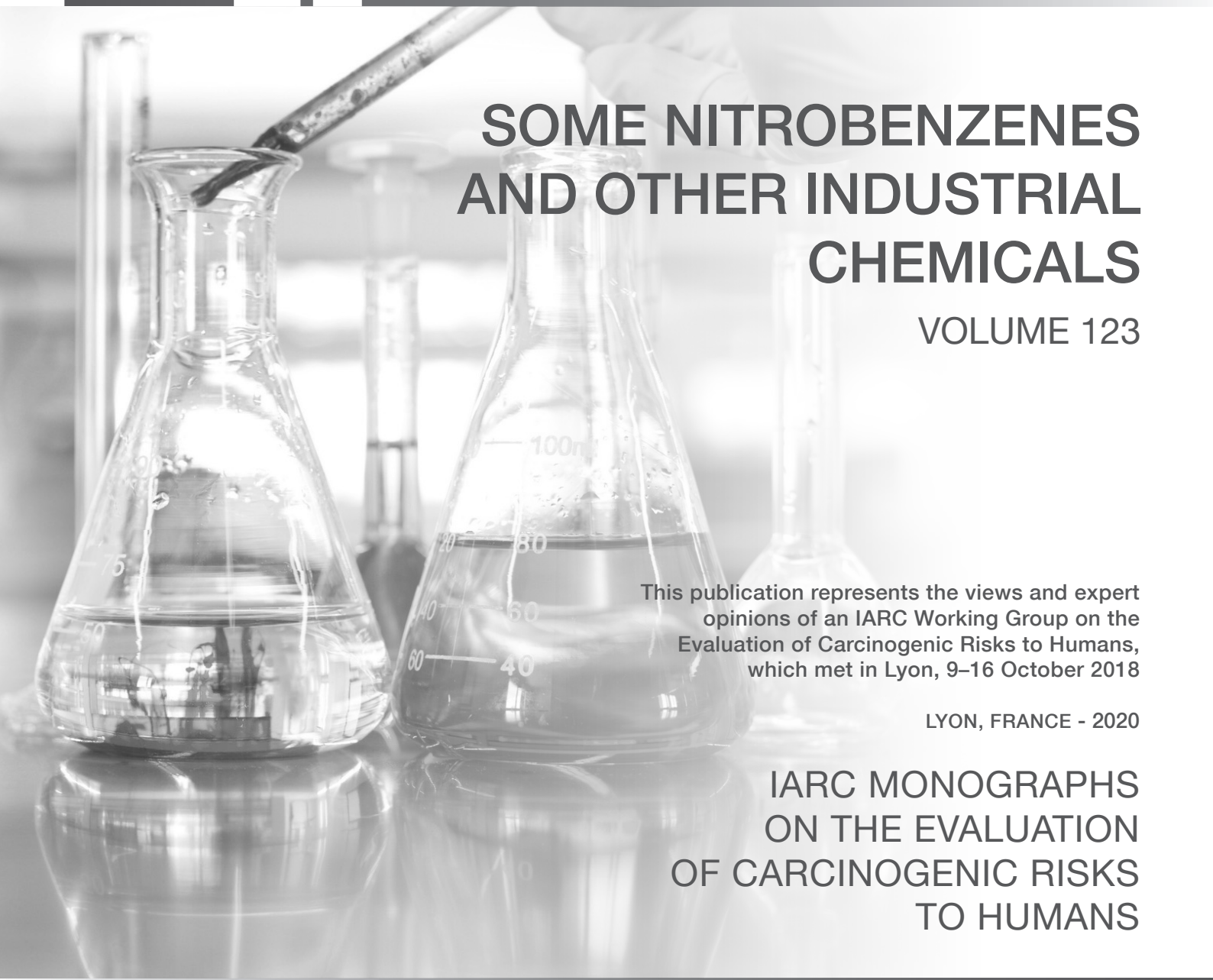
IARC MONOGRAPHS



SOME NITROBENZENES AND OTHER INDUSTRIAL CHEMICALS

VOLUME 123

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International Agency for Research on Cancer

World Health Organization

1,4-DICHLORO-2-NITROBENZENE

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

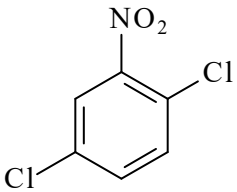
Chem. Abstr. Serv. Reg. No.: 89-61-2

Chem. Abstr. Serv. name:
1,4-dichloro-2-nitrobenzene

IUPAC systematic name:
2,5-dichloronitrobenzene

Synonyms: 2,5-dichloro-1-nitrobenzene; benzene, 1,4-dichloro-2-nitro-; 2,5-DCNB.

