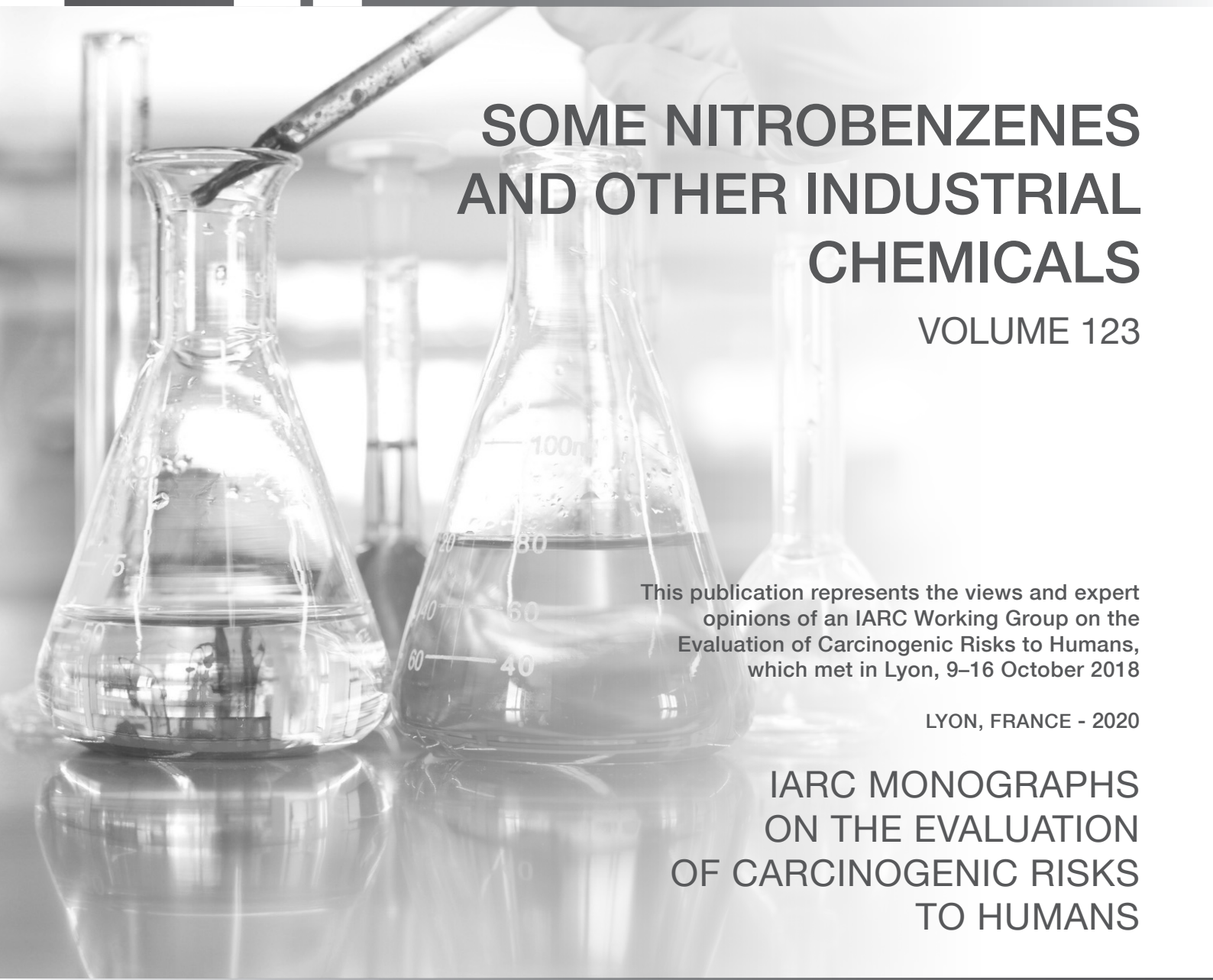
IARC MONOGRAPHS



SOME NITROBENZENES AND OTHER INDUSTRIAL CHEMICALS

VOLUME 123

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International Agency for Research on Cancer

World Health Organization

ORTHO-PHENYLENEDIAMINE AND *ORTHO*-PHENYLENEDIAMINE DIHYDROCHLORIDE

1. Exposure Data

ortho-Phenylenediamine, the parent compound of *ortho*-phenylenediamine dihydrochloride, is a basic compound and will undergo acid–base reactions. *ortho*-Phenylenediamine and its dihydrochloride salt will undergo a pH-dependent acid–base equilibrium in the body.

1.1 *ortho*-Phenylenediamine

1.1.1 Identification of the agent

(a) Nomenclature

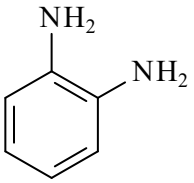
Chem. Abstr. Serv. Reg. No.: 95-54-5

Chem. Abstr. Serv. name: *ortho*-phenylenediamine

IUPAC systematic name: *ortho*-phenylenediamine

Synonyms: 2-aminoaniline; 1,2-benzenediamine; 1,2-diaminobenzene; 1,2-phenylenediamine; 2,3-diaminobenzene; orthamine; *o*-phenylenediamine.

(b) Structural and molecular formula, and relative molecular mass



Molecular formula: $C_6H_8N_2$

Relative molecular mass: 108.14

(c) Chemical and physical properties of the pure substance

Description: solid, crystalline powder, colourless to weak red; can react dangerously with oxidizing agents and concentrated acid (IFA, 2018); colourless monoclinic crystals if pure; technical-grade brownish-yellow crystals or a sandy brown solid when containing oxidized impurities (DFG, 1999)

Density (at 20 °C*)*: 1.14 g/cm^3 (IFA, 2018)

Relative vapour density (air = 1): 3.73 (PubChem, 2018)

Octanol/water partition coefficient (P): log K_{ow} = 0.15 (HSDB, 2013)

Melting point: 99–102 °C (IFA, 2018); 102.1 °C (HSDB, 2013)

Boiling point: 257 °C (IFA, 2018)

Volatility: vapour pressure, 2.06×10^{-3} mm Hg at 25 °C (HSDB, 2013)

Solubility: 4.07×10^{4} mg/L in water at 35 °C; soluble in water and freely soluble in alcohol, chloroform, ether, and benzene (HSDB, 2013)

Flammable limits: lower explosion limit, 1.5 vol% (ILO, 2017)

Flash point: 110 °C (IFA, 2018)

Ignition temperature: 540 °C (IFA, 2018)

Purity (technical grade): 99% or greater (HSDB, 2013)

Dissociation constants (of the conjugated acids BH^+): $pK_a(1) < 2$; $pK_a(2) = 4.47$ (at 25 °C) (HSDB, 2013).

1.1.2 Production and use

(a) Production process

ortho-Phenylenediamine is produced by hydrogenation of 2-nitroaniline, which is obtained by the amination of 2-chloronitrobenzene with ammonia. Commercial hydrogenation is achieved by using palladium catalysts, but iron, hydrazine, or hydrogen sulfide can also be used (Smiley, 2002).

