2-CHLORONITROBENZENE, 3-CHLORONITROBENZENE AND 4-CHLORONITROBENZENE

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

2-Chloronitrobenzene

Chem. Abstr. Serv. Reg. No.: 88-73-3

Chem. Abstr. Name: 1-Chloro-2-nitrobenzene

IUPAC Systematic Name: 1-Chloro-2-nitrobenzene

Synonyms: *ortho*-Chloronitrobenzene; 2-chloro-1-nitrobenzene; 2-CNB; 2-nitrochlorobenzene; *ortho*-nitrochlorobenzene; 1-nitro-2-chlorobenzene

3-Chloronitrobenzene

Chem. Abstr. Serv. Reg. No.: 121-73-3

Chem. Abstr. Name: 1-Chloro-3-nitrobenzene

IUPAC Systematic Name: 1-Chloro-3-nitrobenzene

Synonyms: *meta*-Chloronitrobenzene; 3-chloro-1-nitrobenzene; 3-CNB; 3-nitrochlorobenzene; *meta*-nitrochlorobenzene; 1-nitro-3-chlorobenzene

4-Chloronitrobenzene

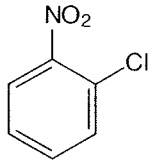
Chem. Abstr. Serv. Reg. No.: 100-00-5

Chem. Abstr. Name: 1-Chloro-4-nitrobenzene

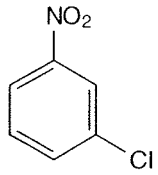
IUPAC Systematic Name: 1-Chloro-4-nitrobenzene

Synonyms: *para*-Chloronitrobenzene; 4-chloro-1-nitrobenzene; 4-CNB; 4-nitrochlorobenzene; *para*-nitrochlorobenzene; 1-nitro-4-chlorobenzene; 4-nitro-1-chlorobenzene; *para*-nitrophenyl chloride

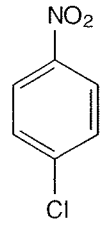
1.1.2 *Structural and molecular formulae and relative molecular mass*



2-Chloronitrobenzene



3-Chloronitrobenzene



4-Chloronitrobenzene

$C_6H_4ClNO_2$ Relative molecular mass: 157.56

1.1.3 *Chemical and physical properties of the pure substance*

2-Chloronitrobenzene

(*a*) *Description*: Monoclinic needles (Lide, 1993)

(*b*) *Boiling-point*: 246 °C (Lide, 1993)

(*c*) *Melting-point*: 34–35 °C (Lide, 1993)

(*d*) *Spectroscopy data*: Infrared (prism [3231, 9802], grating [29726]), ultraviolet (UV) [21893] and nuclear magnetic resonance (proton [10548], C-13 [4218]) spectral data have been reported (Sadtler Research Laboratories, 1980).

(*e*) *Solubility*: Slightly soluble in water (590 mg/L at 20 °C) (BUA, 1992a); soluble in acetone, benzene, diethyl ether and ethanol (Lide, 1993)

(*f*) *Volatility*: Vapour pressure, 0.4 mm Hg [53.3 Pa] at 25 °C; relative vapour density (air = 1), 5.44 (Monsanto Co., 1993a)

(*g*) *Octanol/water partition coefficient (P)*: log P, 2.24 (Hansch *et al.*, 1995)

(*h*) *Conversion factor*: $mg/m^3 = 6.44 \times ppm$[1]

3-Chloronitrobenzene

(*a*) *Description*: Pale yellow, rhombic prisms (from ethanol) (Lide, 1993)

(*b*) *Boiling-point*: 235–236 °C (Lide, 1993)

(*c*) *Melting-point*: 46 °C (Lide, 1993)

(*d*) *Spectroscopy data*: Infrared (prism [3232], grating [15443]), UV [958] and nuclear magnetic resonance (proton [6923], C-13 [6221]) spectral data have been reported (Sadtler Research Laboratories, 1980).

(*e*) *Solubility*: Slightly soluble in water (390 mg/L at 20 °C) (BUA, 1992b); sparingly soluble in cold ethanol; soluble in benzene, carbon disulfide, chloroform, diethyl ether, glacial acetic acid and hot ethanol (Budavari, 1989; Lide, 1993)

(*f*) *Volatility*: Vapour pressure, 1.85 Pa at 25 °C (BUA, 1992b)

(*g*) *Octanol/water partition coefficient (P)*: log P, 2.46 (Hansch *et al.*, 1995)

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[1] Calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming temperature (25 °C) and pressure (101 kPa)

(*h*) *Conversion factor*: $mg/m^3 = 6.44 \times ppm$[1]

4-Chloronitrobenzene

(*a*) *Description*: Monoclinic prisms (Lide, 1993)

(*b*) *Boiling-point*: 242 °C (Lide, 1993)

(*c*) *Melting-point*: 83.6 °C (Lide, 1993)

(*d*) *Spectroscopy data*: Infrared (prism [4683], grating [435]), UV [1290] and nuclear magnetic resonance (proton [10629, V122], C-13 [628]) spectral data have been reported (Sadtler Research Laboratories, 1980).

(*e*) *Solubility*: Slightly soluble in water (243 mg/L at 20 °C) (BUA, 1992b); soluble in acetone, boiling ethanol, diethyl ether and carbon disulfide; sparingly soluble in cold ethanol (Budavari, 1989; Lide, 1993)

(*f*) *Volatility*: Vapour pressure, 0.15 mm Hg [20 Pa] at 30 °C; relative vapour density (air = 1), 5.44 (Monsanto Co., 1993b)

(*g*) *Octanol/water partition coefficient (P)*: log P, 2.39 (Hansch *et al.*, 1995)

(*h*) *Conversion factor*: $mg/m^3 = 6.44 \times ppm$[1]

1.1.4 *Technical products and impurities*

2-Chloronitrobenzene is available commercially at purities of > 99–99.85% (DuPont Chemicals, 1993; Monsanto Co., 1993a; Aldrich Chemical Co., 1994).

3-Chloronitrobenzene is available commercially at purities of 95–98% (Aldrich Chemical Co., 1994).

4-Chloronitrobenzene is available commercially at purities of 99–99.3% (Monsanto Co., 1993b; Aldrich Chemical Co., 1994).

1.1.5 *Analysis*

In evaluating exposure to chloronitrobenzenes, zinc reduction of the nitro group in acidified urine has been used for the routine determination of urinary chloronitrobenzenes at concentrations of 5–50 mg/L. After diazotization and coupling to 1-amino-8-naphthol-2,4-disulfonic acid (Chicago acid), spectrophotometric determination of the primary aromatic amine as a red azo dye was carried out. An alternative method has been described, in which the aromatic nitro compounds are reduced under alkaline conditions using formamidine sulfinic acid (thiourea dioxide) (Koniecki & Linch, 1958).

Mitchell and Deveraux (1978) reported the role of various detectors in gas chromatography in the determination of traces of organic compounds in the atmosphere. For the monochloronitrobenzenes, electron capture detection was approximately 4000 times more sensitive than flame ionization detection. Other selective detectors include thermionic ionization, Hall electrolytic conductivity and mass spectrometry (BUA, 1992b).

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[1] Calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming temperature (25 °C) and pressure (101 kPa)

Simple and accurate high-performance liquid chromatographic (HPLC) methods have been developed for determining 4-chloronitrobenzene in plasma (Yoshida, 1991) and 4-chloronitrobenzene and its metabolites in rat urine (Yoshida, 1993a). Samples are diluted with methanol, centrifuged and the methanol eluate analysed by isocratic reverse-phase HPLC with UV detection. Detection limits for 4-chloronitrobenzene are 0.01 μg/mL in plasma and 0.1 μg/mL in urine.

Selected methods for the analysis of chloronitrobenzenes in various media are presented in Table 1.

