



**SOME NITROBENZENES
AND OTHER INDUSTRIAL
CHEMICALS**

VOLUME 123

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**IARC MONOGRAPHS
ON THE EVALUATION
OF CARCINOGENIC RISKS
TO HUMANS**

2,4-DICHLORO-1-NITROBENZENE

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 611-06-3

Chem. Abstr. Serv. name:

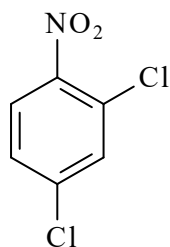
2,4-dichloronitrobenzene

IUPAC systematic name:

2,4-dichloronitrobenzene

Synonyms: 2,4-dichloro-1-nitrobenzene; 1,3-dichloro-4-nitrobenzene; benzene, 2,4-dichloro-1-nitro-; 2,4-DCNB; nitro-*m*-dichlorobenzene.

1.1.2 Structural and molecular formulae, and relative molecular mass



Molecular formula: C₆H₃Cl₂NO₂

Relative molecular mass: 192.00

1.1.3 Chemical and physical properties of the pure substance

Description: solid, crystalline, and pale yellow needles with a faint aromatic odour; the substance can react dangerously with bases, amines, and oxidizing agents ([IFA, 2018](#))

Density: 1.54 g/cm³ at 15 °C ([IFA, 2018](#))

Relative vapour density (air = 1): 6.6 ([PubChem, 2018](#))

Octanol/water partition coefficient (P): log K_{ow} = 3.07 ([IFA, 2018](#))

Henry law constant (at 25 °C): 3.22 × 10⁻⁵ atm m³/mol [3.26 Pa m³/mol] ([HSDB, 2008](#))

Melting point: 34 °C ([IFA, 2018](#))

Boiling point: 258 °C ([IFA, 2018](#))

Volatility: vapour pressure, 1.43 × 10⁻² mm Hg (estimated) [~190 Pa] at 25 °C ([HSDB, 2008](#))

Solubility: entirely soluble in ethanol and ether, and slightly soluble in chloroform ([HSDB, 2008](#)); slightly soluble in water with 188 mg/L at 20 °C ([IFA, 2018](#))

Flammable limits: poorly flammable, prone to combustion ([IFA, 2018](#))

Flash point: 152 °C ([IFA, 2018](#))

Ignition temperature: 500 °C ([IFA, 2018](#)).

1.2 Production and use

1.2.1 Production process

2,4-Dichloro-1-nitrobenzene is produced as a chemical intermediate in closed systems by nitrating 1,3-dichlorobenzene with mixed acid, and separating the product from the resulting isomer mixture ([HSDB, 2008](#)).

1.2.2 Production volume

In 1990, the production volume of 2,4-dichloro-1-nitrobenzene was about 1500 tonnes in Germany and approximately 50 tonnes in Japan ([OECD-SIDS, 1996](#)). It was listed in 2004 and 2007 as a chemical with a high production volume ([OECD, 2004, 2009](#)). Manufacturers in Germany and France were registered with the European Chemical Agency (ECHA) as producers of 2,4-dichloro-1-nitrobenzene for the years 2013 and 2017, respectively ([ECHA, 2018](#)). No other information on recent production volumes of 2,4-dichloro-1-nitrobenzene was available.

1.2.3 Use

2,4-Dichloro-1-nitrobenzene has been used as an intermediate in the manufacture of diazo pigments ([Yamazaki et al., 2006](#)), pharmaceuticals, and agrochemicals (e.g. diazoxide and chlomethoxyfen) ([OECD-SIDS, 1996](#); [HSDB, 2008](#)).

1.3 Methods of measurement and analysis

1.3.1 Air

The only method that has been described for the determination of 2,4-dichloro-1-nitrobenzene in air is part of a multianalyte method for the determination of multiple semivolatile organic compounds. Soxhlet extraction of air filters is followed by solid-phase extraction on

florisil or size-exclusion (gel permeation) chromatography. The final analysis can be conducted by gas chromatography with either electron capture detection or mass spectrometry. Because 2,4-dichloro-1-nitrobenzene can co-elute with some other compounds, particularly (di/tri) chloronitrobenzenes, analysis by gas chromatography with mass spectrometry is recommended. No data on quantitation limits were available for this method ([EPA, 1998](#)).