1.1.2 Structural and molecular formulae, and relative molecular mass



Molecular formula: $C_6H_3Cl_2NO_2$

Relative molecular mass: 192.00

1.1.3 Chemical and physical properties of the pure substance

Description: solid, crystalline, and pale yellow plates and prisms with a faint aromatic odour; the substance can react dangerously with strong bases and strong oxidizing agents (IFA, 2018)

Density (at 22 °C*)*: 1.67 g/cm³ (IFA, 2018)

Octanol/water partition coefficient (P): log K_{ow} = 3.03 (IFA, 2018)

Henry law constant (at 22 °C*)*: 1.52 Pa m³/mol (HSDB, 2013)

Melting point: 56 °C (IFA, 2018)

Boiling point: 267 °C (IFA, 2018)

Volatility: vapour pressure, 0.38×10^{-2} mm Hg [0.51 Pa] at 25 °C (HSDB, 2013)

Solubility: soluble in ethanol, ether, benzene, carbon disulfide; slightly soluble in carbon tetrachloride; 95 mg/L in water at 25 °C; 83 mg/L in water at 20 °C (HSDB, 2013; IFA, 2018)

Flammable limits: lower explosion limit: 2.4 vol% (191 g/m³); upper explosion limit: 8.5 vol% (677 g/m³) (IFA, 2018)

Flash point: 135 °C (IFA, 2018)

Ignition temperature: 465 °C (IFA, 2018).

1.2 Production and use

1.2.1 Production process

Almost-pure 1,4-dichloro-2-nitrobenzene is produced as a chemical intermediate in closed systems by nitrating 1,4-dichlorobenzene at low temperatures (35–65 °C) (HSDB, 2013).

1.2.2 Production volume

1,4-Dichloro-2-nitrobenzene has been listed as a chemical with a high production volume (OECD, 2009). The annual production volume in Japan was 200–1200 tonnes during 1988–1992; in Germany in 1992, it was 2400–2800 tonnes per year (OECD-SIDS, 1996). One manufacturer in the USA is listed in the Hazardous Substances Data Bank as a producer of 1,4-dichloro-2-nitrobenzene (EPA, 2018). Between 1998 and 2002, annual production volume was 10 000–500 000 pounds [~4.5–227 tonnes] in the USA (HSDB, 2013). Annual production volume in and import into Japan was reported to be 2100 tonnes per year in 2000 (Yamazaki et al., 2006). 1,4-Dichloro-2-nitrobenzene is currently manufactured in or imported into the European Economic Area at volumes of 1–10 tonnes per year (ECHA, 2018). No information on current production volumes in other countries or economies was available to the Working Group.

1.2.3 Use

1,4-Dichloro-2-nitrobenzene has been extensively used as an intermediate in the manufacture of diazo pigments. Additional uses have been in the production of agrochemicals and ultraviolet absorbents (OECD-SIDS, 1996). Registered industrial uses in the European Union include the colouring of paper products, chemicals, textiles, leather, and fur (ECHA, 2018).

1.3 Methods of measurement and analysis

1.3.1 Air

No methods have been described for the determination of 1,4-dichloro-2-nitrobenzene in air samples.

1.3.2 Other environmental media

1,4-Dichloro-2-nitrobenzene has been analysed as part of a multianalyte method for the determination of semivolatile nitroaromatics and cyclic ketones by gas chromatography with electron capture detection or nitrogen phosphorus detection in water and soil samples. However, detection might be hampered due to low resolution and potentially accompanying contaminants such as 4-chloro-3-nitrobenzene and 2,4-dichloro-1-nitrobenzene. Sample preparation is usually carried out by ultrasonic extraction using organic solvents such as methylene chloride for water samples. If necessary, further clean-up steps using florisil or size-exclusion (gel permeation) chromatography can be performed. No data on detection or quantitation limits have been reported (EPA, 1996).

1.3.3 Biomarkers

No methods of measurement or analysis have been identified for biomarkers of exposure to 1,4-dichloro-2-nitrobenzene in urine or blood. [The Working Group noted that suitable biomarkers in exposed individuals could include 2,5-dichloroaniline and 4-nitro-2,5-dichlorophenol in urine and haemoglobin adducts of 2,5-dichloroaniline in blood.]

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

1,4-Dichloro-2-nitrobenzene is not known to occur naturally (HSDB, 2013).

If used in the production of dyestuffs, agrochemicals, and coloured consumer products, 1,4-dichloro-2-nitrobenzene can be released through various waste streams (HSDB, 2013; ECHA, 2018) and is considered moderately persistent in the environment (OECD-SIDS, 1996).

With an analytical limit of detection of 20 µg/L, 1,4-dichloro-2-nitrobenzene has not been detected in surface water samples from 21 areas of Japan since 1982 (OECD-SIDS, 1996). The local concentration of 1,4-dichloro-2-nitrobenzene in a bay close to a Japanese manufacturer has been predicted to be 0.8 µg/L based on an estimated release of 8 tonnes of the compound per year, a total effluent of 1.0×10^{10} L per year, and a dilution factor of 1000 (OECD-SIDS, 1996). If released to water, a generic level III fugacity model suggests that most of the 1,4-dichloro-2-nitrobenzene (> 90%) remains in the aqueous system, and about 8% is transported to soil and sediment (OECD-SIDS, 1996). 1,4-Dichloro-2-nitrobenzene remained stable in an aqueous hydrolysis test to abiotic hydrolysis at pH 4–9 at a temperature of 25 °C. A half-life of 34 days has been calculated as a result of decay by photodegradation from water (OECD-SIDS, 1996). Concentrations in fish are 120–820 times greater than in water, suggesting a moderate potential for bioconcentration in aquatic organisms (Deneer et al., 1987; Niimi et al., 1989; Franke et al., 1994).