Table 1. Methods for the analysis of 2-, 3- and 4-chloronitrobenzenes

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Air	Draw sample through solid sorbent tube; desorb with methanol	GC/FID	0.1 μg/sample[a]	Eller (1994) [Method 2005]
	Draw sample through Tenax TA; desorb thermally	GC/FID	< 0.04 μg/L[a,b,c]	Patil & Lonkar (1994)
	Trap in ethanol or isopropanol; reduce to aniline form with zinc/hydrochloric acid; couple with 1,2-naphthoquinone-4-sulfonic acid, disodium salt; extract with carbon tetrachloride; read at 450 nm	Colorimetry	10 μg/L[a]	Dangwal & Jethani (1980)
Water	Degas sample; adsorb on Tenax; desorb thermally	GC/MS	NR[a,b,c]	Braunstein *et al.* (1989)
	Extract sample with dichloromethane or adsorb on Amberlite XAD resin and elute with dichloromethane	GC/ECD	NR[a,b,c]	Feltes *et al.* (1990)
	Liquid–liquid extraction with dichloromethane; dry with anhydrous sodium sulfate; evaporate to dryness; redissolve in methanol	SFC/FID	10 ppm (mg/L)[c] 5 ppm (mg/L)[a]	Ong *et al.* (1992)
Industrial wastewater	Add sodium oxalate/EDTA/perchloric acid solutions; filter; adjust to pH 3.0 with perchloric acid	LC/UV	NR[a]	Nielen *et al.* (1985)
Soil	Extract with methanol; clean-up with solid phase extraction	HPLC/UV	NR[b,c]	Grob & Cao (1990)
		CGC	NR[b,c]	Greco & Grob (1990)
Urine	Reduce to aniline form with zinc/hydrochloric acid; couple with 1,2-naphthoquinone-4-sulfonic acid, disodium salt; extract with carbon tetrachloride; read at 450 nm	Colorimetry	0.6 mg/L[a]	Dangwal & Jethani (1980)

Table 1 (contd)

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Blood	Extract from separated plasma and concentrate simultaneously using 2,2,4-trimethylpentane	GC/ECD	1.0 µg/L[a]	Lewalter & Ellrich (1991)
Fish	Extract sample with acetonitrile; transfer to petroleum ether; clean-up by Florisil column chromatography	GC/ECD	5–25 ppb[b,c] (µg/kg)	Yurawecz & Puma (1983)

GC, gas chromatography; FID, flame ionization detection; MS, mass spectrometry; NR, not reported; ECD, electron capture detection; SFC, capillary supercritical fluid chromatography; EDTA, ethylenediamine tetraacetic acid; LC, liquid chromatography; UV, ultraviolet detection; HPLC, high-performance liquid chromatography; CGC, capillary gas chromatography

[a] 4-Chloronitrobenzene

[b] 3-Chloronitrobenzene

[c] 2-Chloronitrobenzene

1.2 Production and use

1.2.1 *Production*

Continuous or batch nitration of chlorobenzene at 40–70 °C with mixed acids (30% HNO_3 : 56% H_2SO_4 : 14% H_2O) typically gives a 98% yield of an isomer mix consisting of 34–36% 2-chloronitrobenzene, 63–65% 4-chloronitrobenzene and about 1% 3-chloronitrobenzene. The isomers can be separated by a combination of fractional crystallization and distillation (Dunlap, 1981; Booth, 1991).

Chlorination of nitrobenzene at 35–45 °C in the presence of iron [III] chloride gives an isomer mixture containing 86% 3-chloronitrobenzene, 10% 2-chloronitrobenzene and 4% 4-chloronitrobenzene. A continuous process has been described that uses a series of reactors operating at 35–55 °C, with a residence time of 5 h. Purification is achieved by a combination of distillation and crystallization. Final purification of 3-chloronitrobenzene may be achieved chemically by caustic hydrolysis of the residual 2- and 4-chloronitrobenzenes and washing them out as nitrophenols (Booth, 1991).

Production of monochloronitrobenzenes in the United States of America in the 1970s was approximately 70 000 tonnes per year, of which about 25 000 tonnes were the 2-isomer and 45 000 tonnes were the 4-isomer (Dunlap, 1981). Estimated annual production figures in 1985 for 2- and 4-chloronitrobenzenes were 60 000 tonnes in Germany, 40 000 tonnes in the United States and 30 000 tonnes in Japan (Booth, 1991). In Germany, an estimated 1000–3000 tonnes of 3-chloronitrobenzene are produced annually (BUA, 1992b).

2-Chloronitrobenzene is produced by 17 companies in China, four companies in India, three companies each in Germany and Japan, two companies each in France, Italy and the United States, and one company each in Brazil, Poland, the Republic of Korea and the United Kingdom. 4-Chloronitrobenzene is produced by 17 companies in China,

four companies each in India and Japan, three companies in Germany, two companies each in France, Italy and the United States and one company each in Brazil, Poland, the Republic of Korea and Romania. 3-Chloronitrobenzene is apparently produced in small quantities in several countries, including, at least, Germany, India and the United Kingdom (BUA, 1992b; Chemical Information Services, 1994).

1.2.2 *Use*

The chloronitrobenzenes are used almost exclusively as chemical intermediates. 2-Chloronitrobenzene is an important intermediate in the synthesis of colourants. Reduction with iron produces 2-chloroaniline (Fast Yellow G Base) and electrolytic reduction followed by rearrangement of the resulting hydrazo derivative leads to 3,3′-dichlorobenzidine (see IARC, 1982a, 1987a), both of which are important diazo components. Treatment of 2-chloronitrobenzene with aqueous sodium hydroxide at 130 °C produces 2-nitrophenol; treatment with methanol–sodium hydroxide gives 2-nitroanisole (see this volume); and treatment with ethanol–sodium hydroxide gives 2-nitrophenetole. All of these are used as precursors of the derived amines and many other products. Treatment with aqueous ammonia at 175 °C under pressure yields 2-nitroaniline (Fast Orange GR Base). Sulfonation and chlorosulfonation also give important sulfonic acid and sulfonyl chloride derivatives (Booth, 1991).

2-Chloronitrobenzene is also used in the preparation of *ortho*-anisidine (see IARC, 1982b, 1987b) (Fast Red BB Base), *ortho*-phenetidine, 3-amino-4-hydroxybenzenesulfonamide, picric acid, lumber preservatives, diaminophenol hydrochloride (a photographic developer), corrosion inhibitors, pigments and agricultural chemicals (Farris, 1978; Dunlap, 1981; Hathaway *et al.*, 1991; Lewis, 1993).

Most 3-chloronitrobenzene is reduced to 3-chloroaniline (Orange GC Base), a dye intermediate, with minor quantities finding other applications. Crude 3-chloronitrobenzene can be chlorinated exhaustively to give pentachloronitrobenzene, the use of which as a fungicide (Terrachlor) has led to several series of nitro-containing agrochemicals (Booth, 1991).

4-Chloronitrobenzene and its derivatives are also used in many synthetic processes. Common chemical intermediates produced from 4-chloronitrobenzene include 4-chloroaniline (see IARC, 1993), 4-nitrophenol, 4-nitroanisole, *para*-anisidine (IARC, 1982b, 1987b), 4-nitroaniline (Fast Red GC base), 6-chloro-3-nitrobenzenesulfonic acid, 2,4-dinitrochlorobenzene and 3,4-dichloronitrobenzene. 4,4′-Diaminodiphenyl sulfone (Dapsone; see IARC, 1980, 1987c), a drug used to treat leprosy, is synthesized from 4-chloronitrobenzene by one of two methods. In addition, condensation of 4-chloronitrobenzene with 2,4-dichlorophenol gives 2,4-dichloro-4′-nitrodiphenyl ether, which is used as a herbicide (nitrofen; see IARC, 1983, 1987d); 4-chloronitrobenzene can also be reacted with aniline to give 4-nitrodiphenylamine, which on reductive *N*-alkylation gives important antioxidants for rubber (see IARC, 1982c, 1987e; Dunlap, 1981; Budavari, 1989; Booth, 1991; Lewis, 1993).

