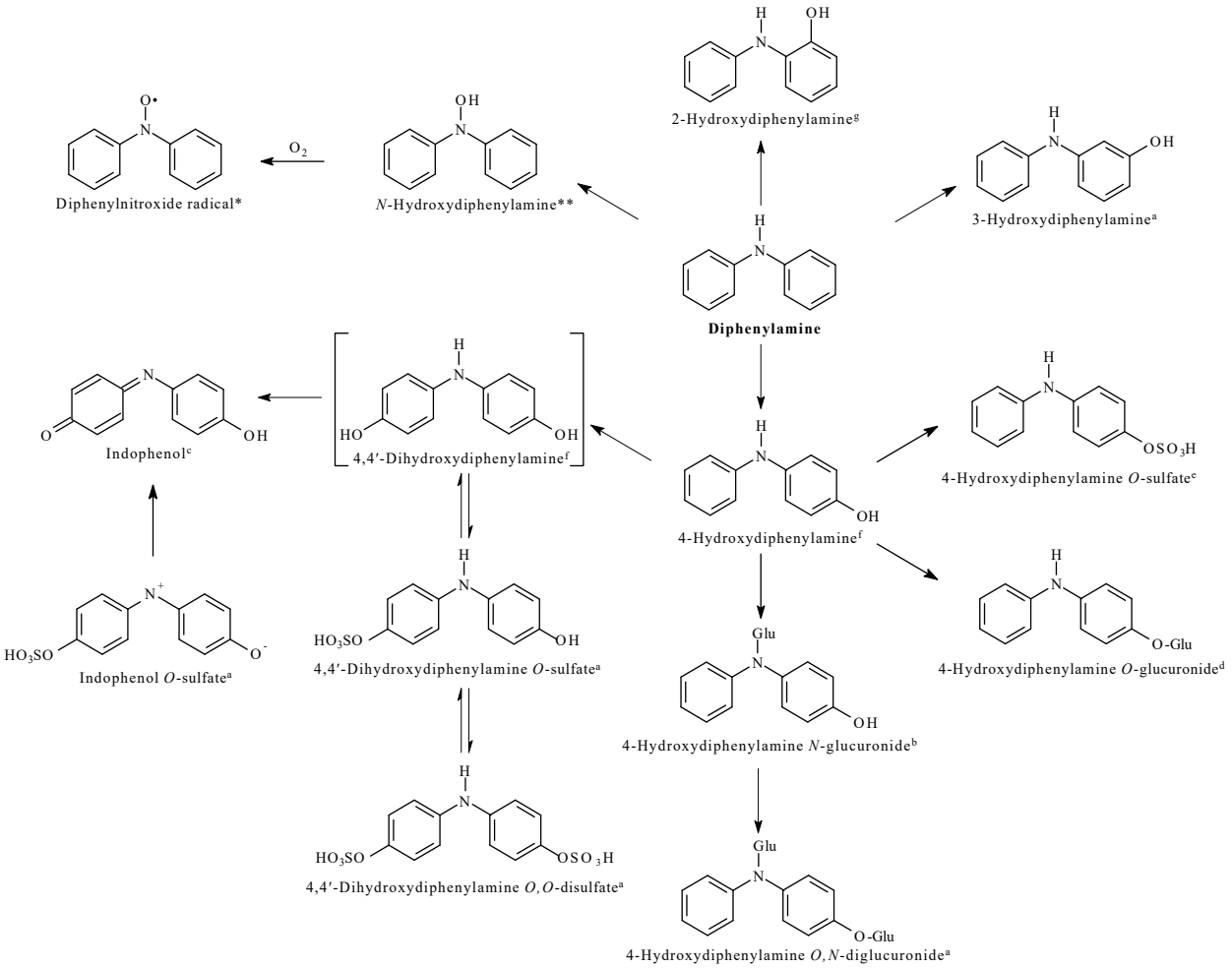
See Fig. 4.1.

Diphenylamine undergoes rapid and extensive metabolism by hydroxylation followed by conjugation (Alexander et al., 1964, 1965; WHO, 1998). A total of 12 metabolites of diphenylamine were identified in rats given oral doses at 5 or 750 mg/kg bw (WHO, 1998), with less than 3% of the administered dose remaining as parent compound in the urine and faeces. Metabolites of diphenylamine in rats include 4-hydroxydiphenylamine, 3-hydroxydiphenylamine, 2-hydroxy-diphenylamine, 4-hydroxydiphenylamine *O*-sulfate, 4-hydroxydiphenylamine *O*-glucuronide, 4-hydroxydiphenylamine *N*-glucuronide, 4-hydroxydiphenylamine *O,N*-diglucuronide, 4,4′-dihydroxydiphenylamine, 4,4′-dihydroxydiphenylamine *O*-sulfate, 4,4′-dihydroxydiphenylamine *O,O*-disulfate, indophenol, and indophenol *O*-sulfate.

Diphenylamine was also shown to be metabolized to 4-hydroxydiphenylamine and 4,4′-dihydroxydiphenylamine in goats, hens, and dogs (DeEds, 1963; WHO, 1998). [The Working Group noted that DeEds (1963) did not provide adequate experimental evidence for their findings in dogs.] 2-Hydroxydiphenylamine was identified as a minor metabolite of diphenylamine in rabbits (Alexander et al., 1964, 1965). 2-Hydroxydiphenylamine was also reported to be a metabolite in rats (WHO, 1998); however, it was not detected in rat urine by Alexander et al. (1965).