No data on the concentration of 1,4-dichloro-2-nitrobenzene and its stability in soil have been found. If released to soil, a generic level III fugacity model suggests that most of the 1,4-dichloro-2-nitrobenzene (> 95%) remains in the soil, and about 3% is transported into the aqueous system (OECD-SIDS, 1996).

No data have been found on the concentration of 1,4-dichloro-2-nitrobenzene in environmental air. If released to air, the compound is susceptible to photodegradation by sunlight as a result of ultraviolet absorption at wavelengths greater than 290 nm (OECD-SIDS, 1996; HSDB, 2013). The half-life in air is estimated to be approximately 1 week. In addition, 1,4-dichloro-2-nitrobenzene is photochemically degraded in the atmosphere in the presence of hydroxyl radicals, with an estimated half-life of approximately 320 days (HSDB, 2013). If released to air, a generic level III fugacity model suggests that approximately 7% and 80% of the released 1,4-dichloro-2-nitrobenzene is transported to water and soil, respectively (OECD-SIDS, 1996).

1.4.2 Occurrence in food

No data on the occurrence of 1,4-dichloro-2-nitrobenzene in food samples were available. However, an average daily intake in humans through drinking-water and consumption of edible fish has been estimated as 2.6×10^{-5} and 1.2×10^{-3} mg/kg per day, respectively (OECD-SIDS, 1996).

1.4.3 Exposure in the general population

Data on exposure of the general population to 1,4-dichloro-2-nitrobenzene were not available to the Working Group. It has previously been reported that 1,4-dichloro-2-nitrobenzene, or dyes and antimicrobials based on the compound, are not known ingredients in consumer products (OECD-SIDS, 1996). However, recent information suggests that manufactured consumer products may contain residues of this substance or coloured products that are based on this substance, including: washing and cleaning products; automobile care products; paints and coatings or adhesives; and long-life materials

for indoor use and with low release rates such as flooring, furniture, toys, curtains, footwear, and paper and cardboard products (ECHA, 2018).

1.4.4 Occupational exposure

Quantitative data on occupational exposure to 1,4-dichloro-2-nitrobenzene were not available to the Working Group; however, the main routes of occupational exposure are expected to be through inhalation in workplaces where 1,4-dichloro-2-nitrobenzene is produced or used as an intermediate in the manufacture of diazo dyes or agrochemical products (HSDB, 2013; IFA 2018). In addition, 1,4-dichloro-2-nitrobenzene is expected to be absorbed via skin; unspecified systemic effects have been observed in workers after repeated dermal exposure (IFA, 2018). Gastrointestinal absorption is also expected based on a kinetic model, and this route might be of importance when inhaled dust particles are transported out of the airways by mucociliary clearance into the gastrointestinal tract (BUA, 1991; IFA, 2018). [The Working Group noted that exposure via ingestion may also arise from inadvertent hand-to-mouth contact.]

Registered industrial uses in the European Union indicate that exposure may also occur in the manufacture of 1,4-dichloro-2-nitrobenzene and its downstream uses, including textile finishing, the manufacture of pulp, paper, and paper products, and as a chemical intermediate (ECHA, 2018).

1.5 Regulations and guidelines

Concerning human health, 1,4-dichloro-2-nitrobenzene is harmful if swallowed (H302, category 4) according to the Globally Harmonized System of Classification and Labelling of Chemicals. The substance may also cause an allergic skin reaction (H317, category 1) and skin and eye irritation (H315, H319, category 2) (ECHA, 2018). Precautionary measures should include avoiding oral uptake and skin contact (IFA, 2018).

No occupational or environmental exposure limit values for 1,4-dichloro-2-nitrobenzene were available to the Working Group.

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

See Table 3.1

3.1 Mouse

Oral administration

In a study that complied with good laboratory practice (GLP), groups of 50 male and 50 female Crj:BDF_1 mice (age, 6 weeks) were randomized by weight and fed diets containing 1,4-dichloro-2-nitrobenzene (purity, > 98.8%) at a concentration of 0, 320, 800, and 2000 ppm for 2 years (104 weeks) (Yamazaki et al., 2006). All mice (except for one male in the control group) underwent complete necropsy. Although survival analysis did not show a difference between groups exposed to 1,4-dichloro-2-nitrobenzene and control groups, the survival rates of the males and females at 2000 ppm tended to be lower after week 65 of treatment, which was attributed to increased tumour-associated mortality. Survival to 2 years for the groups at 0, 320, 800, and 2000 ppm was 27/49, 35/50, 26/50, and 18/50 in males, and 30/50, 27/50, 28/50, and 23/50 in females, respectively. At termination of treatment, the body weights of the males at 800 and 2000 ppm and of the females at 2000 ppm were significantly decreased relative to their respective control groups.