1.3.2 Other environmental media

The United States Environmental Protection Agency (EPA) multianalyte method for the determination of semivolatile organic compounds in air described above can also be applied to water and soil samples. Estimated quantitation limits were reported as 0.01–0.10 mg/L in water and 0.5–1.0 mg/kg in soil when using gas chromatography with mass spectrometry. Recovery from soil samples was low (20–40%) ([EPA, 1998](#)).

1.3.3 Biomarkers

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

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

2,4-Dichloro-1-nitrobenzene is not known to occur naturally ([HSDB, 2008](#)).

2,4-Dichloro-1-nitrobenzene can be released to the environment through various waste streams from industry, where it is used as an intermediate in the manufacture of various

agrochemicals, dyes, and pharmaceuticals ([Meylan & Howard, 1991](#); [HSDB, 2008](#)).

2,4-Dichloro-1-nitrobenzene was detected but not quantified in drinking-water and in effluent from advanced wastewater treatment that was collected in Seattle and Cincinnati, USA, in 1976 and in 1978, respectively ([Lucas, 1984](#)). 2,4-Dichloro-1-nitrobenzene was also detected, but not quantified, in fish collected in the Main river, Germany ([Steinwandter, 1989](#)). Average chloronitrobenzene (isomers not specified) concentrations of 17.3 and 19.2 µg/kg fat were measured in zebra mussels and eels, respectively, collected from the Rhine and Meuse delta ([Hendriks et al., 1998](#)).

Based on its octanol/water partition coefficient, if released into water 2,4-dichloro-1-nitrobenzene will either be adsorbed to the sediment or released by volatilization from water surfaces into the air ([HSDB, 2008](#)). The half-life of the compound in rivers has been predicted as 17 days. For structural reasons (aromaticity), degradation of 2,4-dichloro-1-nitrobenzene by hydrolysis is not likely to be an important process in the environment ([HSDB, 2008](#)). A moderate to high bioconcentration factor of between 80 and 150 has been suggested in various fish and algae ([Freitag et al., 1985](#); [Niimi et al., 1989](#); [Meylan & Howard, 1991](#); [HSDB, 2008](#)).

2,4-Dichloro-1-nitrobenzene has been qualitatively detected in river sediment in Japan ([Tokuda et al., 1985](#)). The compound is expected to have no mobility in soil; biodegradation in soil is estimated to be low (< 0.1%) based on a 5-day biological oxygen demand test and a medium bioaccumulation factor of 310 in activated sludge ([Freitag et al., 1985](#); [HSDB, 2008](#)).

2,4-Dichloro-1-nitrobenzene has not been detected in environmental air. However, volatilization of the compound into the atmosphere is expected to be an important emission process based on its air to water partition coefficient (Henry constant). 2,4-Dichloro-1-nitrobenzene contains chromophores that absorb at wave-

lengths of greater than 290 nm; the compound may therefore degrade by direct photolysis from sunlight ([HSDB, 2008](#)). The mass of 2,4-dichloro-1-nitrobenzene adsorbed on silica gel decreased by 11.2% after irradiation at wavelengths of greater than 290 nm for 17 hours ([Freitag et al., 1985](#)). Reaction with photochemically generated hydroxyl radicals was also reported as an important alternative pathway of degradation, with an estimated half-life of approximately 130 days ([Freitag et al., 1985](#); [HSDB, 2008](#)).

1.4.2 Exposure in the general population

Exposure of the general population to 2,4-dichloro-1-nitrobenzene has not been reported, and the compound is not found in consumer products ([OECD-SIDS, 1996](#)). According to the European Union Registration, Evaluation, Authorisation and Restriction of Chemicals regulation No. 1907/2006 Annex XVII, 2,4-dichloro-1-nitrobenzene is prohibited in consumer goods such as decorative objects or games; exposure of the general population is therefore not expected through the use or handling of these consumer products ([IFA, 2018](#)).