Conjugates of 4-hydroxydiphenylamine were identified as the major metabolites of diphenyl-

Fig. 4.1 Metabolic pathways for diphenylamine



Glu, glucuronate.
[a] Rat.
[b] Rat and dog.
[c] Rat and goat.
[d] Rat, rabbit, goat, and hen.
[e] Rat, rabbit, dog, goat, and hen.
[f] Rat, rabbit, dog, goat, hen and human.
[g] Rat [contradictory findings], rabbit, and hen.
* Microsomal systems in hogs and mice.
** Hydroxylamine derivative as hypothesized metabolic intermediate (Appel et al., 1987; Valvis et al., 1990).
Adapted from WHO (1998).

amine in the urine of rats injected intraperitoneally with 5 mg of diphenylamine (Alexander et al., 1964). In a rabbit, 5 g of diphenylamine was orally administered as a suspension and as a divided dose of 1 g over a period of 9 days. *O*-Sulfate and *O*-glucuronide were detected as the primary conjugates of 4-hydroxydiphenylamine in rabbit urine (Alexander et al., 1965).

O-Sulfate and *N*-glucuronide conjugates of 4-hydroxydiphenylamine and 4,4′-dihydroxydiphenylamine were also detected as the products of metabolism in the urine and faeces of albino rats and beagle dogs in a 2-year feeding study (DeEds, 1963). [The Working Group noted that DeEds (1963) did not provide adequate experimental evidence for their findings.]

Furthermore, N-hydroxylation of diphenylamine was hypothesized as a potential metabolic pathway in rats, rabbits, and cats (Alexander et al., 1964, 1965). [The Working Group noted that it is difficult to detect *N*-hydroxydiphenylamine due to its chemical instability.] Under acidic conditions of urine hydrolysis in vitro, *N*-hydroxydiphenylamine was shown to rearrange to diphenylamine and 4-hydroxydiphenylamine. After a single intraperitoneal injection of 5 mg of *N*-hydroxydiphenylamine in male white rats [the Working Group noted that the strain was not provided], neither *N*-hydroxydiphenylamine nor diphenylamine were detected in the hydrolysed urine. Instead, 4-hydroxydiphenylamine and 4,4′-dihydroxydiphenylamine were detected, possibly due to the chemical rearrangement of *N*-hydroxydiphenylamine in vivo (Alexander et al., 1964, 1965).

Additional evidence for the formation of *N*-hydroxydiphenylamine in vivo is indirect and associated with methaemoglobin formation in rats, mice, and cats after diphenylamine exposure (Alexander et al., 1965; Nomura, 1977). The kinetics of methaemoglobin formation were studied in male ddY mice for 96 hours after intraperitoneal injection with a single dose of diphenylamine at 103 mg/kg bw. Methaemoglobin concentrations in the blood peaked rapidly about 30 minutes after administration and decreased to levels that were similar to those of controls after 90 minutes (Nomura, 1977). No significant formation of methaemoglobin was detected 48 hours after three consecutive days of intraperitoneal injections in male ddY mice (Nomura, 1977). Similarly, methaemoglobin in rat blood was shown to reach peak concentrations 30–35 hours after oral administration (gavage) of diphenylamine at half the median lethal dose (½ LD_{50}) (Volodchenko, 1975). [The Working Group noted that, overall, the N-hydroxylation of diphenylamine in vivo is probable and supported by the evidence of formation of methaemoglobin; however, it has not been chemically detected or conclusively determined (Alexander et al., 1964, 1965; Volodchenko, 1975; Nomura, 1977; Appel et al., 1987; Semak & Pikulev, 1993).]

Acellular assays using hog liver microsomes also showed that diphenylamine is a good substrate for mixed function amine oxidase and can undergo bio-oxidation in the presence of reduced nicotinamide adenine dinucleotide phosphate (NADPH) and molecular oxygen to yield its respective nitroxide free radical, diphenylnitroxide (Valvis et al., 1990). Bio-oxidation was rate-limited by substrate inhibition at higher diphenylamine concentrations although the yield over time was noted to be high (Valvis et al., 1990). Additional acellular assays using mouse microsomes provided more evidence for production of diphenylnitroxide radicals after incubation with diphenylhydroxylamine, a potential metabolite of diphenylamine (Appel et al., 1987). More recently, diphenylamino radical formation was detected after oxidation of diphenylamine in an acellular system (Son & Choi, 2021).

(c) Excretion

Urine is a major route of excretion for diphenylamine in rats, rabbits, dogs, and goats, with bile and faeces contributing to a lesser extent

(DeEds 1963; Alexander et al., 1965; WHO, 1998). Diphenylamine was shown to be excreted primarily as its metabolite 4-hydroxydiphenylamine, its conjugates, and 4,4′-dihydroxydiphenylamine in rat and rabbit urine (DeEds, 1963; Alexander et al., 1964, 1965), in rat bile (Alexander et al., 1964, 1965), and in rat and dog faeces (DeEds, 1963), but not as metabolites in the urine and faeces of goat and hen (WHO, 1998). [The Working Group noted that WHO (1998) contained limited experimental and analytical details.] In rabbits, 2-hydroxydiphenylamine and unchanged diphenylamine were also detected in the urine (Alexander et al., 1965). A 4-day feeding study in a Holstein dairy cow given diphenylamine at 5 ppm showed excretion of 1.4% of the administered dose in the faeces, but no diphenylamine was detected in the urine or milk (Gutenmann & Lisk, 1975). [The Working Group noted that the analytical method used (gas chromatography) could not have detected the metabolites of diphenylamine, which are the primary forms in which diphenylamine is eliminated in urine across species.]