(b) Production volume

ortho-Phenylenediamine was listed as a chemical with a high production volume in 1990, with a production volume in or import into the USA of greater than 1 million pounds [~453 tonnes] (HSDB, 2013). In the USA, the production range was 1–10 million pounds [~453–4535 tonnes] between 1986 and 2002 and in 2006 (HSDB, 2013). Two manufacturers are currently listed in the USA (HSDB, 2013). *ortho*-Phenylenediamine was not listed as a chemical with a high production volume in 2004 (OECD, 2004), but was listed as such in 2009 (OECD, 2009). Annual production and import of *ortho*-phenylenediamine in 2008 in Japan was approximately 2300 tonnes (Matsumoto et al., 2012). It is currently manufactured in or imported into the European Economic Area, but the European Chemicals Agency has no information on annual production or import volume; the registration requirements suggest that this volume is less than 1 tonne (ECHA, 2018). Quantities produced and used elsewhere in the world were not available to the Working Group.

(c) Use

ortho-Phenylenediamine is used as an important precursor (intermediate) in the production of a wide variety of heterocyclic compounds, including synthetic dyes and pigments that are used as colorants for furs and hair dye (HSDB, 2013). Heterocyclic compounds based on *ortho*-phenylenediamine are also widely used in the manufacture of agrochemicals, antioxidants in rubber products, corrosion inhibitors, polyamides, ultraviolet absorbers, and pharmaceuticals (HSDB, 2013).

1.1.3 Methods of measurement and analysis

(a) Air

Early methods for the determination of *ortho*-phenylenediamine in air samples (stationary sampling) relied on its direct collection in acetic anhydride in fritted bubblers, its conversion to the corresponding diacetamide derivative, and analysis by high-performance liquid chromatography (HPLC) and ultraviolet (UV) detection (Burg et al., 1980). To make the method applicable for personal air sampling, Tenax tubes have been used to collect *ortho*-phenylenediamine followed by desorption and derivatization to the corresponding diacetamide derivative (Elia et al., 1982). The limits of detection using HPLC-UV were dependent on the sampling capacity of the Tenax tubes and sampling flow rates. Air concentrations as low as 0.05 mg/m^3 have been analysed using this method (Elia et al., 1982). Another method for the measurement of *ortho*-phenylenediamine in

air samples is available, and relies on the chemical conversion of aromatic amines to amine salts when collected on fibreglass filters coated with dilute sulfuric acid (OSHA, 1991). The filters are extracted with an aqueous ethylenediaminetetraacetic acid (EDTA) solution and the extracts are analysed for the free amine by HPLC-UV. A similar method has been described by the Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK commission) of the German Research Foundation (DFG, 2002) by using a fibreglass filter impregnated with hydrochloric acid rather than sulfuric acid. The adsorbed *ortho*-phenylenediamine is desorbed with a mixture of acetonitrile and aqueous ammonia and analysed by HPLC-UV.

(b) Other environmental media

Several methods are capable of analysing *ortho*-phenylenediamine in water samples. A method based on the induction of chemiluminescence by *ortho*-phenylenediamine in the presence of the superoxide anion radical has been developed to analyse *ortho*-phenylenediamine in shampooing wastewater after dying hair (Zhou et al., 2004). A colorimetric method for the determination of *ortho*-phenylenediamine in water samples, which relies on the formation of silver nanoparticles by the reduction of silver ions in the presence of *ortho*-phenylenediamine and the use of an UV–visible spectrophotometer, has also been described (Li et al., 2015); the limit of detection was approximately 0.2 µmol/L. A method using surface-enhanced Raman spectroscopy has also been developed for the analysis of *ortho*-phenylenediamine in water samples, based on its chemical cyclization with nitrite to benzotriazole (Ma et al., 2015); the limit of detection was reported as 30 nmol/L.

Multiple analytical methods have been described for the determination of *ortho*-phenylenediamine in hair dyes and cosmetic products, including the use of HPLC-UV or diode array detection (Rastogi, 2001; Zhou et al., 2004; Lai et al., 2012) and electrophoresis with amperometric detection (Wang & Huang, 2005; Dong et al., 2008). To prevent oxidation of the target compound, the addition of sodium dithionite has been suggested (Lai et al., 2012). Depending on the method, limits of detection as low as 10 µg/L can be achieved (Dong et al., 2008).

(c) Biomarkers

No biomonitoring methods for the assessment of exposure to *ortho*-phenylenediamine were identified in the literature. [The Working Group noted that measurement of *ortho*-phenylenediamine in urine, although not entirely specific, might serve as a biomarker of exposure; similar to the determination of *para*-phenylenediamine in urine (Gube et al., 2011; Bhandari et al., 2016; Mohamed & Steenkamp, 2016).]

Similar to other amino- and nitroaromatic compounds, *ortho*-phenylenediamine induces the formation of methaemoglobin in blood. Methaemoglobin levels above 1.5% (ACGIH, 2006) or 5% (Leng & Bolt, 2008) have previously been suggested as a general and non-specific biomarker of occupational exposure to aromatic amino- and nitroaromatic compounds.

1.1.4 Occurrence and exposure

(a) Environmental occurrence

ortho-Phenylenediamine is not known to occur naturally in the environment.

Release of *ortho*-phenylenediamine to the environment can occur via multiple waste streams from its industrial use, including in the manufacture of the substance and its use as an intermediate in downstream uses (ECHA, 2018). However, the European Chemicals Agency has no public registered data on routes of release of this substance into the environment (ECHA, 2018).

ortho-Phenylenediamine has been detected in China in samples of tap water and lake water (taken from Lake Xuanwu) at concentrations of 1.8–6.9 µmol/L (Li et al., 2015); however, the concentrations of *ortho*-phenylenediamine were below the limit of detection (30 nmol/L) in tap, lake, reservoir, and river water in Changchun, China (Ma et al., 2015). If released to the aquatic system, the estimated volatilization from water surfaces is low based on its low vapour pressure, moderate solubility in water, and its pK_a values of < 2 and 4.47, indicating that *ortho*-phenylenediamine will exist partially in its cation form at pH values of 5–9 (HSDB, 2013). However, the low octanol/water partition coefficient of *ortho*-phenylenediamine suggests a low potential for bioconcentration in aquatic organisms (HSDB, 2013); treatment of wastewater by ozone has been shown to effectively remove *ortho*-phenylenediamine (Arowo et al., 2016).

No data on the concentrations of *ortho*-phenylenediamine could be found for other environmental media. Based on a model of gas/particle partitioning of semivolatile organic compounds in the atmosphere, if released to air *ortho*-phenylenediamine is expected to exist solely as a vapour in the ambient atmosphere (Bidleman, 1988). It is susceptible to photodegradation by sunlight as a result of UV absorption at wavelengths greater than 290 nm, and can degrade in the atmosphere with an estimated half-life of 0.7 hours (HSDB, 2013). If released to soil, *ortho*-phenylenediamine is expected to have a low mobility as a result of the high reactivity of the aromatic amino group towards mineral contents (Bollag et al., 1978; Adrian et al., 1989). *ortho*-Phenylenediamine is prone to microbial degradation in experimental studies. After 5 days of incubation in an aerobic screening study using activated sludge, 33% of the substance was degraded (Pitter, 1976). A decomposition period of longer than 64 days was determined in a screening test using soil microflora (Alexander & Lustigman, 1966).