1.4.3 Occupational exposure

Measurements of occupational exposure to 2,4-dichloro-1-nitrobenzene were not available to the Working Group. However, exposure may occur through both inhalation and dermal contact at workplaces where 2,4-dichloro-1-nitrobenzene is produced or used as an intermediate ([HSDB, 2008](#)). [The Working Group noted that exposure by ingestion may also arise from inadvertent hand to mouth contact.]

1.5 Regulations and guidelines

Concerning human health, 2,4-dichloro-1-nitrobenzene is suspected of causing cancer (H351, category 2) and of causing genetic defects (H341, category 2) according to the Globally Harmonized System of Classification and Labelling of Chemicals. The substance is also harmful if swallowed (H302, category 4) and toxic if in contact with skin (H311, category 3), and may cause an allergic skin reaction (H317, category 1B). Precautionary measures should include wearing protective clothing (P280) and, if the skin is contaminated, washing with plenty of soap and water (P302, P352) ([IFA, 2018](#)).

No occupational or environmental exposure limit values were available for 2,4-dichloro-1-nitrobenzene in air.

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:BDF₁ mice (age, 6 weeks) were randomized by weight and fed diet containing 2,4-dichloro-1-nitrobenzene (purity, 99.4%) at a concentration of 0, 750, 1500, or 3000 ppm (males) and 0, 1500, 3000, or 6000 ppm (females) for 2 years (104 weeks) ([Kano et al., 2012](#)). All mice (except for one female in the control group) underwent complete necropsy. The survival rates of the males at 3000 ppm and the females at 3000 and

6000 ppm were significantly decreased as a result of increased mortality from tumours attributable to treatment. Survival rates to the end of 2 years for the males at 0, 750, 1500, and 3000 ppm were 35/50, 38/50, 29/50, and 23/50. Survival rates to the end of 2 years for the females at 0, 1500, 3000, and 6000 ppm were 28/49, 28/50, 18/50, and 19/50. Relative to their respective control groups, body weights were significantly decreased in males at 3000 ppm and in females at 3000 and 6000 ppm at the termination of treatment.

In male mice, dietary administration of 2,4-dichloro-1-nitrobenzene caused a significant dose-related increase ($P < 0.01$, Peto trend test) in the incidence of hepatocellular adenoma, hepatocellular carcinoma, hepatoblastoma, and of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined). The incidence of hepatocellular adenoma (18/50, 34/50, 30/50, and 43/50) was significantly increased in males at 1500 ppm ($P < 0.05$, Fisher exact test) and in males at 750 and 3000 ppm ($P < 0.01$, Fisher exact test). The incidence of hepatocellular carcinoma (7/50, 7/50, 11/50, and 15/50) was significantly ($P < 0.05$, Fisher exact test) increased in males at 3000 ppm. The incidence of hepatoblastoma (1/50, 5/50, 16/50, and 27/50) was significantly ($P < 0.01$, Fisher exact test) increased in males at 1500 and 3000 ppm. The incidence of hepatoblastoma in male mice at 750 ppm exceeded the upper bound of the range for historical control groups from this laboratory (10% in this study versus an upper bound of 6% in historical control groups). The incidence of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) (19/50, 39/50, 41/50, and 45/50) was significantly ($P < 0.01$, Fisher exact test) increased in all treated males. Dietary administration of 2,4-dichloro-1-nitrobenzene caused a dose-related increase in peritoneal haemangiosarcoma ($P < 0.01$, Peto trend test) in males. The incidence of peritoneal haemangiosarcoma (1/50, 0/50,