Excretion of diphenylamine is rapid. Urine, faeces, and milk collected cumulatively in goats orally dosed with radiolabelled diphenylamine at 50 mg/kg bw per day for 7 days showed that the administered dose was largely excreted within 24 hours after each dose (WHO, 1998). In Sprague-Dawley rats, up to 72% of the administered dose was reported to be excreted in the urine within 24 hours (WHO, 1998). In male white rats injected with radiolabelled diphenylamine intraperitoneally or intravenously (with bile duct cannulation) at a dose of 5 mg/kg bw, there was 75% recovery of the radiolabel in the urine after 48 hours and 25% in the bile after 6 hours, respectively (Alexander et al., 1965).

4.2 Evidence relevant to key characteristics of carcinogens

4.2.1 Is genotoxic

(a) Humans

(i) Exposed humans

No genotoxicity studies in exposed humans were available to the Working Group. However, Fracasso et al. (1999) detected diphenylamine along with five other chemicals in stationary workplace air samples collected over a 3-hour period and in personal air samples collected over a 2-hour period during a typical work day from five workers employed in different rubber-processing operations. The mutagenic activity of the air samples was determined by a plate incorporation assay using *Salmonella typhimurium* strains TA98NR, TA98, YG1021, and TA100 (Table 4.1).

The results showed direct and indirect frameshift mutagenicity induced by both the ambient and personal air samples. No mutation was induced in the *S. typhimurium* TA100 strain, except for the air sample from one worker. The high levels of mutagenic activity in the ambient and personal air samples compared with negative controls indicate the presence of substances with high genotoxic potency (Fracasso et al., 1999). [The Working Group noted that the air samples contained a mixture of chemicals including diphenylamine; however, it was not possible to conclusively establish a causative link between genotoxicity and exposure to diphenylamine only. Furthermore, the precise concentration of diphenylamine in the air samples and the duration of exposure were not reported.]

(ii) Human cells in vitro

See Table 4.2.

In the study by Ardito et al. (1996), diphenylamine significantly increased the frequency of sister-chromatid exchange in cultured human peripheral blood lymphocytes treated with a non-cytotoxic concentration of 6 μg/mL (but not

Table 4.1 Genetic and related effects of diphenylamine in exposed humans

Test system (species, strain)	End-point	Description of exposed and controls	Results[a]	Comments	Reference
Salmonella typhimurium, TA98NR, TA98, and YG1021	Reverse mutation	Personal air samples collected over a 2 h period during a typical work day from five workers employed in different rubber-processing operations. Control air samples from factories offices included.	(+)	The air samples contained a mixture of chemicals including diphenylamine; however, it was not possible to conclusively establish a causative link between genotoxicity and exposure to diphenylamine only.	Fracasso et al. (1999)
TA100	Reverse mutation	Personal air samples collected over a 2 h period during a typical work day from five workers employed in different rubber-processing operations. Control air samples included.	(–)	The air samples contained a mixture of chemicals including diphenylamine; however, it was not possible to conclusively establish a causative link between genotoxicity and exposure to diphenylamine only.	Fracasso et al. (1999)

[a] (+) or (–), positive or negative in a study of limited quality.

Table 4.2 Genetic and related effects of diphenylamine in human cells in vitro

End-point	Tissue, cell type	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Micronucleus formation	Peripheral blood lymphocytes	+	NT	1.25 µg/mL	Purity, NR; 48 h exposure; statistically significant at this concentration versus the negative and solvent controls.	Santovito et al. (2012)
Sister-chromatid exchange	Peripheral blood lymphocytes	(+)	NT	3.5×10^{-5} M (6 µg/mL), 48 h exposure	Chemical source and purity, NR; increase is small and within 1 SD of control; method inconsistent with OECD test guideline to support clear negatives; S9 from phenobarbital/benzoflavone-induced rat liver.	Ardito et al. (1996)
		(–)	(–)	3.5×10^{-5} M (6 µg/mL), 4 h exposure		

HIC, highest ineffective concentration; LEC, lowest effective concentration; NR, not reported; NT, not tested; OECD, Organisation for Economic Cooperation and Development; S9, 9000 × *g* supernatant; SD, standard deviation.

[a] +, positive; (+) or (–), positive or negative in a study of limited quality.

0.6 µg/mL) for 48 hours. However, no difference was observed when lymphocytes were exposed for 4 hours at 6 µg/mL with and without metabolic activation (Ardito et al., 1996). [The Working Group noted that dose-dependent trends could not be established for diphenylamine owing to cytotoxicity after 48 hours of exposure at higher concentrations (60 µg/mL). The Working Group also noted cautious interpretation of the positive result since the increase, although statistically significant, was small and within only one standard deviation of the control. The number of well-spread metaphases scored in the cultures for each concentration, particularly in the 4-hour treatment group, were 10 times less than that suggested to be required to support a clear negative result (OECD test guideline, TG473; OECD, 2016). Furthermore, the Working Group noted that chemical source and purity were not reported.]