1.3 Occurrence

1.3.1 *Natural occurrence*

Chloronitrobenzenes are not known to occur as natural products.

1.3.2 *Occupational exposure*

Occupational exposures to 4-chloronitrobenzene were measured for 10 workers in a dye factory in Japan producing 4-nitrophenol and *para*-anisidine (Yoshida *et al.*, 1988). The workers packed 4-chloronitrobenzene into bags and also transferred the raw material into a reaction vessel. Personal exposures were monitored for three seasons and averaged 0.31 mg/m^3 in autumn (range, 0.12–0.80 mg/m^3), 0.16 mg/m^3 in winter (range, 0.04–0.66 mg/m^3) and 0.38 mg/m^3 in summer (range, 0.16–0.88 mg/m^3). Workers wore simple dust respirators.

Long-term occupational exposure to aromatic nitro and amino compounds, including 2- and 4-chloronitrobenzenes, has been studied in Japan in 35 male workers involved in the production of dyes or pharmaceuticals. Urinary excretion of diazo metabolites was significantly higher ($p < 0.01$) in exposed workers compared to office workers. The main duties of the exposed workers included loading aromatic nitro and amino compounds from paper bags into reaction vessels, removing the reaction products from the reaction vessels and bagging the reaction products in paper bags. These jobs were carried out in separate rooms in the chemical factories. A few exposed workers carried the bags containing aromatic nitro and amino compounds or reaction products by fork lift truck to the warehouse for storage. The workers wore protective fabric clothing and cotton gloves. When the workers were engaged in the bagging operations, they also wore particulate-filter respirators and polyvinyl chloride-coated cotton gloves. The authors concluded that the workers were exposed to aromatic nitro-amino compounds routinely at more than 0.3 mg/m^3 (as 4-chloronitrobenzene) and that exposure occurred through skin contact and inhalation (Yoshida *et al.*, 1989a).

Patil and Lonkar (1994) described the development of a method for monitoring chloronitrobenzenes in the presence of chlorobenzene in the workplace environment. In order to validate the method, air samples were collected in India at a chemical plant manufacturing chloronitrobenzenes using chlorobenzene as raw material. The relative humidity and temperature of the workplace during the period of investigation were found to vary in the ranges of 50–90% and 20–40 °C, respectively. Concentrations ranged from 0.03 to 0.52 mg/m^3 for 2-chloronitrobenzene, from 0.03 to 0.17 mg/m^3 for 3-chloronitrobenzene and from 0.16 to 0.96 mg/m^3 for 4-chloronitrobenzene.

The National Occupational Exposure Survey conducted between 1981 and 1983 indicated that 2900 and 2950 employees in the United States were potentially exposed to 2-chloronitrobenzene and 4-chloronitrobenzene, respectively. The estimate is based on a survey of companies and did not involve measurements of actual exposure (United States National Institute for Occupational Safety and Health, 1995).

1.3.3 *Environmental occurrence*

The major sources of environmental release of nitro aromatic compounds, such as chloronitrobenzenes, appear to be from chemical plants where they are produced and/or used as intermediates. Minor sources of release into the environment may occur during transport, storage or land burial, and chloronitrobenzenes may form in the environment through the oxidation of man-made aromatic amines or the reaction of nitrogen oxides in highly polluted air with chlorinated aromatic hydrocarbons (Howard, 1989).

Water

In the United States, 2-chloronitrobenzene was detected in river water samples from the Mississippi at Cape Girardeau, MO, and New Orleans, LA, at concentrations of 4–37 μg/L (0.004–0.037 ppm) and 1–2 μg/L (0.001–0.002 ppm), respectively, from September 1957 to April 1959 (Middleton & Lichtenberg, 1960). 2- and 4-Chloronitrobenzenes have been identified but not quantified in drinking-water from New Orleans, LA (Lucas, 1984).

From 1977 to 1982, 2- and 4-chloronitrobenzenes were detected in the River Rhine at levels ranging from less than 0.1 μg/L to 1.0 μg/L and from less than 0.1 μg/L to 0.11 μg/L, respectively. In a related study, 4-chloronitrobenzene was monitored in the River Rhine at a concentration of 1 μg/L, but was not detected in related tap-water (Piet & Morra, 1983). Concentrations of 3-chloronitrobenzene in the River Rhine have generally been less than 0.1 μg/L, although occasional samples have contained up to 1.2 μg/L. Higher concentrations of 2- and 3-chloronitrobenzenes were reported in 1978–79 in some samples from the Dutch section of the River Rhine (BUA, 1992a,b).

Chloronitrobenzenes have been identified in water samples from three sampling points along the River Elbe in Germany. In samples collected near Lauenberg, the concentrations of 2-, 3- and 4-chloronitrobenzenes were 0.2, 0.2 and 0.3 μg/L, respectively; in samples collected near Brokdorf, they were 0.1, 0.04 and 0.1 μg/L, respectively; and in samples collected near Brunsbüttel, they were 0.04, < 0.02 and 0.04 μg/L, respectively (Feltes *et al.*, 1990).

Concentrations of chloronitrobenzenes were measured at five sampling sites on the River Bormida in north-western Italy between September 1989 and February 1990. Twelve samples were taken at each site, and the mean concentrations of 2-, 3- and 4-chloronitrobenzenes were highest near Bistagno (0.48, 0.12 and 0.19 μg/L, respectively) and lowest near the estuary at Alessandria (0.03, 0.01 and 0.01 μg/L, respectively) (Trova *et al.*, 1991)

In Dutch coastal waters, mean concentrations of 11 ng/L (maximum concentration, 50 ng/L) 2-chloronitrobenzene, 4.1 ng/L (maximum concentration, 14 ng/L) 3-chloronitrobenzene and 6.9 ng/L (maximum concentration, 31 ng/L) 4-chloronitrobenzene were detected (van de Meent *et al.*, 1986). Chloronitrobenzenes were also identified as micropollutants in water samples collected in 1986 from the Scheldt Estuary, located in the south-west of the Netherlands and the north-west of Belgium. Dissolved concentrations in water ranged from 0.5 to 2.1 ng/L (median, 1.3 ng/L) for 2-chloronitrobenzene, 0.1 to 1.3 ng/L (median, 0.3 ng/L) for 3-chloronitrobenzene and 0.5 to 2.5 ng/L

(median, 1.4 ng/L) for 4-chloronitrobenzene. Concentrations of suspended particulate matter reached a maximum of 1.9 ng/g for 2-chloronitrobenzene, 1.5 ng/g (median, 0.2 ng/g) for 3-chloronitrobenzene and 1.9 ng/g (median, 0.3 ng/g) for 4-chloronitrobenzene (van Zoest & van Eck, 1991).

Chloronitrobenzenes were identified as pollutants in groundwater samples collected from January to March 1987 in Degrémont, France. 2-Chloronitrobenzene was the primary pollutant, accounting for 70% of the pollution; concentrations ranged from 970 to 1828 µg/L, with an average of 1500 µg/L. Concentrations of 3- and 4-chloronitrobenzenes ranged from 72 to 179 µg/L and 5 to 123 µg/L, respectively (Duguet *et al.*, 1988).

2-Chloronitrobenzene has been identified in treated effluents from an advanced wastewater treatment plant in Orange County, CA, United States (Lucas, 1984). 2-, 3- and 4-Chloronitrobenzenes have also been identified in the effluent from 3-chloronitrobenzene production at concentrations ranging from 1500 to 1800 mg/L (Howard *et al.*, 1976).