Table 3.1 Studies of carcinogenicity with 1,4-dichloro-2-nitrobenzene in experimental animals

Species, strain (sex) Age at start Duration Reference	Route 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 Yamazaki et al. (2006)	Oral > 98.8% Diet 0, 320, 800, 2000 ppm 49, 50, 50, 50 27, 35, 26, 18	*Liver* Hepatocellular adenoma 17/49, 21/50, 20/50, 16/50 Hepatocellular carcinoma 15/49, 15/50, 23/50, 31/50* Hepatoblastoma 1/49, 10/50*, 12/50*, 25/50* Hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) 26/49, 34/50, 41/50*, 45/50*	 NS $P < 0.01$, 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	Principal strengths: covered most of the lifespan; males and females used; multiple doses; well-conducted GLP study; adequate number of mice
Mouse, Crj:BDF$_1$ (F) 6 wk 104 wk Yamazaki et al. (2006)	Oral > 98.8% Diet 0, 320, 800, 2000 ppm 50, 50, 50, 50 30, 27, 28, 23	*Liver* Hepatocellular adenoma 5/50, 5/50, 17/50*, 16/50* Hepatocellular carcinoma 1/50, 3/50, 15/50*, 31/50* Hepatoblastoma 0/50, 0/50, 0/50, 2/50 Hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) 6/50, 8/50, 29/50*, 39/50*	 $P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test $P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test NS $P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	Principal strengths: covered most of the lifespan; males and females used; multiple doses; well-conducted GLP study; adequate number of mice Historical control incidence of hepatoblastoma was 0/1048 female mice

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Route 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 Yamazaki et al. (2006)	Oral > 98.8% Diet 0, 320, 800, 2000 ppm 50, 50, 50, 50 40, 44, 41, 39	*Liver* Hepatocellular adenoma 0/50, 1/50, 0/50, 6/50* Hepatocellular carcinoma 0/50, 0/50, 1/50 (2%), 2/50 (4%) Hepatocellular adenoma or carcinoma (combined) 0/50, 1/50, 1/50, 8/50* *Kidney* Renal cell adenoma 0/50, 0/50, 0/50, 2/50 (4%) Renal cell carcinoma 0/50, 1/50 (2%), 0/50, 1/50 (2%) Renal cell adenoma or carcinoma (combined) 0/50, 1/50, 0/50, 3/50 *Zymbal gland:* adenoma 0/50, 0/50, 0/50, 4/50 (8%)	 $P < 0.01$, Peto trend test; *$P < 0.05$, Fisher exact test NS $P < 0.01$, Peto trend test; *$P < 0.05$, Fisher exact test NS NS $P < 0.05$, Peto trend test $P < 0.01$, Peto trend test	Principal strengths: covered most of the lifespan; males and females used; multiple doses; well-conducted GLP study; adequate number of rats Historical control incidence in 1249 male rats (maximum incidence in any study): hepatocellular carcinoma, 0.2% (2%); renal cell adenoma, 0.16% (2%); renal cell carcinoma, 0.16% (2%); Zymbal gland adenoma, 0.2% (2%)
Rat, F344/DuCrj (F) 6 wk 104 wk Yamazaki et al. (2006)	Oral > 98.8% Diet 0, 320, 800, 2000 ppm 50, 50, 50, 50 38, 35, 39, 34	Any tumour type: no significant increase		Principal strengths: covered most of the lifespan; males and females used; multiple doses; well-conducted GLP study; adequate number of animals

F, female; GLP, good laboratory practice; M, male; NS, not significant; ppm, parts per million; wk, week

In male mice, dietary administration of 1,4-dichloro-2-nitrobenzene caused a significant dose-related increase ($P < 0.01$, Peto trend test) in the incidence of hepatocellular carcinoma, hepatoblastoma, and hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined). The incidence of hepatocellular carcinoma (15/49, 15/50, 23/50, and 31/50) was significantly ($P < 0.01$, Fisher exact test) increased in males at 2000 ppm. The incidence of hepatoblastoma (1/49, 10/50, 12/50, and 25/50) was significantly ($P < 0.01$, Fisher exact test) increased in all exposed groups of males. The incidence of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) (26/49, 34/50, 41/50, and 45/50) was significantly ($P < 0.01$, Fisher exact test) increased in males at 800 and 2000 ppm.

In female mice, dietary administration of 1,4-dichloro-2-nitrobenzene caused a significant dose-related increase ($P < 0.01$, Peto trend test) in the incidence of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined). The incidence of hepatocellular adenoma (5/50, 5/50, 17/50, and 16/50) was significantly ($P < 0.01$, Fisher exact test) increased in females at 800 and 2000 ppm. The incidence of hepatocellular carcinoma (1/50, 3/50, 15/50, and 31/50) was significantly ($P < 0.01$, Fisher exact test) increased in females at 800 and 2000 ppm. The incidence of hepatoblastoma in exposed female mice was not significantly increased (0/50, 0/50, 0/50, and 2/50), although the incidence in females exposed at 2000 ppm (2/50) exceeded the upper bound of the range in historical controls from the laboratory (0%, 0/1048 female mice). The incidence of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) (6/50, 8/50, 29/50, and 39/50) was significantly ($P < 0.01$, Fisher exact test) increased in female mice at 800 and 2000 ppm.