Another study investigated the potential of diphenylamine to induce chromosomal damage within a dose range comparable to that used in the two lowest treatment groups (6 and 0.6 µg/mL) in Ardito et al. (1996) with a similar exposure duration of 48 hours (Santovito et al., 2012). In human peripheral blood lymphocytes, diphenylamine significantly increased the frequency of micronucleus formation at concentrations of 1.25, 2.5, 5, and 10 µg/mL, but not at 0.625 µg/mL, compared with the negative and solvent (1% dimethyl sulfoxide, DMSO) controls. Moreover, diphenylamine was shown to induce an increase in the frequency of micronucleus formation with statistical significance at all treatment concentrations except 1.25 µg/mL when compared with 0.625 µg/mL. None of the tested concentrations were cytotoxic (Santovito et al., 2012).

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.3.

Diphenylamine was reported to give negative results for the induction of micronuclei in the bone marrow of ICR mice exposed at concentrations of 250–1000 mg/kg bw (males) and 375–1500 mg/kg bw (females) (WHO, 1998; European Commission, 2008).

Diphenylamine at concentrations of 1450–2900 µmol/kg bw (plus sodium nitrite) was also shown to lack mutagenic activity in a host-mediated mouse assay when injected intraperitoneally together with *S. typhimurium* TA1950 as a genetic indicator organism (Braun et al., 1977).

(ii) Non-human mammalian cells in vitro

See Table 4.4.

Negative results for DNA single-strand breaks were reported for diphenylamine in Chinese hamster V79 cells (Appel et al., 1987). [The Working Group noted that the doses tested were not indicated.] Diphenylhydroxylamine [*N*-hydroxydiphenylamine], a proposed metabolite of diphenylamine (Section 4.1.2), was shown to cause DNA breaks in Chinese hamster V79 cells (Appel et al., 1987). [The Working Group noted that this was possibly due to its auto-oxidation to the diphenylnitroxide radical.]

Diphenylamine produced negative results for unscheduled DNA synthesis when tested at the highest non-cytotoxic concentration of 100 µM [mol/L] without metabolic activation in cultured rat hepatocytes (Probst et al., 1981). [The Working Group noted the challenges associated with detecting low levels of DNA repair using the autoradiographic method, and the potential ability of a chemical to inhibit DNA repair enzymes, resulting in a negative DNA-repair response.]

Furthermore, diphenylamine was found to be non-mutagenic in the L5178Y mouse lymphoma thymidine kinase ($Tk^{+/-}$) assay in the presence of metabolic activation (Amacher et al., 1980) after 3 hours of treatment at concentrations of up to 6.75×10^{-5} M. Cytotoxicity was observed at higher concentrations (9×10^{-5} M to 21.36×10^{-5} M). In another study reported in

Table 4.3 Genetic and related effects of diphenylamine in non-human mammals in vivo

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LEC or HIC)	Route, duration, dosing regimen	Comments	Reference
Micronucleus formation	Mouse, ICR (M, F)	Bone marrow	(–)	1000 mg/kg bw (M) 1500 mg/kg bw (F)	Oral administration (gavage); 24, 48, and 72 h	Purity, 99.9%	WHO (1998); European Commission (2008)
Mutation (host-mediated assay)	Mouse, NMRI (M)	*S. typhimurium* TA1950 from peritoneal cavity	–	1450–2900 µmol/kg bw (+ sodium nitrite)	Oral administration (gavage) and intraperitoneal injection of bacteria	"Pure" (but % not given)	Braun et al. (1977)
Oxidative DNA damage (8-OHdG)	Rat, Wistar (M)	Liver	+	0.09 mg/kg bw per day	Oral administration (gavage); 10 days	Purity, 99.9%	Lodovici et al. (1997)

bw, body weight; F, female; HIC, highest ineffective concentration; LEC, lowest effective concentration; M, male; 8-OHdG, 8-hydroxy-2′-deoxyguanosine.
[a] +, positive; –, negative; (–), negative in a study of limited quality.

Table 4.4 Genetic and related effects of diphenylamine in non-human mammalian cells in vitro

End-point	Species, cell type	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
DNA single-strand breaks, alkaline elution	Chinese hamster, V79 lung cells	(–)	NT	NR	Chemical source and purity, NR; scant information on analytical methods; dose for diphenylamine not reported, inferred from results for *N*-nitrosodiphenylamine (diphenylamine metabolite).	Appel et al. (1987)
Unscheduled DNA synthesis	F344 rat, hepatocytes	–	NT	100 nmol/mL [100 µM; ~17 µg/mL]	Low levels of DNA repair potentially not detected by autoradiography.	Probst et al. (1981)
Gene mutation, *Tk*$^{+/-}$	Mouse, L5178Y/*Tk*$^{+/-}$ lymphoma cells	NT	–	6.75×10^{-5} M [67.5 µM; ~11.5 µg/mL]	Dose as high as 21.36×10^{-5} M tested; however, cytotoxicity occurred at 9.00×10^{-5} M; S9 from Aroclor-1254-induced male (Sprague Dawley) rat liver.	Amacher et al. (1980)
Gene mutation, *Tk*$^{+/-}$	Mouse, L5178Y/*Tk*$^{+/-}$ lymphoma cells	(+)	(+)	5–80 µg/mL	Weakly positive; dose range cytotoxic and mutation frequency did not increase with dose; effect with or without metabolic activation, NR; purity, ≥ 93%.	WHO (1998)

HIC, highest ineffective concentration; LEC, lowest effective concentration; NR, not reported; NT, not tested; S9, 9000 × *g* supernatant.
[a] –, negative; (+) or (–), positive or negative in a study of limited quality.