Wastewaters discharged from a chloronitrobenzene manufacturing plant in India were found to contain 24–93 mg/L (mean, 71 mg/L) 2-chloronitrobenzene, 1.3–3.0 mg/L (mean, 2.0 mg/L) 3-chloronitrobenzene and 112–227 mg/L (mean, 166 mg/L) 4-chloronitrobenzene (Swaminathan *et al.*, 1987).

1.3.4 *Food*

In the United States, 2-chloronitrobenzene (at concentrations of 0.006–0.24 ppm (mg/kg)), 3-chloronitrobenzene (at 0.057 ppm) and 4-chloronitrobenzene (at 0.008–0.63 ppm) were found in the edible portion of various species of fish taken from the Mississippi River at sampling points 0, 60 and 150 miles south of St Louis, MO. Chloronitrobenzenes were not detected (< 0.005 ppm) in fish taken from the Mississippi River 100 miles north of St Louis (2 samples) or 260–400 miles south of St Louis (3 samples) or in those taken from the Missouri River (6 samples) (Yurawecz & Puma, 1983).

Chlorinated nitrobenzenes, including 2-, 3- and 4-chloronitrobenzenes, have been identified in fish from the River Main in Germany (Steinwandter, 1987)

1.4 Regulations and guidelines

Occupational exposure limits and guidelines for 4-chloronitrobenzene in several countries are given in Table 2.

2. Studies of Cancer In Humans

No data were available to the Working Group.

Table 2. Occupational exposure limits and guidelines for 4-chloronitrobenzene

Country	Year	Concentration (mg/m^3)	Interpretation
Argentina	1991	0.6 (Sk)	TWA
Australia	1993	0.6 (Sk)	TWA
Austria	1993	1 (Sk)	TWA
Belgium	1993	0.64 (Sk)	TWA
Bulgaria[a,b]	1995	0.64 (Sk)	TWA
Canada	1991	3 (Sk)	TWA
Columbia[a,b]	1995	0.64 (Sk)	TWA
Czech Republic[b]	1993	1	TWA
		2	STEL
Denmark	1993	1 (Sk)	TWA
Finland	1993	1 (Sk)	TWA
		3	STEL (15 min)
Germany[b]	1995	None (Sk) IIIB	–
Hungary	1993	1 (Sk)	TWA
		2	STEL
Japan	1993	0.64 (Sk)	TWA
Jordan[a,b]	1995	0.64 (Sk)	TWA
Mexico	1991	1 (Sk)	TWA
		2	STEL (15-min)
Netherlands	1994	1 (Sk)	TWA
New Zealand[a,b]	1995	0.64 (Sk)	TWA
Philippines[a,b]	1995	0.64 (Sk)	TWA
Poland[b]	1993	1	TWA
Republic of Korea[a,b]	1995	0.64 (Sk)	TWA
Russia	1993	0.64 (Sk)	TWA
		1[b]	STEL
Singapore[a,b]	1995	0.64 (Sk)	TWA
Switzerland	1993	1 (Sk)	TWA
		2	STEL
United Kingdom	1995	1 (Sk)	TWA
		2	STEL (15-min)
USA			
ACGIH (TLV)	1995	0.64 (Sk)[c,d]	TWA
OSHA (PEL)	1994	1 (Sk)	TWA
NIOSH (REL)	1994	(Sk,Ca)	LFC

Table 2 (contd)

Country	Year	Concentration (mg/m^3)	Interpretation
Vietnam[a,b]	1995	0.64 (Sk)	TWA

From Arbeidsinspectie (1994); United States National Institute for Occupational Safety and Health (NIOSH) (1994a,b); United States Occupational Safety and Health Administration (OSHA) (1994); American Conference of Governmental Industrial Hygienists (ACGIH) (1995); Deutsche Forschungsgemeinschaft (1995); Health and Safety Executive (1995); United Nations Environment Programme (1995)

Sk, absorption through the skin may be a significant source of exposure; TWA, time-weighted average; STEL, short-term exposure limit; IIIB, suspected of having carcinogenic potential; TLV, threshold limit value; PEL, permissible exposure level; REL, recommended exposure level; LFC, lowest feasible concentration; Ca, potential occupational carcinogen

[a] Follows ACGIH values

[b] Same occupational exposure limit for 2-chloronitrobenzene and/or 3-chloronitrobenzene

[c] Substance identified in the BEI (Biological Exposure Indices) documentation for inducers of methaemoglobin

[d] Substance identified by other sources as a suspected or confirmed carcinogen

3. Studies of Cancer in Experimental Animals

2-Chloronitrobenzene

Oral administration

Mouse: Groups of 25 male and 25 female CD-1 mice (derived from HaM/ICR mice), six to eight weeks of age, were fed diets containing 0 (control), 3000 or 6000 mg/kg (ppm) 2-chloronitrobenzene (97–99% pure) for eight months. After that time, the dietary concentrations were lowered to 0, 1500 and 3000 mg/kg (ppm) for 10 months and mice were then held for three months on control diet prior to terminal examination. Mice that died within the first six months of the study were discarded without necropsy. Complete gross necropsy was carried out on all other animals; certain tissues were examined histopathologically, including those of the lung, liver, spleen, kidney, adrenal glands, heart, urinary bladder, stomach, intestines and reproductive organs; gross lesions and tissue masses were also examined histopathologically. Information on survival or body-weight gain was not reported. The incidence of hepatocellular carcinomas in males (3/18 controls, 7/17 at the low dose and 3/16 at the high dose) was increased at the low dose ($p < 0.025$ versus a pooled control incidence of 7/99, Fisher's exact test) and in females

(0/20 controls, 5/22 at the low dose and 5/19 at the high dose) was increased at the low and high doses ($p < 0.025$ versus concurrent and pooled control incidence of 1/102) (Weisburger *et al.*, 1978). [The Working Group noted the small number of animals and the limited histopathological evaluation and reporting.]

Rat: Groups of 25 male Charles River CD rats (derived from Sprague Dawley), six to eight weeks of age, were fed diets containing 0 (control), 1000 or 2000 mg/kg (ppm) 2-chloronitrobenzene (97–99% pure) for six months. After that time, the dietary concentrations were lowered to 0, 500 and 1000 mg/kg (ppm) for 12 months and rats were then held for six months on control diet prior to terminal examination. Rats that died within the first six months of the study were discarded without necropsy. Complete gross necropsy was carried out on all other animals; certain tissues were examined histopathologically, including those of the lung, liver, spleen, kidney, adrenal glands, heart, urinary bladder, stomach, intestines and pituitaries; gross lesions and tissue masses were also examined histopathologically. Information on survival or body-weight gain and individual tumour incidence was not reported. The total number of animals bearing multiple tumours was increased in low-dose rats (incidence of multiple tumours: controls, 1/22; low-dose rats, 7/22; high-dose rats, 1/19; $p < 0.025$ versus concurrent and pooled control incidence of 14/111, Fisher's exact test) (Weisburger *et al.*, 1978). [The Working Group noted the small number of animals, the short duration of treatment, the limited histopathological evaluation and the inadequate reporting.]