Dietary exposure to 1,4-dichloro-2-nitrobenzene resulted in an increased incidence of non-neoplastic lesions in the liver (hepatocellular hypertrophy with nuclear atypia; centrilobular in males and females at all concentrations, and acidophilic foci in males at 800 and 2000 ppm). [The Working Group noted that the strengths of this well-conducted study that complied with GLP included the use of multiple doses, a large number of mice per group, and testing in males and females.]

3.2 Rat

Oral administration

In a study that complied with GLP, groups of 50 male and 50 female Fischer 344/DuCrj rats (age, 6 weeks) were randomized by weight and fed diets containing 1,4-dichloro-2-nitrobenzene (purity, > 98.8%) at a concentration of 0, 320, 800, or 2000 ppm for 2 years (104 weeks) (Yamazaki et al., 2006). All rats underwent complete necropsy. Survival analysis did not show a difference between the groups exposed to 1,4-dichloro-2-nitrobenzene and control groups. Survival to 2 years for the groups at 0, 320, 800, and 2000 ppm was 40/50, 44/50, 41/50, and 39/50 in males, and 38/50, 35/50, 39/50, and 34/50 in females, respectively. At the termination of treatment, the body weights of all exposed males and of the females at 2000 ppm were significantly decreased relative to their respective control groups.

In male rats, dietary administration of 1,4-dichloro-2-nitrobenzene caused a significant dose-related increase ($P < 0.01$, Peto trend test) in the incidence of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined). The incidence of hepatocellular adenoma (0/50, 1/50, 0/50, and 6/50) was significantly ($P < 0.05$, Fisher exact test) increased in males at 2000 ppm. The incidence of hepatocellular carcinoma was 0/50, 0/50, 1/50 (2%), and

2/50 (4%), respectively. The incidence of hepatocellular carcinoma in the group at the highest dose (2/50) exceeded the upper bound of the range for historical controls (3/1249, 0.2%) in 25 studies that reported a maximum incidence of 2%. The incidence of hepatocellular adenoma or carcinoma (combined) (0/50, 1/50, 1/50, and 8/50) was significantly ($P < 0.05$, Fisher exact test) increased in males at 2000 ppm. Dietary administration of 1,4-dichloro-2-nitrobenzene also caused a significant dose-related increase ($P < 0.05$, Peto trend test) in the incidence of renal cell adenoma or carcinoma (combined) in male rats. By pair-wise comparison, the incidence of renal cell adenoma or carcinoma (combined) (0/50, 1/50, 0/50, and 3/50) was not significantly increased in any exposed group of males. By pair-wise comparison, the incidence of renal cell adenoma (0/50, 0/50, 0/50, and 2/50) was not significantly increased in any exposed group of males. The incidence of renal cell adenoma in males at 2000 ppm (2/50, 4%) exceeded the upper bound of the range for historical controls from this laboratory (4% in this study versus (vs) an upper bound of 2% in historical controls). By pair-wise comparison, the incidence of renal cell carcinoma (0/50, 1/50, 0/50, and 1/50) was not significantly increased in any exposed group of males. The incidence of renal cell carcinoma in any exposed group of males did not exceed the upper bound of the range for historical controls from this laboratory (0–2% in this study vs an upper bound of 2% in historical control groups). Dietary administration of 1,4-dichloro-2-nitrobenzene caused a significant dose-related increase ($P < 0.01$, Peto trend test) in the incidence of adenoma of the Zymbal gland in males. By pair-wise comparison, the incidence of adenoma of the Zymbal gland (0/50, 0/50, 0/50, and 4/50) was not significantly increased in any exposed group of males, but the incidence in males at 2000 ppm exceeded the upper bound of the range for historical control from this laboratory (8% in this study vs an upper bound of 2% in historical controls).

In female rats, dietary administration of 1,4-dichloro-2-nitrobenzene did not cause a significant increase in the incidence of any type of neoplasm.

In male rats, dietary administration of 1,4-dichloro-2-nitrobenzene resulted in an increased incidence of non-neoplastic lesions in the liver (basophilic cell foci in the groups at 800 and 2000 ppm) and in the kidney (chronic progressive nephropathy in all exposed groups; mineralization of the papilla in groups at 800 and 2000 ppm; urothelial hyperplasia of the pelvis in all exposed groups). [The Working Group noted that the strengths of this well-conducted study that complied with GLP included the use of multiple doses, a large number of rats per group, and testing in males and females.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