Table 3.1 Studies of carcinogenicity with 2,4-dichloro-1-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 Kano et al. (2012)	Oral 99.4% Diet 0, 750, 1500, 3000 ppm 50, 50, 50, 50 35, 38, 29, 23	<i>Liver</i> Hepatocellular adenoma	18/50, 34/50*, 30/50**, 43/50*	$P < 0.01$, Peto trend test; * $P < 0.01$, ** $P < 0.05$, Fisher exact test	Principal strengths: well-conducted GLP study; males and females used; multiple doses; adequate number of mice Historical control incidence in 1496 male mice (maximum incidence in any study): hepatoblastoma, 0.7% (6%); peritoneal haemangiosarcoma, 0.2% (4%)
		Hepatocellular carcinoma	7/50, 7/50, 11/50, 15/50*	$P < 0.01$, Peto trend test; * $P < 0.05$, Fisher exact test	
		Hepatoblastoma	1/50, 5/50 (10%), 16/50*, 27/50*	$P < 0.01$, Peto trend test; * $P < 0.01$, Fisher exact test	
		Hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined)	19/50, 39/50*, 41/50*, 45/50*	$P < 0.01$, Peto trend test; * $P < 0.01$, Fisher exact test	
		<i>Peritoneum</i> : haemangiosarcoma	1/50, 0/50, 2/50 (4%), 5/50 (10%)	$P < 0.01$, Peto trend test	
Mouse, Crj:BDF ₁ (F) 6 wk 104 wk Kano et al. (2012)	Oral 99.4% Diet 0, 1500, 3000, 6000 ppm 49, 50, 50, 50 28, 28, 18, 19	<i>Liver</i> Hepatocellular adenoma	8/49, 25/50*, 42/50*, 45/50*	$P < 0.01$, Peto trend test; * $P < 0.01$, Fisher exact test	Principal strengths: well-conducted GLP study; males and females used; multiple doses; adequate number of mice Historical control incidence in 1498 female mice: hepatoblastoma, 0%; peritoneal haemangiosarcoma, 0.4% (maximum incidence in any study, 4%)
		Hepatocellular carcinoma	1/49, 2/50, 11/50*, 21/50*	$P < 0.01$, Peto trend test; * $P < 0.01$, Fisher exact test	
		Hepatoblastoma	0/49, 2/50 (4%), 7/50*, 7/50*	$P < 0.01$, Peto trend test; * $P < 0.01$, Fisher exact test	
		Hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined)	8/49, 28/50*, 43/50*, 48/50*	$P < 0.01$, Peto trend test; * $P < 0.01$, Fisher exact test	

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
Kano et al. (2012) (cont.)		<i>Peritoneum</i> : haemangiosarcoma 0/49, 3/50 (6%), 7/50*, 17/50*	$P < 0.01$, Peto trend test; * $P < 0.01$, Fisher exact test	
Rat, F344/DuCrj (M) 6 wk 104 wk Kano et al. (2012)	Oral 99.4% Diet 0, 750, 1500, 3000 ppm 50, 50, 50, 50 39, 42, 40, 40	<i>Kidney</i> Renal cell adenoma 0/50, 0/50, 3/50 (6%), 26/50* Renal cell carcinoma 0/50, 0/50, 2/50, 23/50* Renal cell adenoma or carcinoma (combined) 0/50, 0/50, 5/50*, 38/50**	$P < 0.01$, 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; * $P < 0.05$, ** $P < 0.01$, Fisher exact test	Principal strengths: well-conducted GLP study; males and females used; multiple doses; adequate number of rats Historical control incidence of renal cell adenoma was 0.1% in 1749 male rats, with a maximum incidence of 2% in any study
		<i>Preputial gland</i> : adenoma 1/50, 4/50, 2/50, 7/50*	$P < 0.05$, Peto trend test; * $P < 0.05$, Fisher exact test	
Rat, F344/DuCrj (F) 6 wk 104 wk Kano et al. (2012)	Oral 99.4% Diet 0, 750, 1500, 3000 ppm 50, 50, 50, 50 35, 44, 38, 43	<i>Kidney</i> Renal cell adenoma 0/50, 0/50, 3/50 (6%), 26/50* Renal cell carcinoma 0/50, 0/50, 0/50, 12/50* Renal cell adenoma or carcinoma (combined) 0/50, 0/50, 3/50, 32/50*	$P < 0.01$, 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	Principal strengths: well-conducted GLP study; males and females used; multiple doses; adequate number of rats Incidence of renal cell adenoma in historical controls was 0.1% in 1597 female rats, with a maximum incidence of 2% in any study

F, female; GLP, good laboratory practice; M, male; ppm, parts per million; wk, week

2/50, and 5/50) was not significantly increased in any exposed males compared with controls. The incidence of peritoneal haemangiosarcoma in males at 3000 ppm exceeded the upper range of historical control groups from this laboratory (10% in this study versus an upper bound of 4% in historical control groups).