4-Chloronitrobenzene

Oral administration

Mouse: Groups of 25 male and 25 female CD-1 mice (derived from HaM/ICR mice), six to eight weeks of age, were fed diets containing 0, 3000 or 6000 mg/kg (ppm) 4-chloronitrobenzene (97–99% pure) for 18 months. The mice were then held for three months on control diet prior to terminal examination. Mice that died within the first six months of the study were discarded without necropsy. Complete gross necropsy was carried out on all other animals; certain tissues were examined histopathologically including those of the lung, liver, spleen, kidney, adrenal glands, heart, urinary bladder, stomach, intestines and reproductive organs; gross lesions and tissue masses were also examined histopathologically. Information on survival or body-weight gain was not reported. The incidence of hepatocellular carcinomas was increased in low-dose males (controls, 1/14; low-dose mice, 4/14; $p < 0.025$ versus pooled control incidence of 7/99, Fisher's exact test; high-dose mice, 0/14). The incidence of vascular tumours was increased in high-dose males (controls, 0/14; low-dose mice, 2/14; high-dose mice, 4/14; $p < 0.025$ versus concurrent and pooled control incidence of 5/99) and in high-dose females (controls, 0/15; low-dose mice, 2/20; high-dose mice, 7/18; $p < 0.025$ versus concurrent and pooled control incidence of 9/102) (Weisburger *et al.*, 1978). [The Working Group noted the small number of animals and the limited histopathological evaluation and reporting.]

Rat: Groups of 25 male Charles River CD rats (derived from Sprague Dawley rats), six to eight weeks of age, were fed diets containing 0, 2000 or 4000 mg/kg (ppm) 4-chloronitrobenzene (97.99% pure) for three months. After that time, the dietary concentrations were lowered to 0, 250 and 500 mg/kg (ppm) for two months, then raised to 0, 500 and 1000 mg/kg (ppm) for 13 months; rats were then held for six months on control diet prior to terminal examination. Rats that died within the first six months of the study were discarded without necropsy. Complete gross necropsy was carried out on all other animals; certain tissues were examined histopathologically, including the lung, liver, spleen, kidney, adrenal glands, heart, urinary bladder, stomach, intestines and pituitaries; gross lesions and tissue masses were also examined histopathologically. Information on survival or body-weight gain was not reported. No increase in tumour incidences was reported (Weisburger *et al.*, 1978). [The Working Group noted the small number of animals, the short duration of dosing and the limited histopathological evaluation and reporting.]

3-Chloronitrobenzene

No data were available to the Working Group.

4. Other Data Relevant for an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

The measurement of urinary excretion of diazo-positive metabolites has been employed for biological monitoring of 4-chloronitrobenzene-exposed workers. The time-weighted average air concentrations in the breathing zones of 10 workers in a dye-producing factory that used 4-chloronitrobenzene were 0.31, 0.16 and 0.38 mg/m^3 in the autumn, winter and summer, respectively. In unexposed controls (laboratory staff), the excretion of diazo-positive metabolites (4-aminophenol equivalents) pre- and post-shift was 0.261 and 0.274 [mg/g] creatinine, respectively. In the exposed workers, the average values ranged from 0.375 to 0.543 [mg/g] creatinine pre-shift and from 0.677 to 1.086 [mg/g] creatinine post-shift. Pre-shift values rose over the working week, which indicates slow elimination and consequent gradual accumulation (Yoshida *et al.*, 1988). [The authors use mg/mg creatinine, but the Working Group considered mg/g creatinine to be the most likely units.]

The excretion of urinary metabolites of 4-chloronitrobenzene has been studied in connection with an outbreak of accidental poisoning with 4-chloronitrobenzene (Yoshida *et al.*, 1992, 1993). In the more extensive study of the outbreak, Yoshida *et al.* (1993) analysed urinary elimination of metabolites for up to 29 days in eight workers. The parent compound was not excreted. However, metabolites were eliminated for the total observation period (total metabolites, 179–1076 mg). The elimination of each metabolite

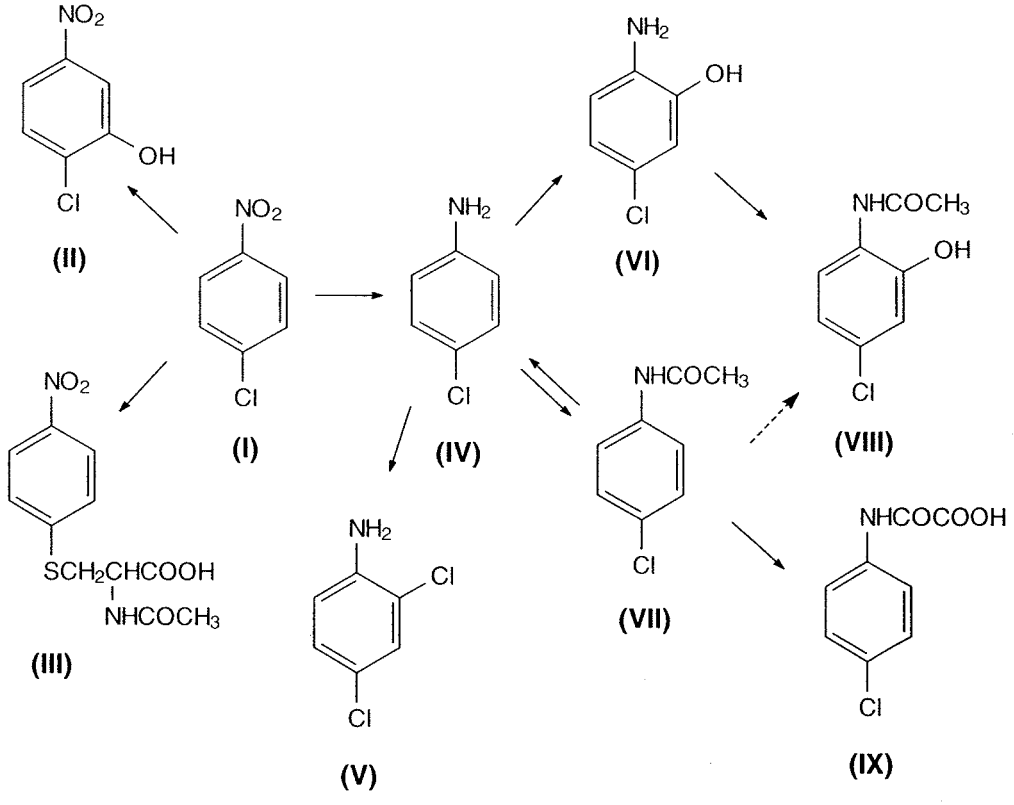
seemed to fit into a one-compartment model with an elimination half-life of about one week. There was a complicated metabolic pattern of considerable interindividual variation, which had three major pathways (see Figure 1):

(1) The most important route of metabolism was glutathione conjugation, which resulted in the excretion of the mercapturic acid, *N*-acetyl-*S*-(4-nitrophenyl)-L-cysteine (48% of the total metabolites).

(2) There was also a slow reduction of the nitro group, resulting in excretion of 4-chloroaniline (29.9%), which was rapidly metabolized further by (a) fast *N*-acetylation to 4-chloroacetanilide and further to 4-chloro-oxanilic acid, (b) ring-hydroxylation to give 2-amino-5-chlorophenol (8.7%) — which was *N*-acetylated into 4-chloro-2-hydroxyacetanilide — and (c) chlorination to give 2,4-dichloroaniline (chlorination was a novel metabolic pathway in humans), which was readily excreted (1.2%).

(3) Finally, there was a slow ring-hydroxylation of the parent compound to give 2-chloro-5-nitrophenol (12.2%).

Figure 1. Metabolic pathway of 4-chloronitrobenzene in humans



From Yoshida *et al.* (1993)

I, 4-Chloronitrobenzene; II, 2-chloro-5-nitrophenol; III, *N*-acetyl-*S*-(4-nitrophenyl)-L-cysteine; IV, 4-chloroaniline; V, 2,4-dichloroaniline; VI, 2-amino-5-chlorophenol; VII, 4-chloroacetanilide; VIII, 4-chloro-2-hydroxyacetanilide; and IX, 4-chloro-oxanilic acid.

Dotted line: It was not clear whether VIII was formed by hydroxylation of VII.

No data concerning the absorption, distribution, metabolism and excretion of 2- and 3-chloronitrobenzenes in humans were available to the Working Group.