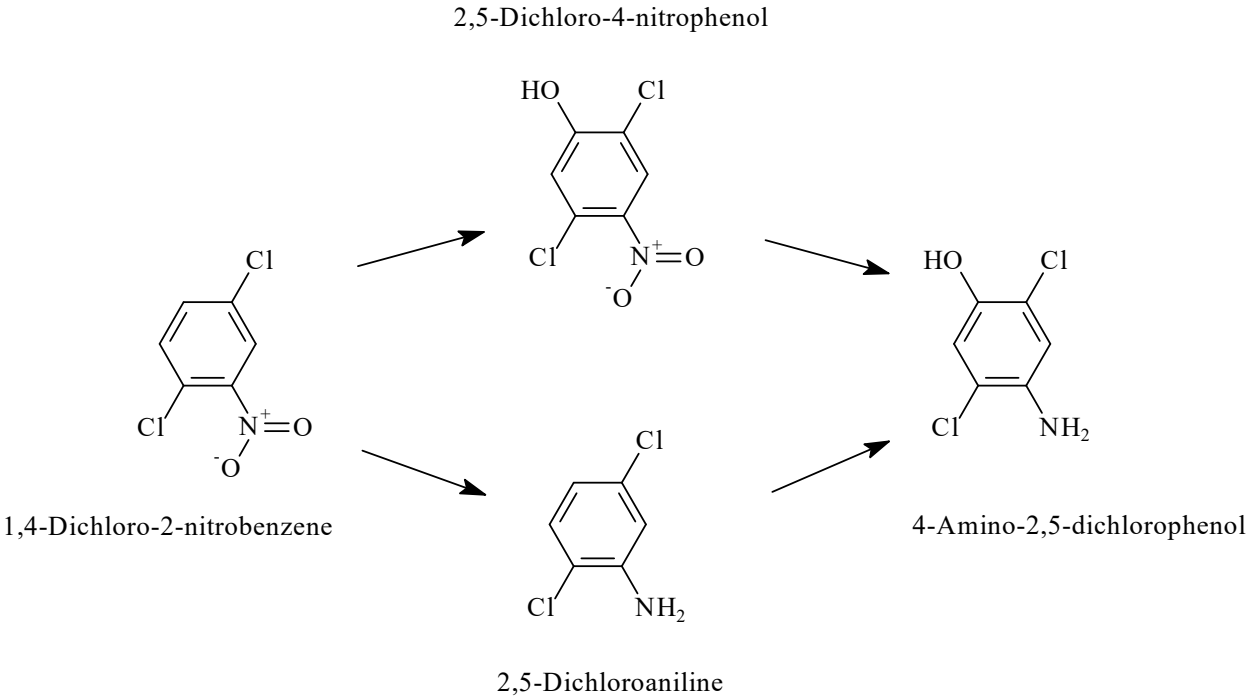
4.1.1 Humans

No data were available to the Working Group.

4.1.2 Experimental systems

Data on the absorption, distribution, metabolism, and excretion of 1,4-dichloro-2-nitrobenzene in experimental systems were sparse. On the basis of studies of metabolism and other studies, gastrointestinal absorption of 1,4-dichloro-2-nitrobenzene occurs. For example, Bray et al. (1957) gave a group of 6–10 doe rabbits (weight, 2–3 kg) an aqueous oral suspension of 1,4-dichloro-2-nitrobenzene at a dose of 0.4 g/kg body weight (bw). After exposure to 1,4-dichloro-2-nitrobenzene, rabbit

Fig. 4.1 Reported urinary metabolites of 1,4-dichloro-2-nitrobenzene observed in rabbits after oral exposure to 1,4-dichloro-2-nitrobenzene



Adapted with permission from *Biochemical Journal*. Bray HG, James SP, Thorpe WV, The metabolism of 2:4-, 2:5- and 3:4-dichloronitrobenzene in the rabbit, 1957; Volume 65, issue 3: 483-490 © The Biochemical Society (Bray et al., 1957)

urine samples were collected and metabolites identified using paper chromatography and absorption spectra. Some oxidative metabolites were excreted as mercapturic acid (9–33%), glucuronide (8–56%), and sulfate (3–21%) metabolites. The main urinary metabolites of 1,4-dichloro-2-nitrobenzene observed in rabbits after exposure were 2,5-dichloroaniline (13%), *N*-acetyl-*S*-(4-chloro-2-nitrophenyl)-L-cysteine (2%), and 4-amino-2,5-dichlorophenol (1%). Fig. 4.1 shows a partial metabolic scheme for 1,4-dichloro-2-nitrobenzene.

Ohnishi et al. (2004) identified the urinary metabolites of 1,4-dichloro-2-nitrobenzene in three male Fischer 344/DuCrj rats given a diet containing 1,4-dichloro-2-nitrobenzene at 1% for 2 days. Individual urine samples were collected for 24 hours using metabolic cages, and samples were subsequently pooled yielding a single sample. Urine samples were analysed using a variety of analytical chemistry methods including liquid chromatography with tandem mass spectrometry. The main urinary metabolite was an *N*-acetylcysteine conjugate, *N*-acetyl-*S*-(4-chloro-3-nitrophenyl)-L-cysteine.

4.2 Mechanisms of carcinogenesis

This section summarizes the available evidence for the key characteristics of carcinogens (Smith et al., 2016). 1,4-Dichloro-2-nitrobenzene has only been studied in a small number of assays related to genotoxicity.