In female mice, dietary administration of 2,4-dichloro-1-nitrobenzene caused a significant dose-related increase ($P < 0.01$, Peto trend test) in the incidence of hepatocellular adenoma, hepatocellular carcinoma, hepatoblastoma, and of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined). The incidence of hepatocellular adenoma (8/49, 25/50, 42/50, and 45/50) was significantly ($P < 0.01$, Fisher exact test) increased in all groups of treated females. The incidence of hepatocellular carcinoma (1/49, 2/50, 11/50, and 21/50) was significantly ($P < 0.01$, Fisher exact test) increased in females at 3000 and 6000 ppm. The incidence of hepatoblastoma (0/49, 2/50, 7/50, and 7/50) was significantly ($P < 0.01$, Fisher exact test) increased in females at 3000 and 6000 ppm. The incidence of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) (8/49, 28/50, 43/50, and 48/50) was significantly ($P < 0.01$, Fisher exact test) increased in all groups of treated females. Dietary administration of 2,4-dichloro-1-nitrobenzene caused a significant dose-related increase in the incidence of peritoneal haemangiosarcoma ($P < 0.01$, Peto trend test) in females. The incidence of peritoneal haemangiosarcoma (0/49, 3/50, 7/50, and 17/50) was significantly ($P < 0.01$, Fisher exact test) increased in females at 3000 and 6000 ppm.

Exposure to 2,4-dichloro-1-nitrobenzene resulted in increases in the incidence of non-neoplastic lesions in the liver (hepatocellular hypertrophy (centrilobular) in all groups of treated males and in females exposed at 6000 ppm; acidophilic foci in females exposed at 3000 and 6000 ppm). [The Working Group noted that the strengths of this well-conducted

GLP study included the use of multiple doses, the 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 diet containing 2,4-dichloro-1-nitrobenzene (purity, 99.4%) at a concentration of 0, 750, 1500, or 3000 ppm for 2 years (104 weeks) ([Kano et al., 2012](#)). All rats underwent complete necropsy. Survival analysis did not show a difference between groups exposed to 2,4-dichloro-1-nitrobenzene and control groups. Survival rates to the end of 2 years for the groups at 0, 750, 1500, and 3000 ppm were 39/50, 42/50, 40/50, and 40/50 in males, and 35/50, 44/50, 38/50, and 43/50 in females. Relative to their respective control groups, body weights were significantly decreased in all treated males and in the females exposed at 1500 and 3000 ppm at the termination of treatment.

In male rats, dietary administration of 2,4-dichloro-1-nitrobenzene caused a significant dose-related increase ($P < 0.01$, Peto trend test) in the incidence of renal cell adenoma, renal cell carcinoma, and renal cell adenoma or carcinoma (combined). The incidence of renal cell adenoma (0/50, 0/50, 3/50, and 26/50) was significantly ($P < 0.01$, Fisher exact test) increased in males at 3000 ppm. The incidence of renal cell carcinoma (0/50, 0/50, 2/50, and 23/50) was significantly ($P < 0.01$, Fisher exact test) increased in males at 3000 ppm. The incidence of renal cell adenoma or carcinoma (combined) (0/50, 0/50, 5/50, and 38/50) was significantly increased in males at 1500 ppm ($P < 0.05$, Fisher exact test) and at 3000 ppm ($P < 0.01$, Fisher exact test). Dietary administration of 2,4-dichloro-1-nitrobenzene caused a significant dose-related increase

($P < 0.05$, Peto test) in the incidence of adenoma of the preputial gland in males. The incidence of adenoma of the preputial gland (1/50, 4/50, 2/50, and 7/50) was significantly ($P < 0.05$, Fisher exact test) increased in males at 3000 ppm.