4.1.2 *Experimental systems*

Dermal absorption studies with 2- and 4-chloronitro[^{14}C]benzenes were conducted in male Fischer 344 rats using dermal applications of [0.0325, 0.32, and 3.25 mg/cm^2] (0.65, 6.5 and 65 mg/kg bw, respectively) (United States National Toxicology Program, 1993). At all dose levels, 33–40% of 2-chloronitrobenzene and 51–62% of 4-chloronitrobenzene were absorbed from the skin within 72 h, based upon measurements of eliminated radioactivity. The absorbed radioactivity was excreted in urine and faeces. Urinary excretion of 4-chloronitrobenzene-derived radioactivity was substantially greater than that of 2-chloronitrobenzene-derived radioactivity over 72 h (43–45% versus 21–28% of the dose). Approximately 5–12% of 4-chloronitrobenzene-derived radioactivity were excreted in faeces, while 11–15% of 2-chloronitrobenzene-derived radioactivity were excreted via this route.

In studies by oral administration, rats were given 2, 20 or 200 mg/kg bw 2-chloronitrobenzene or 4-chloronitrobenzene, and urine and faeces were collected for up to 72 h. At all doses, 61–77% of the 2-chloronitrobenzene and 73–78% of the 4-chloronitrobenzene were absorbed. Both isomers were rapidly metabolized and excreted primarily in the urine. At the lower doses, about 6% and 3% of the administered doses of 2-chloronitrobenzene and about 23% and 5% of the administered doses of 4-chloronitrobenzene were found in tissues after 24 and 72 h, respectively. For 2-chloronitrobenzene, most tissues contained less than 0.1% of the dose administered. Most of the radioactivity was in the liver, followed by fat, muscle and kidney. For 4-nitrochlorobenzene, the highest concentrations of ^{14}C at 24 h were found in fat, followed by blood cells, kidney, liver and spleen. HPLC analysis of urine revealed the presence of up to 23 metabolites from 2-chloronitrobenzene and 25 metabolites from 4-chloronitrobenzene [metabolites unspecified] (United States National Toxicology Program, 1993).

In repeated-dose studies, groups of four young adult (10–12 weeks of age) or geriatric (19–20 months of age) male Fischer 344 rats received 65 mg/kg bw 2-chloronitro[^{14}C]-benzene or 4-chloronitro[^{14}C]benzene by gavage on days 1, 5 and 9. Unlabelled parent compound was administered in corn oil at the same dose on days 2, 3, 4, 6, 7, 8, 10 and 11. In young adult rats after 0, 4 or 8 days of pretreatment with 2-chloronitrobenzene, ^{14}C was excreted primarily in urine for the first 24 h. Radioactivity was excreted rapidly in both urine and faeces following pretreatment. Approximately 5% of the administered radioactivity was found in the tissues 72 h after the day 9 dose, and most was in the liver. Similar results were reported for 4-chloronitrobenzene. However, with 4-chloronitrobenzene, most of the radioactivity was found in blood cells and fat. The absorption, metabolism and excretion of 2-chloronitrobenzene and 4-chloronitrobenzene were not greatly affected by age. Geriatric rats absorbed, metabolized and cleared these compounds as readily as young adults at the 65-mg/kg dose. Only very minor variations in rates of excretion in urine and faeces were noted (United States National Toxicology Program, 1993).

Bray *et al.* (1956) examined the metabolism of the three isomers of chloronitrobenzenes in rabbits. Doe rabbits were given [route unspecified] 0.1 g/kg [whether diet or body weight not specified] 2-chloronitrobenzene and 0.2 g/kg 3- and 4-chloronitrobenzenes. Urine was collected over 24-h periods until metabolites were no longer excreted (usually after 48 h). The main metabolic processes were reduction and hydroxylation. For each isomer, nearly the entire dose was excreted in urine as the chloroaniline or derivatives of phenolic metabolites. For all three isomers, about 10% of the administered dose was excreted as chloroaniline. The phenols formed were excreted mainly as conjugates with sulfuric and glucuronic acids (66% of the administered dose for 2-chloronitrobenzene, 51% for 3-chloronitrobenzene and 40% for 4-chloronitrobenzene).

Yoshida *et al.* (1991) identified the urinary metabolites of 4-chloronitrobenzene in rats by gas chromatography–mass spectrometry. Male Sprague-Dawley rats were given a single intraperitoneal injection of 100 mg/kg bw 4-chloronitrobenzene and urine was collected from 8 to 24 h after dosage. Rats excreted eight urinary metabolites: 4-chloroaniline, 2,4-dichloroaniline, 4-nitrothiophenol, 2-chloro-5-nitrophenol, 2-amino-5-chlorophenol, 4-chloroformanilide, 4-chloro-2-hydroxyacetanilide and a small amount of 4-chloroacetanilide. Only trace amounts of unchanged 4-chloronitrobenzene were detected.

Rickert and Held (1990) studied the metabolism of the radiolabelled isomers of chloronitrobenzenes by isolated male Fischer 344 rat hepatocytes. Incubation of rat hepatocytes with 2-chloronitro[^{14}C]benzene resulted in the detection of 2-chloroaniline (19.2% of total radioactivity), 2-chloroaniline-*N*-glucuronide (14.2% of total radioactivity and *S*-(2-nitrophenyl)glutathione (13.3% of total radioactivity). Metabolism of 3-chloronitro[^{14}C]benzene by isolated male Fischer 344 rat hepatocytes produced 3-chloroaniline as the major metabolite (30.9% of total radioactivity). Much smaller quantities of 3-chloroacetanilide (16.7%) and 3-chloroaniline-*N*-glucuronide (6.7%) were also produced by this isomer. No glutathione conjugate was detected. 4-Chloronitro[^{14}C]-benzene was metabolized to 4-chloroaniline (15.4% of total radioactivity), *S*-(4-nitrophenyl) glutathione (10.4%) and 4-chloroacetaniline (16.3%). Studies of metabolism with hepatic microsomes demonstrated that the formation of the chloroanilines from all isomers of the chloronitrobenzenes was mediated by cytochrome P450-dependent metabolism; formation was inhibited by SKF 525-A, metyrapone and carbon monoxide.

4.2 Toxic effects

4.2.1 *Humans*

Eleven longshoremen were poisoned with 4-chloronitrobenzene due to the accidental opening of bags. It was assumed that both inhalation and skin absorption occurred. The symptoms included headache, palpitation, dizziness, nausea, vomiting and poor appetite. On physical examination they had cyanosis. Laboratory tests revealed methaemoglobinaemia, anaemia, reticulocytosis and Heinz bodies (Yoshida *et al.*, 1992, 1993).

No data concerning the toxic effects of 2-chloronitrobenzene (or 3-chloronitrobenzene) in humans were available to the Working Group.

4.2.2 *Experimental systems*

Vernot *et al.* (1977) determined the acute toxicity of 2-chloronitrobenzene and 4-chloronitrobenzene. In male Sprague-Dawley rats, single oral LD_{50} values for 2-chloronitrobenzene and 4-chloronitrobenzene were 270 and 810 mg/kg bw, respectively. For male CF-1 mice, acute oral LD_{50} values were reported as 140 mg/kg bw for 2-chloronitrobenzene and 1410 mg/kg bw for 4-chloronitrobenzene.

In a teratogenicity study with New Zealand white rabbits, 8/18 died during treatment with 4-chloronitrobenzene at 40 mg/kg bw per day on gestation days 7–19 (Nair *et al.*, 1985).