(b) Exposure in the general population

The presence of *ortho*-phenylenediamine in some hair dyes indicates that selected individuals within the general population will be exposed to the compound. Exposure is more likely in countries outside the European Union, where the use of *ortho*-phenylenediamine as an ingredient of hair dyes was banned in 2007 (EPA, 2016). The occurrence of *ortho*-phenylenediamine in hair dye has been evaluated in several studies in China and Italy; no *ortho*-phenylenediamine was detected in the majority of these studies (Tokuda et al., 1986; Gennaro et al., 1990; Wu et al., 2011; Lai et al., 2012). However, the analysis of hair dye samples of different colours from a local supermarket in Shanghai, China yielded concentrations of up to 109 µmol/L in six out of the eight samples (Dong et al., 2008). *ortho*-Phenylenediamine was consistently below the limit of quantification in 30 hair dye creams (15 each from Japan and China, six different colorants) (Zhong et al., 2012).

ortho-Phenylenediamine occurs as a degradation product of thiophanate methyl and benomyl, two benzimidazole fungicides (HSDB, 2013). [The Working Group noted that individuals may be potentially exposed to *ortho*-phenylenediamine in agricultural settings in which these fungicides have been applied.]

The United States Environmental Protection Agency (EPA) estimated a median daily intake of 3.62×10^{-8} mg/kg body weight (bw) per day for *ortho*-phenylenediamine in the general population, with a 95th percentile of 3.98×10^{-6} mg/kg bw per day (EPA, 2018). [The Working Group noted that the data forming the basis of this estimate (diet, drinking-water, etc.) could not be located.]

(c) Occupational exposure

Quantitative occupational exposures to *ortho*-phenylenediamine have not been reported in the literature. However, approximately 3000

workers who participated in the National Occupational Exposure Survey of the United States National Institute of Occupational Safety and Health (NIOSH), conducted between 1981 and 1983, were potentially exposed to *ortho*-phenylenediamine (HSDB, 2013). According to the 2006 Toxic Substances Control Act Inventory Update Reporting data, the number of people potentially exposed to *ortho*-phenylenediamine in United States workplaces is 1000 or greater (HSDB, 2013).

Occupational exposure is expected to occur primarily through inhalation in workplaces where *ortho*-phenylenediamine is produced or used as an intermediate in the manufacture of other products, such as heterocyclic compounds used in dyes, agrochemicals, and corrosion inhibitors (HSDB, 2013; IFA, 2018). Inhalation may be particularly likely if the substance is heated, as a result of release vapours or sublimation dusts (IFA, 2018). Skin intake may also occur, although a low rate of penetration of the epidermis is expected based on a permeability constant of 0.45 mm per hour (Bronaugh & Congdon, 1984). [The Working Group noted that inadvertent ingestion may also occur.]

Clinical records over a 7-year period (1975–1982) were evaluated for 27 workers in a phenylenediamine manufacturing chemical plant in the USA (EPA, 2016). Haemoglobin and oxygen saturation levels among these employees did not differ from normal levels. However, specific exposure data in this workplace, such as air concentrations of the individual phenylenediamine isomer(s), were unknown.

1.1.5 Regulations and guidelines

According to the Globally Harmonized System of Classification and Labelling of Chemicals, *ortho*-phenylenediamine is suspected of causing cancer (H351, category 2) and mutagenic defects (H341, category 2). *ortho*-Phenylenediamine is also toxic if swallowed (H301, category 3), inhaled (H332, category 4), or taken up via the skin (H312, category 4), and can cause skin sensitization (H317, category 1) and eye irritation (H319, category 2) (ECHA, 2018).

A threshold limit value averaged over 8 hours for *ortho*-phenylenediamine of 0.1 mg/m^3 has been assigned by several national authorities worldwide (ACGIH, 2001; Matsumoto et al., 2012; IFA, 2018). Short-term limit values of 0.4, 0.2, and 10 mg/m^3 have been assigned in Austria, Denmark, and Romania (15-minute average value), respectively (IFA, 2018).

1.2 *ortho*-Phenylenediamine dihydrochloride

1.2.1 Identification of the agent

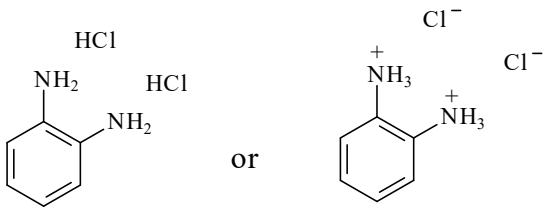
(a) Nomenclature

Chem. Abstr. Serv. Reg. No.: 615-28-1

Chem. Abstr. Serv. name: *ortho*-phenylenediamine dihydrochloride

Synonyms: 1,2-phenylenediamine dihydrochloride; benzene-1,2-diamine dihydrochloride; *o*-phenylenediamine dihydrochloride; 1,2-benzenediamine dihydrochloride.

(b) Structural and molecular formulae, and relative molecular mass



Molecular formula: $C_6H_{10}Cl_2N_2$

Relative molecular mass: 181.06

(c) Chemical and physical properties of the pure substance

Description: slightly yellow crystalline solid (IFA, 2018)

Melting point: 258 °C (Chemspider, 2018)

Density: 1.17 g/cm^3 (EPA, 2018)

Solubility: very soluble in water, 0.405 mol/L (EPA, 2018)

Octanol/water partition coefficient (P): log K_{ow} = 0.131 (EPA, 2018)

Stability: combustible substance, poorly flammable; the substance decomposes to hydrogen chloride and nitrogen oxides, and can react dangerously with oxidizing agents (IFA, 2018)

Impurities: none known.

1.2.2 Production and use

ortho-Phenylenediamine dihydrochloride is used as a chemical laboratory reagent, and as an intermediate in the manufacture of dyes, coatings, and photographic chemicals (Matsumoto et al., 2012; OEHHA, 2018).

Less than 1 tonne of *ortho*-phenylenediamine dihydrochloride is manufactured or used in the European Union (ECHA, 2018). Quantities produced and used elsewhere in the world are unknown.

1.2.3 Methods of measurement and analysis

No specific measurement methods are available for *ortho*-phenylenediamine dihydrochloride.

1.2.4 Occurrence and exposure

ortho-Phenylenediamine dihydrochloride does not occur naturally. Accidental environmental releases of this chemical are likely to accumulate in water; contamination of air and soil are less likely. Exposure outside the workplace is unlikely to occur (HSDB, 2013).

Occupational exposure may arise from inhalation of aerosols, skin contact, and inadvertent ingestion (HSDB, 2013).

1.2.5 Regulations and guidelines

ortho-Phenylenediamine dihydrochloride is covered by generic regulations relating to hazardous chemicals, although it is not registered under the European Union Registration, Evaluation, Authorisation and Restriction of Chemicals regulations (ECHA, 2018). There are no occupational exposure limits for *ortho*-phenylenediamine dihydrochloride.