In female rats, dietary administration of 2,4-dichloro-1-nitrobenzene caused a significant dose-related increase ($P < 0.01$, Peto trend test) in the incidence of renal cell adenoma, renal cell carcinoma, and renal cell adenoma or carcinoma (combined). The incidence of renal cell adenoma (0/50, 0/50, 3/50, and 26/50) was significantly ($P < 0.01$, Fisher exact test) increased in females at 3000 ppm. The incidence of renal cell carcinoma (0/50, 0/50, 0/50, and 12/50) was significantly ($P < 0.01$, Fisher exact test) increased in females at 3000 ppm. The incidence of renal cell adenoma or carcinoma (combined) (0/50, 0/50, 3/50, and 32/50) was significantly ($P < 0.01$, Fisher exact test) increased in females at 3000 ppm.

Exposure to 2,4-dichloro-1-nitrobenzene resulted in an increased incidence of non-neoplastic lesions in the kidney (atypical tubular hyperplasia in all treated males and females; chronic progressive nephropathy in all treated males; chronic progressive nephropathy in females exposed at 750 and 1500 ppm; eosinophilic droplets in the proximal tubules in all treated males and females; mineralization of the papilla in all treated males; and urothelial hyperplasia of the pelvis in all treated males). [The Working Group noted that the strengths of this well-conducted GLP study included the use of multiple doses, the 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.

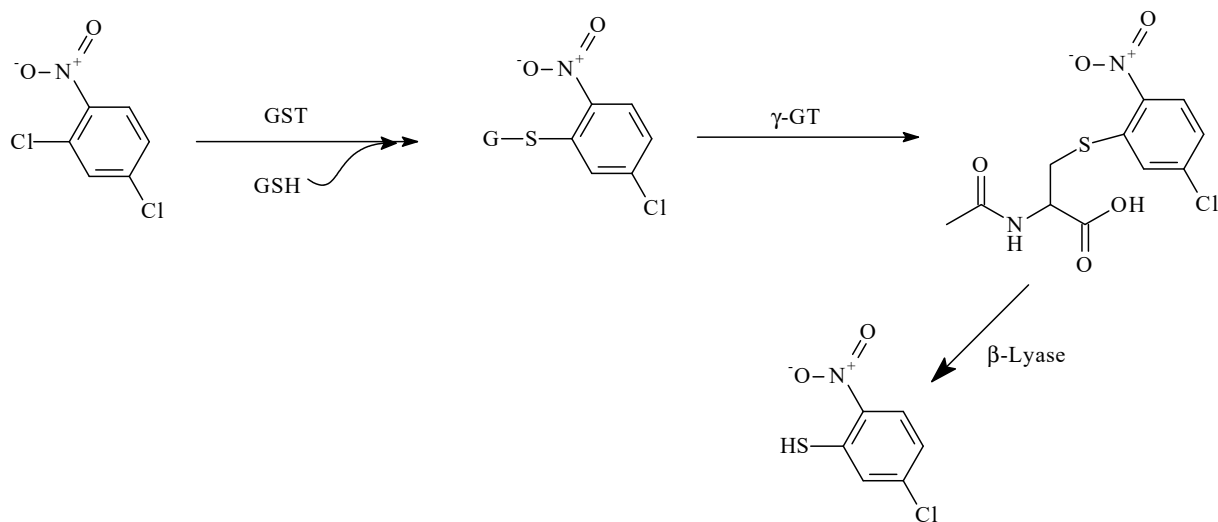
4.1.2 Experimental systems

Data concerning the absorption, distribution, metabolism, and excretion of 2,4-dichloro-1-nitrobenzene in experimental systems were sparse.

[Botham et al. \(1987\)](#) evaluated the dermal uptake of ^{14}C -radiolabelled 2,4-dichloro-1-nitrobenzene in mice. Mice were dermally exposed by application of 50 μL of a solution containing 13 μmol of 2,4-dichloro-1-nitrobenzene suspended in acetone:olive oil (4:1). Dermal absorption was estimated to be 45% and 71% of the applied dose after 24 hours and 72 hours, respectively.