Nair *et al.* (1986a) exposed male and female Sprague Dawley rats by inhalation to 2-chloronitrobenzene atmospheres at concentrations of 0, 10, 30 and 60 mg/m^3 (measured values, 0, 9.9, 30 and 59 mg/m^3) for 6 h per day on five days per week for four weeks. A dose-related increase in blood methaemoglobin levels was observed after two weeks of exposure. The increase was statistically significant at the mid- and high-exposure concentrations compared to control animals. Four weeks of exposure to 30 and 60 mg/m^3 produced a significant increase in blood methaemoglobin levels and a decrease in haemoglobin, haematocrit and red blood cell count values in both sexes. Increases in liver, kidney and spleen weights were also observed at 30 and 60 mg/m^3. Only the spleen was found to have microscopic changes, such as an increase in extramedullary haematopoiesis, at exposures of 30 and 60 mg/m^3. In addition, there was an increase in haemosiderosis in the spleen at all doses of 2-chloronitrobenzene.

In a similar study, Nair *et al.* (1986b) also examined the inhalation toxicity of 4-chloronitrobenzene in male and female Sprague-Dawley rats. Animals were exposed to concentrations of 0, 5, 15 and 45 mg/m^3 (measured values, 6, 19 and 46 mg/m^3) 4-chloronitrobenzene in air for 6 h per day on five days per week for four weeks. 4-Chloronitrobenzene caused dose-dependent cyanosis. Cyanosis of the conjunctival and nasal area was observed at all concentration levels, while rats in the mid- and high-exposure groups were cyanotic over the entire body. 4-Chloronitrobenzene produced significant methaemoglobinaemia at the two-week interval in mid-exposure females (11.5% ± 5.1 versus 2.7 ± 2.8 for controls) and in high-exposure males (14.0 % ± 9.2 versus 3.2 % ± 2.7) and females (14.9 % ± 1.6 versus 2.7 % ± 2.8). After four weeks of exposure, 4-chloronitrobenzene produced a significant increase in methaemoglobin levels in female rats exposed to mid and high doses and in male rats exposed to low, mid and high doses. This isomer also produced statistically significant reductions in red blood cell counts, haematocrit and haemoglobin at both 15 and 45 mg/m^3 in both males and females exposed for two and four weeks. A significant increase in white blood cell counts was seen in mid- and high-dose females and high-dose males after two weeks of exposure. At four weeks, white blood counts were elevated in high-dose males and females. Splenic enlargement, accompanied by an increase in absolute and relative spleen weights, was observed in male rats exposed to the high dose of 4-chloronitrobenzene. Mean and relative liver weights were also significantly increased in high-dose male rats. Microscopic examination revealed that high-dose exposure also increased the incidence of splenic congestion and extramedullary haematopoiesis and haemosiderosis.

Nishida *et al.* (1982) observed increased methaemoglobin levels, increased osmotic fragility of erythrocytes and increased formation of Heinz bodies in rabbits [sex unspecified] following a single subcutaneous dose of 50–200 mg/kg bw 4-chloronitrobenzene.

Watanabe *et al.* (1976) examined the toxicity of the three isomers of chloronitrobenzene in male Wistar rats injected intraperitoneally with 100 μmol/kg bw [15.8 mg/kg bw] 2-, 3- or 4-chloronitrobenzenes. Animals were killed 5 h later to determine methaemoglobin levels. 3-Chloronitrobenzene was the most potent of the isomers in the production of methaemoglobinaemia (31.9 ± 5.8% versus 20.6 ± 6.9% for 2-chloronitrobenzene and 16.3 ± 7.5% for 4-chloronitrobenzene). Methaemoglobin formation was also studied *in vitro*. Both 3- and 4-chloronitrobenzenes produced levels of methaemoglobin that were significantly greater than control levels.

In a study conducted by Yoshida *et al.* (1989b) male Fischer 344 rats were injected intraperitoneally with 1.0 mmol [158 mg]/kg bw 4-chloronitrobenzene. Urine was collected for 24 h and rats were killed 48 h after the injection to remove kidneys and collect blood. 4-Chloronitrobenzene had no effect on the urea nitrogen content of blood but produced a significant elevation in urine output and a significant increase in urinary *N*-acetyl-β-D-glucosaminidase activity. 4-Chloronitrobenzene caused no significant histopathological change in renal tubular epithelial cells. The compound caused a significant elevation in methaemoglobin levels as compared to controls (5.4% ± 0.5 versus 1.1% ± 0.2). 4-Chloronitrobenzene caused no significant change in serum aspartate aminotransferase or alanine aminotransferase activities.

Two-week and 13-week whole-body exposure studies of 2-chloronitrobenzene and 4-chloronitrobenzene were conducted in male and female Fischer 344 rats and in male and female B6C3F1 mice (United States National Toxicology Program, 1993). During the studies, rats and mice were exposed to 0, 1.1, 2.3, 4.5, 9 or 18 ppm [0, 6.4, 14.8, 29, 58 or 116 mg/m^3] 2-chloronitrobenzene vapour or 0, 1.5, 3, 6, 12 or 24 ppm [9.6, 19.3, 38.6, 77 or 155 mg/m^3] 4-chloronitrobenzene vapour for 6 h per day on five days per week, excluding weekends and holidays, for either 12 exposure days or 13 weeks.

Male and female rats exposed to 2-chloronitrobenzene for two weeks had exposure-related increases in absolute and relative liver weights. Absolute and relative spleen weights were increased in males and females in the 18-ppm groups, while relative kidney weights were slightly increased in males exposed to 18 ppm. In this exposure group, haemosiderin deposition was observed in the spleens and in the portal and central areas of the livers of all rats. Mice also demonstrated exposure-related increases in liver weights and increases in spleen weights at 18 ppm after two weeks' exposure to 2-chloronitrobenzene. Mice, especially in the 18 ppm-exposure group, had liver changes such as coagulative necrosis and granulomatous inflammation. Mice also had increased haematopoietic cell proliferation and haemosiderin deposition in the spleen at exposure concentrations as low as 4.5 ppm (United States National Toxicology Program, 1993)

In the rats exposed to 2-chloronitrobenzene by inhalation for 13 weeks, elevated methaemoglobin concentrations were detected in all treated rats; indeed, elevated methaemoglobin levels were detected in all treated male rats by day 23. Histopatho-

logical changes in male and female rats included increased basophilia of centrilobular hepatocytes, pigmentation and regeneration of the proximal convoluted tubules of the kidney and hyperplasia of the nasal cavity epithelia. Exposure of mice to 2-chloronitrobenzene for 13 weeks produced hepatocellular necrosis, cytomegaly, mineralization and chronic inflammation of the liver after exposure to 18 ppm. Mice also demonstrated increased haematopoietic activity (United States National Toxicology Program, 1993)

Exposure to 4-chloronitrobenzene for two weeks induced exposure-related increases in absolute and relative spleen and liver weights in both male and female rats. Exposure to this isomer also produced splenic enlargement and dark discoloration in all males and females exposed to 12 or 24 ppm and microscopic changes in the spleen, such as an increase in haematopoiesis, in male and female rats exposed to 6 ppm and above. Microscopic changes in the proximal convoluted tubules of the kidneys of males and females in the 12 and 24 ppm groups — hyaline droplet nephropathy in male rats and an increase in haemosiderin deposition in female rats — were also noted (United States National Toxicology Program, 1993).

Mice exposed to 4-chloronitrobenzene for two weeks showed a dose-related increase in absolute and relative liver and spleen weights. Microscopic changes in the spleen, including increased haematopoietic cell proliferation and pigmentation, occurred in all animals exposed to 12 and 24 ppm (United States National Toxicology Program, 1993).

More severe methaemoglobinaemia occurred in rats exposed to 4-chloronitrobenzene for 13 weeks as compared to those exposed to 2-chloronitrobenzene. Spleen weights were also increased in exposed rats. Lesions in the spleen, liver and kidney were similar to those described in the two-week study (United States National Toxicology Program, 1993).