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

See Table 3.1

3.1 Mouse

3.1.1 Oral administration in drinking-water

To assess the carcinogenicity of *ortho*-phenylenediamine, in a study that complied with good laboratory practice (GLP), randomized groups of 50 male and 50 female Crj:BDF$_1$ mice (age, 6 weeks) were given drinking-water containing *ortho*-phenylenediamine dihydrochloride (purity, 99.5%) at a concentration of 0, 500, 1000, or 2000 ppm (0, 46, 94, or 177 mg/kg bw per day) in males and 0, 1000, 2000, or 4000 ppm (0, 106, 200, or 391 mg/kg bw per day) in females for 2 years (104 weeks). All animals underwent complete necropsy. The survival of male and female mice was not affected in exposed groups;

Table 3.1 Studies of carcinogenicity with *ortho*-phenylenediamine dihydrochloride in experimental animals

Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Mouse, Crj:BDF$_1$ (M) 6 wk 104 wk Matsumoto et al. (2012)	Oral *ortho*-Phenylenediamine dihydrochloride, 99.5% Drinking-water 0, 500, 1000, 2000 ppm 50, 50, 50, 50 38, 38, 42, 39	*Liver* Hepatocellular adenoma 12/50, 25/50*, 34/50*, 35/50* Hepatocellular carcinoma 6/50, 9/50, 12/50, 10/50 Hepatocellular adenoma or carcinoma (combined) 18/50, 29/50*, 39/50**, 38/50** *Gall bladder*: papillary adenoma 0/46, 2/50, 4/49, 5/47*	 $P < 0.01$, Peto trend test; $^*P < 0.01$, Fisher exact test NS $P < 0.01$, Peto trend test; $^*P < 0.05$, $^{**}P < 0.01$, Fisher exact test $P < 0.05$, Peto trend test; $^*P < 0.05$, Fisher exact test	Principal strengths: well-conducted GLP study; stratified randomization of animals; males and females used Historical control incidence for 1296 mice (range): hepatocellular adenoma, 17.8% (4–34%); hepatocellular carcinoma, 20.4% (2–42%) (JBRC, 2004a) Gall bladder papillary adenoma was not observed in 1296 male historical controls
Mouse, Crj:BDF$_1$ (F) 6 wk 104 wk Matsumoto et al. (2012)	Oral *ortho*-Phenylenediamine dihydrochloride, 99.5% Drinking-water 0, 1000, 2000, 4000 ppm 50, 50, 50, 50 24, 29, 28, 34	*Liver* Hepatocellular adenoma 6/50, 22/50*, 23/50*, 34/50* Hepatocellular carcinoma 1/50, 4/50, 11/50*, 17/50* Hepatocellular adenoma or carcinoma (combined) 6/50, 23/50*, 31/50*, 41/50* *Gall bladder*: papillary adenoma 0/50, 1/50, 5/50*, 3/50	 $P < 0.01$, Peto trend test; $^*P < 0.01$, Fisher exact test $P < 0.01$, Peto trend test; $^*P < 0.01$, Fisher exact test $P < 0.01$, Peto trend test; $^*P < 0.01$, Fisher exact test $^*P < 0.05$, Fisher exact test	Principal strengths: well-conducted GLP study; stratified randomization of animals; males and females used Historical control incidence for 1298 mice (range): hepatocellular adenoma, 5.1% (0–10%); hepatocellular carcinoma, 2.5% (0–8%) (JBRC, 2004a) Gall bladder papillary adenoma was not observed in 1298 female historical controls

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Mouse, albino CD-1 derived from HaM/ICR mice (Charles River) (M) 6–8 wk 21 mo Weisburger et al. (1978), Sontag (1981)	Oral *ortho*-Phenylenediamine dihydrochloride; among the 21 tested chemicals in the study, most were 97–99% pure Diet 0, 6872, 13 743 (time-weighted average), 0 (pooled control) mg/kg diet for 18 mo, then control diet for 3 mo 25, 25, 25, 99 NR	*Liver* Hepatocellular carcinoma 0/14, 5/17*, 3/14, 7/99	*$P < 0.025$ (vs concurrent or pooled controls), Fisher exact test	Principal strengths: mice randomly allocated to groups; males and females used Principal limitations: only two dose groups; lack of reported details on histopathology; only 25 mice per group The doses were increased from 4000 and 8000 mg/kg diet after 5 mo to 8000 and 16 000 mg/kg diet for the next 13 mo Histopathology was only conducted on mice surviving until 6 mo
Mouse, albino CD-1 derived from HaM/ICR mice (Charles River) (F) 6–8 wk 21 mo Weisburger et al. (1978), Sontag (1981)	Oral *ortho*-Phenylenediamine dihydrochloride; among the 21 tested chemicals in the study, most were 97–99% pure Diet 0, 6872, 13 743 (time-weighted average), 0 (pooled control) mg/kg diet for 18 mo, then control diet for 3 mo 25, 25, 25, 102 NR	*Liver:* hepatocellular carcinoma 1/15, 6/18*, 6/15**, 1/102	*$P < 0.025$ (vs pooled control), **$P < 0.025$ (vs concurrent or pooled controls), Fisher exact test	Principal strengths: mice randomly allocated to groups; males and females used Principal limitations: only two dose groups; lack of reported details on histopathology; only 25 mice per group The doses were increased from 4000 or 8000 mg/kg diet after 5 mo to 8000 or 16 000 mg/kg diet for the next 13 mo Histopathology was only conducted on mice surviving until 6 mo

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Rat, F344/DuCrj (M) 6 wk 104 wk Matsumoto et al. (2012)	Oral *ortho*-Phenylenediamine dihydrochloride, 99.5% Drinking-water 0, 500, 1000, 2000 ppm 50, 50, 50, 50 41, 36, 42, 42	*Liver* Hepatocellular adenoma 3/50, 2/50, 12/50*, 15/50**	$P < 0.01$, Peto trend test; $^{*}P < 0.05$, $^{**}P < 0.01$, Fisher exact test	Principal strengths: well-conducted GLP study; stratified randomization of animals; males and females used Historical control incidence for 1499 rats (range): hepatocellular adenoma, 1.4% (0–6%); hepatocellular carcinoma, 0.3% (0–2%); hepatocellular adenoma or carcinoma (combined), 1.7% (0–6%) Historical control incidence of urinary bladder transitional cell papilloma, 6/1498 (maximum of 2% in any study) Historical control incidence of thyroid follicular adenoma (1493 animals), 0.8% (range, 0–4%) (JBRC, 2004b) In rats at the highest dose, the incidence of urinary bladder transitional cell papilloma and transitional cell carcinoma exceeded the historical control range
		Hepatocellular carcinoma 1/50, 1/50, 6/50, 10/50*	$P < 0.01$, Peto trend test; $^{*}P < 0.01$, Fisher exact test	
		Hepatocellular adenoma or carcinoma (combined) 4/50, 3/50, 16/50*, 22/50*	$P < 0.01$, Peto trend test; $^{*}P < 0.01$, Fisher exact test	
		Urinary bladder Transitional cell papilloma 1/50, 0/50, 0/50, 6/50 (12%)	$P < 0.01$, Peto trend test	
		Transitional cell carcinoma 1/50, 0/50, 0/50, 4/50 (8%)	$P < 0.05$, Peto trend test	
		Transitional cell papilloma or carcinoma (combined) 2/50, 0/50, 0/50, 10/50*	$P < 0.01$, Peto trend test; $^{*}P < 0.05$, Fisher exact test	
		Thyroid Follicular adenoma 0/50, 1/50, 0/50, 4/50 (8%)	$P < 0.01$, Peto trend test	
		Follicular adenocarcinoma 1/50, 0/50, 1/50, 1/50	NS	