Table 4.1 Genetic and related effects of 1,4-dichloro-2-nitrobenzene in non-human mammalian cells in vitro

End-point	Species, tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Chromosomal aberrations	Chinese hamster lung, CHL/IU	(+)	–	LEC, 24 µg/mL (–S9) HIC, 94 µg/mL (+S9)	Cytotoxic at 0.15 mg/mL (–S9)	MHW Japan (1994), cited in OECD-SIDS (1996)

HIC, highest ineffective concentration; LEC, lowest effective concentration; S9, 9000 × *g* supernatant

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

Table 4.2 Genetic and related effects of 1,4-dichloro-2-nitrobenzene 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			
Salmonella typhimurium TA1535/pSK1002	Mutation, other (SOS)	+	NT	1000 µg/mL		Jin & Qian (1991)
Salmonella typhimurium TA1535/pSK1002	Reverse mutation	+	NT	5000 µg/mL		Jin & Qian (1991)
Salmonella typhimurium TA100	Reverse mutation	+	+	5000 µg/plate	Cytotoxic at 1250 µg/plate (+/–S9)	MHW Japan (1994), cited in OECD-SIDS (1996)
Salmonella typhimurium TA1535	Reverse mutation	+	–	LEC, 78 µg/plate (–S9) HIC, 5000 µg/plate (+S9)	Cytotoxic at 1250 µg/plate (+/–S9)	MHW Japan (1994), cited in OECD-SIDS (1996)
Salmonella typhimurium TA98, TA1537	Reverse mutation	–	–	5000 µg/plate	Cytotoxic at 1250 µg/plate (+/–S9)	MHW Japan (1994), cited in OECD-SIDS (1996)
Escherichia coli WP2 *uvr*A	Reverse mutation	–	–	5000 µg/plate	Cytotoxic at 1250 µg/plate (+/–S9)	MHW Japan (1994), cited in OECD SIDS (1996)

HIC, highest ineffective concentration; LEC, lowest effective concentration; S9, 9000 × *g* supernatant

[a] +, positive; –, negative; NT, not tested

4.2.1 Genetic and related effects

See Table 4.1 and Table 4.2

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

No data in non-human mammals in vivo were available to the Working Group.

A test for chromosomal aberration was conducted in cultured Chinese hamster lung (CHL/IU) cells (MHW Japan, 1994, as cited in OECD-SIDS, 1996). [The Working Group noted that results were available from a secondary source, but were unable to obtain the primary source documentation to determine whether positive results occurred as a result of cytotoxicity.]

Jin & Qian (1991) reported that 1,4-dichloro-2-nitrobenzene (at concentrations of ≥ 1000 µg/mL) induces *umu* expression and is mutagenic in *Salmonella typhimurium* strain TA1535 with the plasmid pSK1002.

Additional limited data from secondary sources were also available to the Working Group. An assay for reverse gene mutation was conducted, using the pre-incubation method. 1,4-Dichloro-2-nitrobenzene yielded positive results in *S. typhimurium* strain TA100 with and without metabolic activation, and in strain TA1535 without metabolic activation. Negative results were obtained in *S. typhimurium* (strain TA98 and TA1537) and in *Escherichia coli* WP2 *uvrA* with and without metabolic activation, at concentrations of up to 5 mg per plate (MHW Japan, 1994, as cited in OECD-SIDS, 1996). [The Working Group noted that these results were available from a secondary source, but was unable to obtain the primary source documentation to determine whether positive results occurred as a result of cytotoxicity.]

4.3 Other adverse effects

4.3.1 Humans

No data were available to the Working Group.

4.3.2 Experimental systems

Other adverse effects of 1,4-dichloro-2-nitrobenzene that may be related to carcinogenicity (see Section 3) include toxicity in the liver, kidney, lymphohaematopoietic system, and the male reproductive tract in rodents. [The Working Group noted that carcinogenic effects were seen in mouse and rat liver.]

The most sensitive end-points observed in mice and rats after 14-day and 13-week exposure to 1,4-dichloro-2-nitrobenzene included hepatic effects in male and female mice and rats, and renal effects in male rats only (Yamazaki et al., 2005a, b). Mice exposed at the highest doses demonstrated a reduced terminal body weight and increased incidence of mortality. Treatment-related histopathological changes were seen in the liver, kidney, testes, and haematopoietic systems. Centrilobular single-cell hepatic necrosis and increased hepatocyte mitosis were seen after exposure to 1,4-dichloro-2-nitrobenzene. Increased incidences of proximal tubule hyaline droplets and granular casts suggestive of α_{2u}-globulin nephropathy were also observed in male rats orally exposed to 1,4-dichloro-2-nitrobenzene. Although these studies suggest that α_{2u}-globulin nephropathy occurs in male rats after exposure to 1,4-dichloro-2-nitrobenzene (Yamazaki et al., 2005a, b), the findings are incomplete with respect to fulfilling all criteria for concluding that 1,4-dichloro-2-nitrobenzene operates through response associated with α_{2u}-globulin in male rats (IARC, 1999). In particular, criteria not met included chemical identification of the protein accumulating in tubule cells as α_{2u}-globulin, and reversible binding of 1,4-dichloro-2-nitrobenzene or a metabolite to α_{2u}-globulin.