WHO (1998), diphenylamine was found to give weak positive results in the L5178Y ($Tk^{+/-}$) assay over a concentration range of 5–80 µg/mL. [The Working Group noted that the exposure duration and whether this effect was observed with or without metabolic activation were not specified. The report also noted that the dose range was cytotoxic, and the mutation frequency did not increase with dose.]

(iii) Non-mammalian experimental systems

See Table 4.5.

Wakabayashi et al. (1982) found that diphenylamine at 1.0 µmol/plate induced mutations in *S. typhimurium* TA98 but only in the presence of the co-mutagen norharman and with metabolic activation. No mutagenic activity was reported for diphenylamine (without norharman) in the presence or absence of metabolic activation in *S. typhimurium* TA98 or TA100. Epler et al. (1978) also reported that diphenylamine with metabolic activation was not mutagenic in *S. typhimurium* TA100. [The Working Group noted that the dose was not clearly reported in this study.] Similarly, diphenylamine at 100 µg/plate was not found to be mutagenic in *S. typhimurium* TA1538, with or without metabolic activation (Ferretti et al., 1977). [The Working Group noted that positive and negative controls were not included in this study.]

Diphenylamine (dissolved in ethanol) was not mutagenic at a concentration of 3 µmol/plate when spot-tested in *S. typhimurium* TA98, TA100, TA1535, and TA1537, with or without metabolic activation (Florin et al., 1980). [The Working Group noted that, although diphenylamine was reported to be non-mutagenic, there were challenges interpreting the results, and that diphenylamine precipitated at this concentration.] However, Zeiger et al. (1988) tested diphenylamine in similar *S. typhimurium* strains (TA97, TA98, TA100, and TA1535) over a range of concentrations with and without metabolic activation and conclusively determined it to be non-mutagenic. Another study also reported a lack of a mutagenic response with diphenylamine in a modified Ames test in gradient plates and at concentrations ranging from approximately 0.1 to 1000 µg/mL (McMahon et al., 1979; Probst et al., 1981). The bacterial strains tested were *S. typhimurium* (G46, C3076, D3052, TA1535, TA1537, TA1538, TA100, and TA98) and *Escherichia coli* (WP2 and WP2 *uvrA*$^-$) with and without metabolic activation (McMahon et al., 1979; Probst et al., 1981). [The Working Group noted that it was not clear whether negative and positive controls were tested or whether cytotoxicity occurred concurrently in this study. The chemical source but not the purity was reported.] McGregor et al. (1980) also reported a negative mutagenic response with diphenylamine in bacterial and yeast systems. The test systems included *S. typhimurium* (TA1535, TA1537, TA1538, TA98, and TA100), *E. coli* (W3110/polA$^+$ and p3478/polA$^-$), and *Saccharomyces cerevisiae* (D5), with and without metabolic activation (McGregor et al., 1980). [The Working Group noted that no sufficient information on the experimental specifications or dose of the diphenylamine tested were provided.] Moreover, diphenylamine tested negative for mutagenicity in another short-term assay, the SOS chromotest, conducted in *E. coli* PQ37, with and without metabolic activation (von der Hude et al., 1988). Kubo et al. (2002) reported a negative mutagenic response for diphenylamine (1 mM) in *S. typhimurium* strains TA98 and TA100, with and without metabolic activation. Diphenylamine at concentration ranges of 6.67–333 µg/plate and 10–667 µg/plate did not induce mutations in *S. typhimurium* strains TA98 and TA100, TA1535, TA1537, and TA1538 (WHO, 1998). [The Working Group noted that it was not clear whether this effect was with or without metabolic activation.]

Comet assays conducted in haemocytes of adult fatmucket mussels (*Lampsilis siliquoidea)* showed greater percentage of tail DNA when exposed to diphenylamine for 28 days;