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Rat, F344/DuCrj (F) 6 wk 104 wk Matsumoto et al. (2012)	Oral *ortho*-Phenylenediamine dihydrochloride, 99.5% Drinking-water 0, 250, 500, 1000 ppm 50, 50, 50, 50 41, 38, 44, 41	*Liver* Hepatocellular adenoma 1/50, 3/50, 15/50*, 36/50* Hepatocellular carcinoma 0/50, 0/50, 4/50, 18/50* Hepatocellular adenoma or carcinoma (combined) 1/50, 3/50, 19/50*, 44/50* *Thyroid* Follicular adenoma 1/50, 0/50, 1/50, 0/50 Follicular adenocarcinoma 0/50, 0/50, 1/50, 0/50 *Urinary bladder* Transitional cell papilloma 1/50, 0/50, 1/50, 1/50 Transitional cell carcinoma 0/50, 0/50, 0/50, 0/50	 $P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test $P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test $P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test NS NS NS –	Principal strengths: well-conducted GLP study; stratified randomization of animals; males and females used Historical control incidence for 1447 rats (range): hepatocellular adenoma, 1.2% (0–6%); hepatocellular carcinoma, 0.1% (0–2%); hepatocellular adenoma or carcinoma (combined), 1.3% (0–8%) Historical control incidence of urinary bladder transitional cell papilloma is 8/1445 with a maximum of 2% in any study (JBRC, 2004b)

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Rat, Charles River CD (M) 6–8 wk 24 mo Weisburger et al. (1978)	Oral *ortho*-Phenylenediamine dihydrochloride; among the 21 tested chemicals in the study, most were 97–99% pure Diet 0, 2000, 4000, 0 (pooled control) mg/kg diet for 18 mo, then control diet for 6 mo 25, 25, 25, 111 NR	*Liver*: hepatocellular carcinoma 0/16, 0/14, 5/16*, 2/111	*$P < 0.025$ (vs simultaneous or pooled control), Fisher exact test	Principal strengths: rats randomly allocated to groups Principal limitations: only males were used; only two dose groups; lack of reported details on histopathology; only 25 rats per group Histopathology conducted only on rats surviving until 6 mo

F, female; GLP, good laboratory practice; M, male; mo, month; NR, not reported; NS, not significant; ppm, parts per million; vs, versus; wk, week

survival rates were 38/50, 38/50, 42/50, and 39/50 in males, and 24/50, 29/50, 28/50, and 34/50 in females. Terminal body weights were decreased significantly in male and female mice for all exposed groups (Matsumoto et al., 2012; see also JBRC, 2004a).

There was a statistically significant ($P < 0.01$, Peto trend test) positive trend and significant increase in the incidence of hepatocellular adenoma in all exposed male mice (12/50, 25/50, 34/50, and 35/50; $P < 0.01$, Fisher exact test) and in all exposed female mice (6/50, 22/50, 23/50, and 34/50; $P < 0.01$, Fisher exact test). The incidence of hepatocellular adenoma or carcinoma (combined) (18/50, 29/50, 39/50, and 38/50) was significantly increased in male mice at 500 ppm ($P < 0.05$, Fisher exact test) and at 1000 and 2000 ppm ($P < 0.01$, Fisher exact test), with a significant positive trend ($P < 0.01$, Peto trend test). The incidence of hepatocellular adenoma or carcinoma (combined) (6/50, 23/50, 31/50, and 41/50) was significantly ($P < 0.01$, Fisher exact test) increased in all exposed female mice, with a significant positive trend ($P < 0.01$, Peto trend test). Female mice also showed a significant ($P < 0.01$, Peto trend test) positive trend for hepatocellular carcinoma (1/50, 4/50, 11/50, and 17/50), the incidence of which was significantly ($P < 0.01$, Fisher exact test) increased in those exposed at 2000 and 4000 ppm. The increased incidence of hepatocellular carcinoma in exposed male mice (6/50, 9/50, 12/50, and 10/50) was not significant. In male mice, the incidence of hepatocellular adenoma in historical controls (1296 animals) (JBRC, 2004a) was 17.8% (range, 4–34%), and the incidence of hepatocellular carcinoma in historical controls was 20.4% (range, 2–42%). [The Working Group noted that the incidence of hepatocellular carcinoma in males did not exceed the range for historical controls.]

In both male and female mice, *ortho*-phenylenediamine dihydrochloride caused a significant increase in the incidence of papillary adenoma of the gall bladder for males exposed at the highest dose (0/46, 2/50, 4/49, and 5/47; $P < 0.05$, Fisher exact test) and for females exposed at the intermediate dose (5/50 compared with 0/50 controls; $P < 0.05$, Fisher exact test) with a significant positive trend ($P < 0.05$, Peto trend test). Matsumoto et al. (2012) stated that papillary adenoma of the gall bladder was not observed in historical controls of the JBRC database of 1296 male and 1298 female Crj:BDF_1 mice.

Significant increases in the incidence of non-neoplastic lesions were observed for most of the treated groups in the liver (acidophilic cell foci and basophilic cell foci for male and female mice, and clear cell foci in female mice) and, for some of the treated groups, in the gall bladder (papillary hyperplasia in male and female mice). [The Working group noted that this was a well-conducted GLP study, with stratified randomization of the animals and the use of historical controls for tumours.]

3.1.2 Oral administration in diet

In a study by Weisburger et al. (1978), randomized groups of 25 male and 25 female albino CD-1 mice (age, 6–8 weeks) were fed diet containing *ortho*-phenylenediamine dihydrochloride (21 chemicals were tested in the study; purity of most, 97–99%) at a concentration of 0, 6872, or 13 743 mg/kg diet (time-weighted average) (Sontag, 1981) for 18 months. Mice were first exposed at 0, 4000, or 8000 mg/kg diet for 5 months, then at 0, 8000, or 16 000 mg/kg diet for another 13 months, and were then kept for 3 months on control diet. Only mice older than 6 months were necropsied, resulting in reported groups of 14, 17, and 14 males, and of 15, 18, and 15 females. There was a pooled control group of 99 males and 102 females [no additional details provided]. [No information on body weights was provided.] Tissues examined histopathologically included all grossly abnormal organs, tumour masses, lung, liver, spleen, kidney, adrenal gland, heart, urinary bladder, stomach, intestines, and

reproductive organs (Weisburger et al., 1978). For male mice, the incidence of hepatocellular carcinoma in the group exposed at the lower dose (5/17, 29%) was significantly increased compared with controls (0/14 concurrent controls and 7/99 (7%) pooled controls; $P < 0.025$, Fisher exact test for both comparisons). For female mice, there was a significant increase in the incidence of hepatocellular carcinoma for the group exposed at the lower dose – 6/18 (33%) compared with 1/102 (1%) pooled controls; $P < 0.025$, Fisher exact test – and for the group exposed at the higher dose – 6/15 (40%) compared with 1/15 (7%) concurrent or 1/102 (1%) pooled controls; $P < 0.025$ for both comparisons, Fisher exact test. [The Working Group noted that the limitations of this study included the small number of mice at the start and the small number necropsied; the use of only two dose groups; and the limited histopathological examination and reporting.]

3.2 Rat

3.2.1 Oral administration in drinking-water

To assess the carcinogenicity of *ortho*-phenylenediamine, in a GLP study Matsumoto et al. (2012) (see also JBRC, 2004b) gave randomized groups of 50 male and 50 female Fischer 344/DuCrj rats (age, 6 weeks) drinking-water containing *ortho*-phenylenediamine dihydrochloride (purity, 99.5%) at a concentration of 0, 500, 1000, or 2000 ppm in males (0, 22, 42, or 86 mg/kg bw per day), and 0, 250, 500, or 1000 ppm in females (0, 18, 33, or 58 mg/kg bw per day) for 2 years (104 weeks). All animals underwent complete necropsy. The survival of male and female rats was not affected in exposed groups; survival rates in males were 41/50, 36/50, 42/50, and 42/50, and in females were 41/50, 38/50, 44/50, and 41/50. Terminal body weights were decreased significantly in male rats in all exposed groups and in female rats exposed at the two higher concentrations.