[Bray et al. \(1957\)](#) treated three groups of six doe rabbits (weight, 2–3 kg) with 2,4-dichloro-1-nitrobenzene at a dose of 200, 300, or 400 mg/kg body weight (bw) by aqueous oral suspension. Pronounced anorexia was observed in rabbits at the highest dose. After exposure to 2,4-dichloro-1-nitrobenzene, urine samples were collected and metabolites identified using paper chromatography and absorption spectra. Some oxidative metabolites were excreted as mercapturic acid and phenols. The main urinary metabolites observed in rabbits were *N*-acetyl-*S*-(5-chloro-2-nitrophenyl)-*L*-cysteine (23% of dose) and 2,4-dichloroaniline as the acetyl conjugate (1%).

[Ohnishi et al. \(2009\)](#) identified the urinary metabolites of 2,4-dichloro-1-nitrobenzene in three male Fischer 344/DuCrj rats fed diet containing 2,4-dichloro-1-nitrobenzene at a con-

Fig. 4.1 Putative steps in the metabolism of 2,4-dichloro-1-nitrobenzene in rats

GSH, glutathione; GST, glutathione S-transferase; γ -GT, γ -glutamyltransferase
Adapted from [Ohnishi et al. \(2009\)](#)

centration of 1% for 2 days. Individual samples of urine 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 the *N*-acetylcysteine conjugate *N*-acetyl-S-(5-chloro-2-nitrophenyl)-L-cysteine. [Ohnishi et al. \(2009\)](#) proposed the metabolic scheme depicted in [Fig. 4.1](#) for 2,4-dichloro-1-nitrobenzene in rats.

4.2 Mechanisms of carcinogenesis

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

4.2.1 Genetic and related effects

See [Table 4.1](#)

(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 chromosomal aberration test was conducted using cultured Chinese hamster lung (CHL/IU) cells. [The Working Group noted that the results were available from a secondary source, but were unable to obtain the primary source documentation.] Structural chromosomal aberrations and polyploidy were not observed in CHL/IU cells after exposure in vitro to 2,4-dichloro-1-nitrobenzene at concentrations of up to 140 $\mu\text{g}/\text{mL}$, whether in the presence or absence of an exogenous metabolic activation system ([MHW Japan, 1996](#), as cited in [OECD-SIDS, 1996](#)).

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

Test system	End-point	Results ^a		Concentration (LEC or HIC)	Comments on study quality	Reference
		Without metabolic activation	With metabolic activation			
Chinese hamster lung, CHL	Chromosomal aberrations	–	–	140 µg/mL	Concentrations used: 0, 40, 70, 140 µg/mL	MHW Japan (1996) , cited in OECD-SIDS (1996)
<i>Salmonella typhimurium</i> TA100	Reverse mutation	+	+	3.3 µg/plate		Haworth et al. (1983)
<i>Salmonella typhimurium</i> TA1535	Reverse mutation	–	–	100 µg/plate	Toxicity at 215 µg/plate	Haworth et al. (1983)
<i>Salmonella typhimurium</i> TA1537	Reverse mutation	–	+	10 µg/plate	Toxicity at 215 µg/plate	Haworth et al. (1983)
<i>Salmonella typhimurium</i> TA98	Reverse mutation	–	+	10 µg/plate		Haworth et al. (1983)

HIC, highest ineffective concentration; LEC, lowest effective concentration

^a +, positive; –, negative

Non-mammalian experimental systems

2,4-Dichloro-1-nitrobenzene gave positive results in *Salmonella typhimurium* strains TA100, TA1537, and TA98, and negative results in strain TA1535 ([Haworth et al., 1983](#)).