Table 4.5 Genetic and related effects of diphenylamine 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			
Lampsilis siliquoidea	DNA damage (comet assay), haemocytes	(+)	NT	0.3 µg/g dw of sediment	Purity, > 95%; no dose-dependent effect; statistical significance attained only at 0.3 but not 2.6, 4.6, 6.6, or 11.6 µg/g dw.	Prosser et al. (2017)
Saccharomyces cerevisiae, D5	Mitotic recombination	(–)	(–)	NR	Purity, NR; dose, NR; S9 from Aroclor-1254-induced male rat liver.	McGregor et al. (1980)
Salmonella typhimurium, TA98 and TA100	Reverse mutation	–	–	1.0 µmol/ plate (without norharman)	Purity, NR; S9 from male rat liver.	Wakabayashi et al. (1982)
TA100	Reverse mutation	NT	(–)	NR	S9 from Aroclor-1254-induced male rat liver.	Epler et al. (1978)
TA1535, TA1537, TA1538, TA98, and TA100	Reverse mutation	(–)	(–)	NR	Purity, NR; liver S9.	McGregor et al. (1980)
TA98, TA100, TA1535, and TA1537	Reverse mutation	(–)	(–)	3 µmol/plate	Precipitation of diphenylamine; S9 from Aroclor-1254-induced male (Sprague-Dawley) rat liver.	Florin et al. (1980)
G46, C3076, D3052, TA1535, TA1537, TA1538, TA100, and TA98	Reverse mutation	(–)	(–)	0.1 µg/mL to 1000 µg/mL	Purity, NR; controls, NR; cytotoxicity, NR; S9 from Aroclor-1254-induced male (Fischer) rat liver.	McMahon et al. (1979); Probst et al. (1981)
TA97, TA98, TA100, and TA1535	Reverse mutation	–	–	333 µg/plate	Dose inferred from secondary reference; S9 from Aroclor-1254-induced rat or hamster liver.	Zeiger et al. (1988)
TA98 and TA100	Reverse mutation	–	–	1 mM	Purity, NR; rat liver S9.	Kubo et al. (2002)
TA98, TA100, TA1535, TA1537, and TA1538	Reverse mutation	(–)	(–)	667 µg/plate	Purity, 99.9%.	WHO (1998)
TA1538	Reverse mutation	(–)	(–)	100 µg/plate	Single dose; purity, NR; no replicates; no positive or negative controls.	Ferretti et al. (1977)
Escherichia coli, W3110/ polA$^+$ and p3478/polA$^-$	Reverse mutation	(–)	(–)	NR	Purity, NR; dose, NR.	McGregor et al. (1980)

Table 4.5 (continued)

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
PQ37, SOS chromotest	DNA damage	(–)	(–)	Limit of solubility or 100 mM	Purity, NR; concentration tested not clear.	von der Hude et al. (1988)
WP2 and WP2 *uvrA*$^-$	Reverse mutation	(–)	(–)	0.1 µg/mL to 1000 µg/mL	Purity, NR; controls, NR; cytotoxicity evaluations, NR.	McMahon et al. (1979); Probst et al. (1981)

dw, dry weight; HIC, highest ineffective concentration; LEC, lowest effective concentration; NT, not tested; NR, not reported; S9, 9000 × *g* supernatant.

[a] –, negative; (+) or (–), positive or negative in a study of limited quality.

however, statistical significance was reported only at the lowest evaluated concentration of 0.3 µg/g dry weight (dw) of sediment (Prosser et al., 2017). No dose–response related effects were observed across the remaining doses of 2.6, 4.6, 6.6, and 11.6 µg/g dw of sediment measured at the start of the experiment. [The Working Group noted that these concentrations translate to 39.56 µg/L in the overlying water for the highest dose group. Water (containing diphenylamine) was replenished in the tanks after 14 days. The Working Group also noted that the diphenylamine concentrations in the water decreased after exposure initiation within each 14-day period.]

4.2.2 Induces oxidative stress

(a) Humans

(i) Exposed humans

No studies were available to the Working Group.

(ii) Human cells in vitro

Diphenylamine at concentrations of 10^{-4} and 10^{-5} M significantly induced increased superoxide anion production by phagocytosing human blood-derived polymorphonuclear leukocytes (Vandenbroucke-Grauls et al., 1984). Furthermore, diphenylamine (at concentrations greater than 0.05 mM) was shown to enhance lipid peroxidation via an intermediate nitrogen-based radical by increasing lipid hydroperoxide formation and oxygen consumption in erythrocytes obtained from healthy donors, therefore contributing to peroxidative stress (Sugihara et al., 1993).

(b) Experimental systems

(i) Non-human mammals in vivo

Diphenylamine was shown to induce oxidative stress in male Wistar rats exposed at a dose of 0.09–1.4 mg/kg bw per day for 10 days by gavage, as determined by the presence of 8-hydroxy-2′-deoxyguanosine (8-OHdG) in liver DNA, a biomarker of oxidative DNA damage (Lodovici et al., 1997) (see Table 4.3).

Regional glutathione concentrations in the kidney cortex were found to be reduced 1 hour after a single oral dose of diphenylamine of 200, 400, or 600 mg/kg bw, and 4 hours after 400 and 600 mg/kg bw in male Syrian hamsters. However, no significant changes in glutathione levels were observed in the renal outer medulla or papilla at any dose tested (Lenz, 1996). [The Working Group noted that measurements of glutathione concentration in the renal papilla might not be reflective of oxidative stress at the capillary endothelium (i.e. decreased renal papillary glutathione levels may not correlate with renal papillary necrosis).]

Diphenylamine induced microsomal and cytosolic glutathione *S*-transferase (GST) activities by 2- and 1.3-fold, respectively, compared with controls, in male albino rats given a single oral dose at one third of the LD_{50} (Semak & Pikulev, 1993). [The Working Group noted that the strain was not reported, and the dose was unclear.]