There was a statistically significant positive trend ($P < 0.01$, Peto trend test) and significant increase in the incidence of hepatocellular adenoma in male rats – 3/50, 2/50, 12/50 ($P < 0.05$, Fisher exact test), and 15/50 ($P < 0.01$, Fisher exact test) – and in female rats – 1/50, 3/50, 15/50 ($P < 0.01$, Fisher exact test), and 36/50 ($P < 0.01$, Fisher exact test). The incidence of hepatocellular carcinoma was significantly ($P < 0.01$, Fisher exact test) increased in males (1/50, 1/50, 6/50, and 10/50) and females (0/50, 0/50, 4/50, and 18/50) at the highest dose, with a significant positive trend ($P < 0.01$, Peto trend test). The incidence of hepatocellular adenoma or carcinoma (combined) was significantly ($P < 0.01$, Fisher exact test) increased in males (4/50, 3/50, 16/50, and 22/50) and females (1/50, 3/50, 19/50, and 44/50) at the two higher doses, with a significant positive trend ($P < 0.01$, Peto trend test).

In male rats only, there were statistically significant positive trend for transitional cell papilloma of the urinary bladder (1/50, 0/50, 0/50, and 6/50 (12%); $P < 0.01$, Peto trend test), transitional cell carcinoma of the urinary bladder (1/50, 0/50, 0/50, and 4/50; $P < 0.05$, Peto trend test), and transitional cell papilloma or carcinoma (combined) (2/50, 0/50, 0/50, and 10/50; $P < 0.01$, Peto trend test); the incidence of transitional cell papilloma or carcinoma (combined) at the highest dose (10/50, 20%) was significantly ($P < 0.05$, Fisher exact test) increased compared with controls (2/50, 4%). JBRC historical control incidence in males for transitional cell papilloma of the urinary bladder (JBRC, 2004b) was 6/1498 (0.4%; range, 0–2%). [The Working Group noted that, although Matsumoto et al. (2012) stated that the incidence of transitional cell carcinoma of the urinary bladder exceeded the historical control range in the group exposed at the highest dose, no numerical values were provided.]

For male rats, the incidence of follicular adenoma of the thyroid (0/50, 1/50, 0/50, and 4/50 (8%)) showed a statistically significant positive trend ($P < 0.01$, Peto trend test). For follicular adenoma of the thyroid in males, the historical incidence was 13/1493 (0.8%; range, 0–4%) (JBRC, 2004b).

Significant increases in the incidence of non-neoplastic lesions in the liver were observed for some of the dose groups (basophilic cell foci for male and female rats, and clear cell foci in male rats) and urinary bladder (papillary and/or nodular hyperplasia in male rats). [The Working group noted that this was a well-conducted GLP study, with stratified randomization of the animals and the availability of historical controls for tumours.]

3.2.2 Oral administration in diet

Randomized groups of 25 male Charles River CD rats (age, 6–8 weeks) were fed diet (Purina laboratory chow) containing *ortho*-phenylenediamine dihydrochloride (21 chemicals were tested in the study; purity of most, 97–99%) at a concentration of 0, 2000, or 4000 mg/kg diet for 18 months, then 6 months on control diet (Weisburger et al., 1978). Only rats older than 6 months were necropsied, resulting in reported groups of 16, 14, and 16 males. There was a pooled control group of 111 male rats [no additional details provided]. [No information on body weights was provided.] Tissues examined histopathologically included all grossly abnormal organs, tumour masses, lung, liver, spleen, kidney, adrenal gland, heart, urinary bladder, stomach, intestines, reproductive organs, and pituitaries (Weisburger et al., 1978). The incidence of hepatocellular carcinoma in the group at the higher dose (5/16, 31%) was significantly increased ($P < 0.025$, Fisher exact test) compared with concurrent (0/16) and pooled (2/111, 2%) controls. The incidence of hepatocellular carcinoma in the group at the lower dose was 0/14. [The Working Group noted that the limitations of the study included the small number of animals at the start and the small number necropsied; the use of only two dose groups; and the limited histopathological examination and reporting.]

3.2.3 Subcutaneous injection

Four groups of five Wistar-King rats [age and sex not reported] were injected subcutaneously with *ortho*-phenylenediamine [purity not reported] in 0.5 mL distilled water at a dose of 0 (control) or 45 mg/kg bw in one experiment, and 0 (control) or 90 mg/kg bw in a second experiment, every second day, for 17 months. No tumours of the skin or subcutaneous tissue were observed in any of the groups (Saruta et al., 1962). [The Working Group noted the very limited reporting of the study, the use of only one dose, and the very low number of animals, making the study impossible to interpret. The Working Group considered the study inadequate for the evaluation.]

4. Mechanistic and Other Relevant Data

No data were available on *ortho*-phenylenediamine dihydrochloride, other than as reported from the chronic bioassays (see Section 4.3, "Other adverse effects").

Data available on *ortho*-phenylenediamine are summarized in the following sections.

4.1 Absorption, distribution, metabolism, and excretion

No data in humans or in experimental animals were available.

A study in vitro using human abdominal skin showed that *ortho*-phenylenediamine binds and is absorbed by skin (Bronaugh & Congdon, 1984).

4.2 Mechanisms of carcinogenesis

This section summarizes the available evidence for the key characteristics of carcinogens (Smith et al., 2016), on whether *ortho*-phenylenediamine: is genotoxic, and induces oxidative stress.

4.2.1 Genetic and related effects

See Table 4.1, Table 4.2, Table 4.3, and Table 4.4

(a) Humans

(i) Exposed humans

No data were available to the Working Group.

(ii) Human cells in vitro

DNA damage (as assessed using the comet assay), chromosomal aberrations, and sister-chromatid exchanges were induced by *ortho*-phenylenediamine (0–40 mM) in human lymphocytes in a study by Cebulska-Wasilewska et al. (1998).

(b) Experimental systems

ortho-Phenylenediamine did not increase somatic gene mutations in mouse embryos as assessed using the mammalian spot test (Gocke et al., 1983). Exposure to *ortho*-phenylenediamine by intraperitoneal injection induced micronuclei in the bone marrow of mice, Chinese hamsters, and guinea-pigs (Wild et al., 1980). A similar result was also seen in mice after oral exposure to *ortho*-phenylenediamine (Wild et al., 1980).

In rat hepatocytes, *ortho*-phenylenediamine gave positive results in an assay for unscheduled DNA synthesis (Thompson et al., 1983). Asgård et al. (2013) performed several assays for genotoxicity in mouse lymphoma cells with *ortho*-phenylenediamine and reported positive results in assays for DNA damage and mutation.

In plants, *ortho*-phenylenediamine induced DNA damage, detectable using the comet assay, or a change in phenotypic expression (Gichner et al., 1994, 2001; Xiao & Ichikawa, 1998; Gichner, 2003).