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

[Kano et al. \(2012\)](#) evaluated the chronic toxicity of 2,4-dichloro-1-nitrobenzene administered in the diet to male and female Fischer 344/DuCrj rats and Crj:BDF₁ mice for 2 years. In rats, survival rate was unaffected by exposure to 2,4-dichloro-1-nitrobenzene. Terminal body weights decreased in all exposed male rats (decreases of 7–15%) and in female rats exposed at 1500 ppm and above (decreases of 8–14%). Increased absolute and relative liver weights were seen in all exposed male rats and in female rats at the two higher doses. Absolute kidney weights were also increased in all groups exposed to 2,4-dichloro-1-nitrobenzene, and relative kidney weight and blood urea nitrogen concentrations were significantly increased in all exposed male rats and in female rats at 1500 ppm and above. Rats exposed to 2,4-dichloro-1-nitrobenzene also developed atypical hyperplasia and eosinophilic droplets in the proximal tubule. The severity of chronic progressive nephropathy increased in a dose-related manner in male rats, and incidence

was increased in female rats at 750 and 1500 ppm. An increased incidence of urothelial hyperplasia in the renal pelvis and renal papilla mineralization was observed in male rats exposed to 2,4-dichloro-1-nitrobenzene.

In mice, unlike in rats, survival rates decreased after chronic exposure to 2,4-dichloro-1-nitrobenzene at 3000 ppm and above, attributed to fatal tumours. Similarly to rats, terminal body weights decreased in mice. This effect was seen in male mice at 1500 ppm and above (decreases of 10–27%) and in females exposed at 1500 ppm and above (decreases of 8–40%). Increased absolute and relative liver weights were observed in male mice exposed at 1500 ppm and above and in all treated female mice. Histological changes observed in male and female mice given 2,4-dichloro-1-nitrobenzene included pigment deposition and respiratory metaplasia (with increased numbers of submucosal glands) in the olfactory epithelium. An increased incidence of eosinophilic globules in the olfactory and respiratory epithelia was reported in exposed female mice, and an increased incidence of eosinophilic globules in the nasopharynx was observed in male and female mice ([Kano et al., 2012](#)).

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

2,4-Dichloro-1-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 primarily

as an intermediate in the manufacture of diazo pigments, pharmaceuticals, and agrochemicals.

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. It has been detected in water and soil.

Occupational exposure is expected to occur primarily through inhalation in workplaces where 2,4-dichloro-1-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 found.

According to European Union regulations, 2,4-dichloro-1-nitrobenzene is not permitted in consumer goods such as decorative objects and games. Quantitative information on exposure in the general population was not found.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

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

In female mice, 2,4-dichloro-1-nitrobenzene caused a significant positive trend in the incidence and an increase in the incidence

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

In male rats, 2,4-dichloro-1-nitrobenzene caused a significant positive trend in the incidence and an increase in the incidence of renal cell adenoma, renal cell carcinoma, and of renal cell adenoma or carcinoma (combined). In male rats, 2,4-dichloro-1-nitrobenzene caused a significant positive trend in the incidence and an increase in the incidence of preputial gland adenoma.

In female rats, 2,4-dichloro-1-nitrobenzene caused a significant positive trend in the incidence and an increase in the incidence of renal cell adenoma, renal cell carcinoma, and of renal cell adenoma or carcinoma (combined).

5.4 Mechanistic and other relevant data

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

Concerning the key characteristics of carcinogens, there is *weak* evidence that 2,4-dichloro-1-nitrobenzene is genotoxic. No data in humans or in non-human mammals *in vivo* were available. 2,4-Dichloro-1-nitrobenzene gave negative results in the presence or absence of metabolic activation for chromosomal aberrations performed in a single study in Chinese hamster lung cells. In one study, 2,4-dichloro-1-nitrobenzene gave positive results

in various *Salmonella typhimurium* strains in the presence or absence of metabolic activation.

Exposure to 2,4-dichloro-1-nitrobenzene resulted in toxicity in rodent liver and kidney, and in the mouse respiratory system. No data for humans were available. In the chronic bioassay, both male and female rats exposed to 2,4-dichloro-1-nitrobenzene by diet developed atypical hyperplasia and eosinophilic droplets in the proximal tubule. Chronic progressive nephropathy occurred in male and female rats exposed to 2,4-dichloro-1-nitrobenzene.

6. Evaluation

6.1 Cancer in humans

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

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

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

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