Oral administration of diphenylamine at a dose of 400, 600, or 800 mg/kg bw per day in peanut oil for 9 days induced renal papillotoxicity in male Syrian hamsters. Exposure to diphenylamine in DMSO, which is a potent scavenger of oxygen-free radicals, inhibited this effect. Pre-treatment of hamsters with DMSO significantly reduced the renal toxicity at day 3 (Lenz & Carlton, 1991).

(ii) Non-human mammalian experimental systems

As discussed in Section 4.1.2, the formation of the diphenylnitroxide free radical has been reported in mammalian microsomal systems treated with diphenylamine in vitro (Appel et al., 1987; Valvis et al., 1990). The rate of oxygen consumption during diphenylamine bio-oxidation was found to be nonlinear and exhibited substrate

inhibition kinetics at diphenylamine concentrations greater than approximately 250 nmol/mL (Valvis et al., 1990).

4.2.3 Evidence relevant to other key characteristics

(a) Humans

Regarding immunosuppression, in studies conducted in vitro with natural killer (NK) cells enriched from human lymphocytes (effector) and a human myeloid leukaemia cell line (target), diphenylamine reduced NK cell activity in a dose-dependent manner with almost no activity observed at a concentration of 1 mM. Diphenylamine was shown to noncompetitively inhibit the kinetics of NK-mediated target cell lysis. However, it did not affect the effector–target cell binding at 1 mM but instead considerably reduced the level and activity of intracellular lysosomal enzymes (Verhoef & Sharma, 1983).

(b) Experimental systems

Regarding immortalization, pre-treatment of normal rat kidney cells with diphenylamine at concentrations of 2.5–20 µg/mL without metabolic activation did not increase the frequency of viral transformation by murine sarcoma virus. With metabolic activation, an increase of 2.5-fold in the frequency of transformation by murine sarcoma virus was induced by diphenylamine; however, this was not found to be statistically significant when compared with controls (Wilson & Khoobyarian, 1982). [The Working Group noted that the mechanisms of chemical carcinogenesis for this assay system were not clearly defined.]

Regarding alterations in cell proliferation, cell death, or nutrient supply, in male and female Fischer 344 rats exposed to diphenylamine at a dose of 1000 mg/kg bw per day for 28 days, necrosis and degeneration of the kidney tubules and erosion of the forestomach were induced (Yoshida et al., 1989). These changes were associated with increased blood leukocyte counts, bone marrow hyperplasia, and forestomach hyperplasia. [The Working Group considered that the increase in leukocytes and bone marrow hyperplasia, which increased the leukocyte counts, were a secondary response to tissue necrosis and degeneration in the kidney and forestomach erosion, and the forestomach hyperplasia was an indicator of mucosal repair in the forestomach.] Exposure of male Sprague-Dawley rats to diphenylamine at 1% in the feed also induced hyperplasia of the tubular cells in the collecting ducts of the kidney at 5 weeks (Evan & Gardner, 1976; Evan et al., 1978). Gershbein (1975) reported that diphenylamine accelerated the rate of liver regeneration in partially hepatectomized male rats treated via the diet (0.5%) for 10 continuous days, when compared with controls. [The Working Group noted that the rat strain was not reported.]

4.2.4 High-throughput in vitro toxicity screening data evaluation

The analysis of the in vitro bioactivity of the agents reviewed in *IARC Monographs* Volume 130 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). Diphenylamine was one of thousands of chemicals tested across the large assay battery of the Tox21 and ToxCast research programmes of the US EPA and the United States National Institutes of Health. Detailed information about the chemicals tested, assays used, and associated procedures for data analysis is publicly available (US EPA, 2021). A supplementary table (Annex 2, Supplementary material for Section 4, Mechanistic Evidence, web only; available from: https://publications.iarc.fr/611) provides a summary of the findings (including the assay name, the corresponding key characteristic, the resulting "hit calls" both positive and negative, and any reported caution flags) for diphenyl-

amine. The results were generated with the software "kc-hits" (key characteristics of carcinogens – high-throughput screening discovery tool) (available from: https://gitlab.com/i1650/kc-hits) using the US EPA ToxCast and Tox21 assay data and the curated mapping of key characteristics to assays available at the time of the evaluations performed for the present monograph. Findings and interpretations from these high-throughput assays for diphenylamine are discussed below.

After mapping against the key characteristics of carcinogens, the ToxCast/Tox21 database contained 294 assays in which diphenylamine was tested. Of these, diphenylamine was found to be active and without caution flags in 14 assays relevant to the key characteristics of carcinogens. [The Working Group noted that the cytotoxic limit for diphenylamine is 8.97 μM.]

Diphenylamine was active in six assays mapped to key characteristic 8 (KC8), "modulates receptor-mediated effects". These assays included: activation of the estrogen response element with a half-maximal activity concentration (AC_{50}) of 52.3 μM; the peroxisome proliferator-activated response element (AC_{50}, 17.7 μM); the pregnane X receptor (PXR) response element (AC_{50}, 35.2 μM); and the human estrogen receptor α (AC_{50}, 47.3 μM). The PXR assay was conducted in the HepG2 cell line; all other assays were conducted in a metabolically enhanced HG19 variant of the HepG2 cell line. Diphenylamine was active in one assay with metabolically competent HepaRG cells that measured changes in the expression of the transcription factors for CYP2B6 ($AC_{50,}$ 23.8 μM). The chemical was also active in one assay with a human adrenal gland cell line, H295R (AC_{50}, 26.3 μM).