Numerous studies have assessed the mutagenicity of *ortho*-phenylenediamine in bacteria. Positive results have been seen in *Salmonella typhimurium* with (but not without) metabolic activation in strains TA98 (Voogd et al., 1980; Thompson et al., 1983; Gentile et al., 1987; Watanabe et al., 1990), TA100 (Thompson et al., 1983; Gentile et al., 1987), and TA1538 (Ames et al., 1975; Thompson et al., 1983), as well as in strains TA1537, D3052, and G46 (Thompson et al., 1983), and YG1024 (Wagner et al., 1997). Negative results were observed in strains TA100 and TA1537 (with metabolic activation) and in *Klebsiella pneumoniae* (tested only without metabolic activation) in a study by Voogd et al. (1980).

4.2.2 Oxidative stress

In the presence of Cu(II), *ortho*-phenylenediamine (100 µM) induced DNA damage in a *TP53* gene fragment. These lesions occurred at cytosine and guanine residues in a site reported to be a mutation hotspot region of the *TP53* gene (Murata et al., 2006).

ortho-Phenylenediamine caused Cu(II)-mediated damage to calf thymus DNA, measured by 8-oxo-7,8-dihydro-2-deoxyguanosine formation, and superoxide dismutase enhanced this oxidative DNA damage (Murata et al., 2006).

4.3 Other adverse effects

4.3.1 Humans

No data were available to the Working Group.

4.3.2 Experimental systems

In chronic bioassays with *ortho*-phenylenediamine dihydrochloride (Matsumoto et al., 2012) and a study with *ortho*-phenylenediamine (Saruta et al., 1962), effects that may be related

Table 4.1 Genetic and related effects of *ortho*-phenylenediamine in human cells in vitro

End-point	Tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
DNA strand breaks	Lymphocytes	+	NT	NR	Cytotoxicity, NR	Cebulska-Wasilewska et al. (1998)
Chromosomal aberrations	Lymphocytes	+	NT	NR	Cytotoxicity, NR; treated whole blood, then analysed the lymphocytes	Cebulska-Wasilewska et al. (1998)
Sister-chromatid exchange	Lymphocytes	+	NT	NR	Cytotoxicity, NR; treated whole blood, then analysed the lymphocytes	Cebulska-Wasilewska et al. (1998)

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

[a] +, positive

Table 4.2 Genetic and related effects of *ortho*-phenylenediamine in non-human mammals in vivo

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Reference
Mutation (spot test)	Mouse, C57BL/6JHan × T hybrid (M, F)	Whole body hair	–	196 mg/kg bw	Single intraperitoneal injection on the 10th day of pregnancy	Gocke et al. (1983)
Micronucleus formation	Mouse, NMRI (M, F)	Bone marrow (total PE)	+	108 mg/kg bw	Intraperitoneal injection, 2×, 24 h interval or oral, 2×, 24 h interval	Wild et al. (1980)
Micronucleus formation	Chinese hamster (M, F)	Bone marrow (total PE)	+	216 mg/kg bw	Intraperitoneal injection, 2×, 24 h interval	Wild et al. (1980)
Micronucleus formation	Guinea-pig, albino (M, F)	Bone marrow (total PE)	+	108 mg/kg bw	Intraperitoneal injection, 2×, 24 h interval	Wild et al. (1980)

bw, body weight; F, female; HID, highest ineffective dose; LED, lowest effective dose; M, male; PE, polychromatic erythrocytes

[a] +, positive; –, negative

Table 4.3 Genetic and related effects of *ortho*-phenylenediamine in non-human mammalian cells in vitro

End-point	Species, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Unscheduled DNA synthesis	Rat, Fischer F334, primary hepatocytes	+	NT	50 µM	Positive results were at non-cytotoxic concentrations	Thompson et al. (1983)
DNA oxidation	Mouse lymphoma, L5178Y 3.7.2c	+	+	0.08 mM	Under the condition of hOGG1 treatment	Asgård et al. (2013)
DNA strand breaks	Mouse lymphoma, L5178Y 3.7.2c	+	+	0.08 mM (–S9) and 0.12 mM (+S9)		Asgård et al. (2013)
Mutation/*Tk*	Mouse lymphoma, L5178Y 3.7.2c	+	+	0.08 mM		Asgård et al. (2013)

HIC, highest ineffective concentration; hOGG1, human 8-oxoguanine DNA *N*-glycosylase; HPLC, high-performance liquid chromatography; LEC, lowest effective concentration; NT, not tested

[a] +, positive

Table 4.4 Genetic and related effects of *ortho*-phenylenediamine 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			
Tradescantia clone BNL4430, stamen	DNA damage/ other	+	NT	10 mM		Xiao & Ichikawa (1998)
Tradescantia clone 4430, stamen	DNA damage/ other	+	NT	50 mM		Gichner et al. (1994)
Nicotiana tabacum var. Petit Havana SR1-wildtype, *CAT1AS* mutant	DNA damage/ other	–	NT	8 mM		Gichner (2003)
Nicotiana tabacum var. Petit Havana SR1-wildtype, *CAT1AS* mutant	DNA damage/ other	+	NT	8 mM		Gichner (2003)
Nicotiana tabacum var. xanthi, chlorophyll-deficient	DNA damage/ other	+	NT	0.01 mM		Gichner et al. (2001)
Nicotiana tabacum var. xanthi	DNA damage/ other	+	NT	0.01 mM		Gichner et al. (2001)
Salmonella typhimurium TA98	Reverse mutation	–	+	1 mg/L		Voogd et al. (1980)
Salmonella typhimurium TA98, TA100, TA1537, TA1538, D3052, G46	Reverse mutation	–	+	0.1–100 µg/mL		Thompson et al. (1983)
Salmonella typhimurium TA98, TA100	Reverse mutation	–	+	10 µg per plate	Both mammalian S9 and plant S9 were used for metabolic activation; DMSO was the solvent	Gentile et al. (1987)
Salmonella typhimurium TA98	Reverse mutation	–	+	10 µg per plate		Watanabe et al. (1990)
Salmonella typhimurium TA100, TA1537	Reverse mutation	–	–	1 mg/L		Voogd et al. (1980)
Salmonella typhimurium TA1538	Reverse mutation	NT	+	10–100 µg per plate		Ames et al. (1975)
Salmonella typhimurium YG1024	Reverse mutation	NT	+	20–100 µM	This mutation activity was dose-dependently increased, and enhanced by paraoxon	Wagner et al. (1997)
Klebsiella pneumoniae	Reverse mutation	–	NT	4.6 mM		Voogd et al. (1980)

8-oxodG, 8-oxo-7,8-dihydro-2-deoxyguanosine; DMSO, dimethyl sulfoxide; HIC, highest ineffective concentration; LEC, lowest effective concentration; NT, not tested

[a] +, positive; –, negative

to carcinogenicity were observed in the liver, kidney, and the haematological system (see also Section 3).

In the study in rats with chronic exposure to drinking-water containing *ortho*-phenylenediamine dihydrochloride, Matsumoto et al. (2012) observed increased absolute and relative liver weight, haematological changes including decreased mean corpuscular volume, mean corpuscular haemoglobin, and haemoglobin concentration, as well as clinical chemistry alterations suggestive of renal and hepatic injury. Gross pathology revealed liver nodule formation, and microscopic evaluations showed renal changes including chronic nephropathy, renal papillary necrosis with mineralization, and urothelial hyperplasia of the renal pelvis.