Yamazaki et al. (2006) also performed a 2-year study of toxicity in groups of 50 male and 50 female mice and rats fed diet containing 1,4-dichloro-2-nitrobenzene at a concentration of 320, 800, or 2000 ppm. As for the shorter-term studies, relative liver weight was increased in rats at 320 ppm and above and in mice at 800 ppm and above. Centrilobular hepatocyte hyperplasia with nuclear atypia was increased in mice in all dose groups. Relative kidney weights were also increased in exposed male rats and male mice. Lesions consistent with chronic progressive nephropathy were seen more frequently in male rats. An increased incidence of urothelial hyperplasia in the renal pelvis and of renal papilla mineralization was seen in male rats at 320 ppm and above, and 800 ppm and above, respectively. An increased incidence of renal haemosiderin deposition and increased numbers of bone marrow erythroblasts occurred in male mice at 2000 ppm.

4.4 Data related 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

1,4-Dichloro-2-nitrobenzene has been classified as a chemical with a high production volume, although data on current production volumes and locations are limited. It is used as an intermediate in the manufacture of diazo pigments, agrochemicals, and ultraviolet absorbents.

The compound is not known to occur naturally, but it can be released to the environment as a by-product of manufacturing and downstream uses.

Some coloured consumer products may contain residues of 1,4-dichloro-2-nitrobenzene, although quantitative information on exposure in the general population was not found.

Occupational exposure is expected to occur primarily through inhalation in workplaces where 1,4-dichloro-2-nitrobenzene is produced or used as an intermediate in the manufacture of other products; exposure may also occur through skin contact or inadvertent ingestion. Quantitative information on exposure in occupational settings was not available to the Working Group.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

1,4-Dichloro-2-nitrobenzene was tested for carcinogenicity in well-conducted good laboratory practice (GLP) studies of oral exposure by diet from the same laboratory, one in male and female mice and one in male and female rats.

In male mice, 1,4-dichloro-2-nitrobenzene caused a significant positive trend in the incidence and an increase in the incidence of hepatocellular carcinoma, hepatoblastoma, and of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined).

In female mice, 1,4-dichloro-2-nitrobenzene caused a significant positive trend in the incidence and an increase in the incidence of hepatocellular adenoma, hepatocellular carcinoma, and of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined). Two female mice at the highest dose developed hepatoblastoma; no hepatoblastomas have been reported in female mice in the historical database of the laboratory.

In male rats, 1,4-dichloro-2-nitrobenzene caused a significant positive trend in the incidence

and an increase in the incidence of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined). In male rats exposed at the highest dose, the incidence of hepatocellular carcinoma exceeded the upper bound of the range for historical controls for the laboratory. In male rats, 1,4-dichloro-2-nitrobenzene caused a significant positive trend in the incidence of renal cell adenoma or carcinoma (combined) and of adenoma of the Zymbal gland. In male rats exposed at the highest dose, the incidence of adenoma of the Zymbal gland exceeded the upper bound of the range for historical controls for the laboratory.

In female rats exposed to 1,4-dichloro-2-nitrobenzene, there was no significant increase in the incidence of any neoplasm.

5.4 Mechanistic and other relevant data

No data on absorption, distribution, metabolism, or excretion of 1,4-dichloro-2-nitrobenzene in humans were available. In rodents, 1,4-dichloro-2-nitrobenzene is absorbed after oral exposure and is metabolized to aniline and phenol metabolites; these metabolites can undergo secondary mercapturic acid, glucuronide, sulfate, and *N*-acetylcysteine conjugation.

Concerning the key characteristics of carcinogens, there is *weak* evidence that 1,4-dichloro-2-nitrobenzene is genotoxic. No data in exposed humans or in non-human mammals in vivo were available. In a single test, 1,4-dichloro-2-nitrobenzene gave positive results solely in the absence of metabolic activation for chromosomal aberrations in Chinese hamster lung cells, but concurrent cytotoxicity could not be ruled out. 1,4-Dichloro-2-nitrobenzene gave positive results in some, but not all, tested *Salmonella typhimurium* strains in the absence or presence of metabolic activation.

Exposure to 1,4-dichloro-2-nitrobenzene resulted in toxicity in the liver, kidney, lymphohaematopoietic system, and male reproductive tract in rodents. No data in exposed humans were available. Increased incidences of proximal tubule hyaline droplets and granular casts, suggestive of α_{2u}-globulin nephropathy, were also observed in male rats orally exposed to 1,4-dichloro-2-nitrobenzene by diet; however, the criteria established by IARC for considering the induction of kidney tumours to have occurred by a response associated with α_{2u}-globulin were not met.

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of 1,4-dichloro-2-nitrobenzene.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 1,4-dichloro-2-nitrobenzene.

6.3 Overall evaluation

1,4-Dichloro-2-nitrobenzene is *possibly carcinogenic to humans (Group 2B).*

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