In addition, diphenylamine was active in eight assays mapped to KC10, "alters cell proliferation, cell death, or nutrient supply"; however, these assays reported a loss of cell viability.

5. Summary of Data Reported

5.1 Exposure characterization

Diphenylamine is a High Production Volume chemical that is predominantly used in lubricants and greases, hydraulic fluids, metal working fluids, dyes and textile treatment products, including leather and fur. It is also used as an intermediate in the manufacture of other substances, including antioxidants in the rubber and elastomer industries. In addition, it is applied in agriculture to prevent scalding on apples and pears. Use of diphenylamine in agriculture is prohibited in the European Union; however, it is frequently applied to post-harvest fruit in agricultural markets in the USA.

The most relevant occupational exposure routes are respiratory and dermal. The main source of occupational exposure to diphenylamine is during its production and further processing. Pesticide mixers, loaders, and applicators can be exposed to diphenylamine during and after regular use in agriculture and other settings.

Environmental exposure to diphenylamine occurs through the air, in sediment around military bases, sewage, residential dust, electronic waste dust, fruit storage facilities, eggs, water, and fruit (both for infants and other ages). The main route of exposure for the general population is oral intake of diphenylamine, primarily through ingestion of fruit and vegetables. The second is dermal exposure through the use of lubricants.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

Treatment with diphenylamine caused an increase in the incidence of either malignant neoplasms or an appropriate combination of benign and malignant neoplasms in two species.

Diphenylamine was administered by oral administration (in the feed) in one study in male and female Crj:BDF_1 mice. In males, diphenylamine caused an increase in the incidence of haemangioma or haemangiosarcoma (combined) of the liver, of the spleen, and of all organs combined. In females, diphenylamine caused an increase in the incidence of histiocytic sarcoma of the uterus.

Diphenylamine was administered by oral administration (in the feed) in one study in male and female F344/DuCrj rats. In males, diphenylamine caused an increase in the incidence of haemangiosarcoma and haemangioma or haemangiosarcoma (combined) of the spleen and of all organs combined, and of fibroma or fibrosarcoma (combined) of the subcutis. In females, diphenylamine caused an increase in the incidence of adenocarcinoma and adenoma or adenocarcinoma (combined) of the uterus and mononuclear cell leukaemia of the spleen.

5.4 Mechanistic evidence

In two human subjects administered a single oral dose, diphenylamine was absorbed and excreted in the urine as parent compound and/or metabolites. Studies in rats, rabbits, goats, cows, dogs, and laying hens treated with diphenylamine by oral administration showed absorption, tissue distribution without appreciable accumulation, metabolism, and rapid excretion primarily in the urine. In in vitro microsomal systems exposed to diphenylamine, the formation of the diphenylnitroxide free radical has been reported.

Overall, the mechanistic evidence for diphenylamine regarding the key characteristics of carcinogens ("is genotoxic", "induces oxidative stress", "is immunosuppressive", and "alters cell proliferation, cell death, or nutrient supply") is suggestive but incoherent across different experimental systems. There were no studies in humans with exposure specifically attributable to diphenylamine only.

The mechanistic evidence that diphenylamine is genotoxic is suggestive but incoherent across different experimental systems. Diphenylamine gave positive results for micronucleus formation in human peripheral blood lymphocytes in vitro in one study but negative results in the bone marrow of mice in another study. In a few studies, diphenylamine with and without metabolic activation gave negative results for mutagenicity in non-human mammalian systems in vitro and in non-mammalian experimental systems including multiple strains of bacteria. The mechanistic evidence that diphenylamine causes oxidative stress is suggestive based on two studies with positive results in human cells in vitro, four studies with positive results in rodents, and one positive result in vitro using mammalian microsomes.

The mechanistic evidence is also suggestive for the key characteristics "is immunosuppressive" and "alters cell proliferation, cell death, or nutrient supply" based on a few studies. Regarding immunosuppression, diphenylamine reduced human natural killer cell activity in a dose-dependent manner in vitro in one study. Regarding alterations in cell proliferation, cell death, or nutrient supply, diphenylamine induced hyperplasia in several tissues of rats in two studies, and one study reported that diphenylamine accelerated rat liver regeneration.

Diphenylamine was found to be mostly without effects in the assay battery of the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes in the USA.

6. Evaluation and Rationale

6.1 Cancer in humans

There is *inadequate evidence* in humans regarding the carcinogenicity of diphenylamine.

6.2 Cancer in experimental animals

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

6.3 Mechanistic evidence

There is *limited mechanistic evidence*.

6.4 Overall evaluation

Diphenylamine is *possibly carcinogenic to humans (Group 2B)*.

6.5 Rationale

The Group 2B evaluation for diphenylamine is based on *sufficient evidence* for cancer in experimental animals. This *sufficient evidence* in experimental animals is based on an increased incidence of either malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in two species. The evidence regarding cancer in humans is *inadequate* because no studies were available. The mechanistic evidence was *limited* as the findings regarding key characteristics of carcinogens across experimental systems, including in some studies using human cells in vitro, were suggestive, but incoherent.

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