In the study in mice with chronic exposure to drinking-water containing *ortho*-phenylenediamine dihydrochloride, Matsumoto et al. (2012) observed haematological changes and clinical chemistry changes suggestive of hepatic injury. Mice also developed hepatic nodules in addition to hydronephrosis. Non-neoplastic hepatic lesions, including the presence of increased numbers of clear, acidophilic, and basophilic cell foci, were observed in treated mice. Hepatocellular hyperplasia was not observed in mice. Increased eosinophilic changes in the respiratory and olfactory epithelium, and nasopharynx and nasal glandular metaplasia, were observed in female mice. Hydronephrosis was increased in all exposed females.

Saruta et al. (1962) exposed rats to *ortho*-phenylenediamine by subcutaneous injection and observed decreased haemoglobin values and decreased numbers of erythrocytes and leukocytes in blood after 10 months. Histopathological alterations occurred in the liver, spleen, kidney, and lung. [The Working Group noted that the manuscript implies a total dose of 90 mg/kg bw in the form of two injections at 45 mg/kg bw given 24 hours apart, although this is not explicitly stated.]

4.4 Data relevant to comparisons across agents and end-points

See the monograph on 2-chloronitrobenzene in the present volume.

5. Summary of Data Reported

5.1 Exposure data

ortho-Phenylenediamine, the parent compound of *ortho*-phenylenediamine dihydrochloride, is a basic compound and will undergo acid–base reactions. *ortho*-Phenylenediamine and its dihydrochloride salt will undergo a pH-dependent acid–base equilibrium in the body.

ortho-Phenylenediamine has been listed as a chemical with a global high production volume. *ortho*-Phenylenediamine and *ortho*-phenylenediamine dihydrochloride are manufactured in and/or imported into the European Economic Area in small amounts; information on quantities produced and used elsewhere in the world was not available. *ortho*-Phenylenediamine is used as an intermediate in the production of chemicals used to produce agrochemicals, antioxidants in rubber products, corrosion inhibitors, ultraviolet adsorbers, pharmaceuticals, and dyes and pigments used for colouring furs and hair dyes. *ortho*-Phenylenediamine dihydrochloride is primarily used as a chemical laboratory reagent and as an intermediate in the manufacture of dyes, coatings, and photographic chemicals.

ortho-Phenylenediamine and *ortho*-phenylenediamine dihydrochloride are not known to occur naturally, although they may be released to the environment as a by-product of production and downstream uses; accumulation in water is most likely. *ortho*-Phenylenediamine has been detected in tap and lake water in China.

Occupational exposure to both agents is expected to occur primarily through inhalation in workplaces where they are produced or

used as an intermediate in the manufacture of other products; exposure through inadvertent ingestion may also occur. Exposure to *ortho*-phenylenediamine may also occur through skin contact.

The general population may be exposed to *ortho*-phenylenediamine through the use of hair dyes that contain this agent. Such exposure is more likely in countries outside the European Union, where the use of *ortho*-phenylenediamine in hair dyes has been banned since 2007.

No other quantitative data on exposure in the general population were available for these agents.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

ortho-Phenylenediamine was tested as its dihydrochloride for carcinogenicity in the same laboratory in two well-conducted good laboratory practice (GLP) oral exposure studies by drinking-water: one in male and female mice, and one in male and female rats. There were also two limited oral exposure studies by diet from another laboratory: one in male and female mice, and one in male rats.

In male mice, *ortho*-phenylenediamine dihydrochloride induced a significant positive trend in the incidence and an increase in the incidence of hepatocellular adenoma, hepatocellular adenoma or carcinoma (combined), and papillary adenoma of the gall bladder in the study of oral exposure by drinking-water. The study of oral exposure by diet reported a significant increase in the incidence of hepatocellular carcinoma.

In female mice, *ortho*-phenylenediamine dihydrochloride induced a significant positive trend in the incidence and a significant increase in the incidence of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined) in the study of oral exposure by drinking-water; there was also a significant increase in the incidence of papillary adenoma of the gall bladder. The study of oral exposure by diet reported a significant increase in the incidence of hepatocellular carcinoma.

In male rats, *ortho*-phenylenediamine dihydrochloride induced a significant positive trend in the incidence and a significant increase in the incidence of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined) in the study of oral exposure by drinking-water. In the same study, *ortho*-phenylenediamine dihydrochloride induced a significant positive trend in the incidence of transitional cell papilloma, transitional cell carcinoma, and transitional cell papilloma or carcinoma (combined) of the urinary bladder, and of follicular adenoma of the thyroid. A significant increase in the incidence of transitional cell papilloma or carcinoma (combined) of the urinary bladder was also observed. The study of oral exposure by diet reported a significant increase in the incidence of hepatocellular carcinoma.

In female rats, *ortho*-phenylenediamine dihydrochloride induced a significant positive trend in the incidence and a significant increase in the incidence of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined) in the study of oral exposure by drinking-water.

5.4 Mechanistic and other relevant data

No studies evaluating absorption, distribution, metabolism, or excretion of *ortho*-phenylenediamine dihydrochloride or *ortho*-phenylenediamine in humans or experimental animals were available. No mechanistic studies of exposure to

ortho-phenylenediamine dihydrochloride were available.

Concerning the key characteristics of carcinogens, there is *strong* evidence that *ortho*-phenylenediamine is genotoxic based upon positive results in mammals, in mammalian cells in vitro, and in non-mammalian experimental systems including plants and prokaryotes. No data in exposed humans were available. In cultured human lymphocytes (one study), DNA strand breaks, chromosomal aberrations, and sister-chromatid exchanges were observed. In one study in non-human mammals conducted in vivo, the frequency of bone marrow micronuclei was increased after exposure by intraperitoneal injection (hamster, guinea-pig, and mouse) or orally (mouse). *ortho*-Phenylenediamine increased the frequency of unscheduled DNA synthesis in a study in rat hepatocytes, and gave positive results in assays for DNA damage and mutation in mouse lymphoma cells. Metabolic activation of *ortho*-phenylenediamine resulted in mutagenicity in multiple strains of *Salmonella typhimurium*, although in one study negative results were reported in two strains. *ortho*-Phenylenediamine induced DNA damage in multiple plant species. In one study of oxidative stress providing *weak* evidence, *ortho*-phenylenediamine in the presence of Cu(II) induced DNA damage in a human *TP53* gene fragment and calf thymus DNA.

Long-term effects observed in rats after acute exposure to *ortho*-phenylenediamine by subcutaneous injection included toxicity to the haematological, hepatic, and renal systems. Long-term effects of oral exposure to *ortho*-phenylenediamine dihydrochloride by drinking-water in rats and mice included toxicity to the haematological, hepatic, and renal systems. Histological changes were also seen in the mouse upper respiratory tract.

6. Evaluation and Rationale

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of *ortho*-phenylenediamine and its dihydrochloride salt.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of *ortho*-phenylenediamine dihydrochloride.

6.3 Overall evaluation

ortho-Phenylenediamine and its dihydrochloride salt are *possibly carcinogenic to humans (Group 2B).*

6.4 Rationale

ortho-Phenylenediamine is the parent compound of *ortho*-phenylenediamine dihydrochloride. A pH-dependent acid–base equilibrium exists between the two compounds, and the Working Group considered that in vivo studies on either compound were informative about the carcinogenic hazard of both.

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