Mice exposed to 4-chloronitrobenzene for 13 weeks showed microscopic changes in liver and spleen similar to those observed after two weeks of inhalation exposure. In addition, male and female mice showed increased haematopoiesis and haemosiderin deposition in bone marrow while, in female mice, squamous-cell hyperplasia of the forestomach epithelium was noted (United States National Toxicology Program, 1993).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Groups of 24 Sprague-Dawley rats were administered 0, 5, 15 or 45 mg/kg bw per day 4-chloronitrobenzene by gavage on gestation days 6–19. The dams receiving the highest dose had reduced weight gain and increased spleen weights. There was no significant difference in the mean number of implantations or viable fetuses after administration of 5 and 15 mg/kg bw per day when compared with controls. The highest dose was associated with an increased number of resorptions per dam (5.6 ± 5.8, compared with 0.5 ± 0.7 in controls). The frequency of skeletal malformations was increased in the

group receiving the highest dose (29 skeletal malformations in 10 litters) compared with controls (two malformations in two litters). The predominant skeletal malformation was angulated ribs occurring either alone or in association with misshapen forelimb bones. The frequency of external and soft-tissue malformations was similar between treated and untreated groups, and the types of these malformations were considered to be representative of sporadic isolated occurrences. Groups of 18 New Zealand rabbits were also administered 0, 5, 15 or 40 mg/kg bw per day by gavage on gestation days 7–19. However, eight of the 18 rabbits dosed with 40 mg/kg bw per day chloronitrobenzene died and therefore this treatment was abandoned. No effect of the lower doses on maternal or fetal toxicity or on teratogenesis was observed (Nair *et al.*, 1985).

In a reproduction study of 4-chloronitrobenzene, 0.1, 0.7 or 5.0 mg/kg bw per day were administered by gavage in corn oil to groups of 15 male and 30 female Sprague-Dawley rats for about 14 weeks prior to mating, during mating and during gestation and lactation (Nair *et al.*, 1989). F_1 parental rats were dosed similarly from prior to mating to throughout lactation. Other than a slight decrease in the pregnancy rate and male fertility index in the F_0 generation receiving 5 mg/kg bw per day, no reproductive effect was observed in the F_0 or the F_1 generation. [This study is described only in the form of an abstract.]

The United States National Toxicology Program (1993) studied the effects of inhalation of 2-chloronitrobenzene or 4-chloronitrobenzene for 13 weeks on sperm morphology and vaginal cytology in Fischer 344/N rats and B6C3F1 mice. Groups of 10 male and 10 female rats and 10 male and 10 female mice were exposed to 0, 4.5, 9 and 18 ppm [29, 58 and 116 mg/m^3] 2-chloronitrobenzene or 0, 6, 12 and 24 ppm [39, 77 and 155 mg/m^3] 4-chloronitrobenzene vapour through whole-body exposure for 6 h plus T_{90} per day on five days per week. (T_{90} signifies the time following the start of exposure for the vapour concentration to read 90% of the final stable concentration in the chamber. T_{90} was 20–25 min for 2-chloronitrobenzene and 15–18 min for 4-chloronitrobenzene.) In male rats exposed to the highest dose of 2-chloronitrobenzene, the left cauda epididymal weight, the number of spermatid heads per testes and the spermatid count were significantly lower than those of controls. No significant change occurred in exposed females. In mice, sperm motility was significantly decreased in all groups of exposed males, but again no significant change occurred in female mice. In male rats exposed to 4-chloronitrobenzene at the highest concentration, atrophy of the seminiferous tubules was observed, together with reductions in spermatid count, spermatozoa concentration and sperm motility. The oestrus cycle length was decreased in all groups of female rats exposed to the compound. In mice, no significant change occurred in males exposed to 4-chloronitrobenzene, but the oestrus cycle length of females exposed to the highest concentration was significantly increased.

Continuous breeding studies in CD-1 Swiss mice were performed to determine whether the relatively mild changes seen in these assays would be indicative of significant deficits in fertility in more exhaustive studies (United States National Toxicology Program, 1993). Groups of 20 breeding pairs received 40, 80 or 160 mg/kg bw per day 2-chloronitrobenzene or 62.5, 125 or 250 mg/kg bw per day 4-chloronitrobenzene in corn oil by gavage for seven days prior to cohousing and for 98 days of continuous breeding.

Forty breeding pairs received the corn oil vehicle only. These doses were up to approximately one-third to one-half of those that caused mortality in two-week range-finding studies. No noteworthy effect of 2-chloronitrobenzene on reproductive performance or outcome was observed. 4-Chloronitrobenzene produced significant and progressive deficits in fertility in the F_0 generation; the number of high-dose breeding pairs delivering litters declined by the second litter, and the difference was significant for the third and fourth litters. The average number of litters per pair decreased slightly with increasing dose. Weight gains of F_1 and F_2 pups were lower than those of the control pups.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see also Table 3 and Appendices 1 and 2)

2-Chloronitrobenzene gave negative results in the *Escherichia coli* SOS-chromotest. Mutagenic activity was reported for strain TA100 of *Salmonella typhimurium* when tested in the presence of induced hamster or rat-liver S9. Conflicting responses were obtained with TA98. 2-Chloronitrobenzene did not induce sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* when administered to adults either by feeding or by injection or to larvae by feeding. 2-Chloronitrobenzene injected intraperitoneally into Swiss CD-1 mice produced DNA single-strand breaks in the liver, kidney and brain. 2-Chloronitrobenzene induced sister chromatid exchange and chromosomal aberrations in Chinese hamster ovary CHO cells.

Studies with 3-chloronitrobenzene were negative in the *E. coli* SOS-chromotest and in *S. typhimurium* mutagenicity assays. Negative results were also obtained in Chinese hamster ovary cell tests for the induction of chromosomal aberrations and sister chromatid exchange.

4-Chloronitrobenzene gave negative results in the *E. coli* SOS-chromotest, but was mutagenic in *S. typhimurium* when tested in the presence and absence of induced liver S9. 4-Chloronitrobenzene did not induce sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* when administered to adults either by feeding or by injection or to larvae by feeding. 4-Chloronitrobenzene injected intraperitoneally into male Swiss CD-1 mice induced DNA single-strand breaks in liver, kidney and brain. In non-proliferating cultured rat hepatocytes, 4-chloronitrobenzene induced DNA single-strand breaks at 5 mM which were almost completely repaired within 24 h. At 50 mM only about one-half of the induced breaks were repaired within 48 h and most repair occurred during the second day (Cesarone *et al.*, 1984). In Chinese hamster ovary cells, 4-chloronitrobenzene induced sister chromatid exchange in the presence of S9 and chromosomal aberrations with and without S9, but the positive response for chromosomal aberrations occurred only at doses that were severely toxic.

Table 3. Genetic and related effects of chloronitrobenzenes

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
2-Chloronitrobenzene				
PRB, Prophage induction, SOS repair test, DNA strand breaks, cross links or related damage	–	–	NR	von der Hude *et al.* (1988)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	NR	Gilbert *et al.* (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	38	Haworth *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	50	Suzuki *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	0	630	Shimizu *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	38	US National Toxicology Program (1993)
SA3, *Salmonella typhimurium* TA1530, reverse mutation	–	–	NR	Gilbert *et al.* (1980)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	NR	Gilbert *et al.* (1980)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	128	Haworth *et al.* (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	0	630	Shimizu *et al.* (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	NR	Gilbert *et al.* (1980)
SA7, *Salmonella typhimurium* TA1537 reverse mutation	–	–	128	Haworth *et al.* (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	0	630	Shimizu *et al.* (1983)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	NR	Gilbert *et al.* (1980)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	+	0	80	Shimizu *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	NR	Gilbert *et al.* (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	(+)	77	Haworth *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–[c]	50	Suzuki *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	0	80	Shimizu *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	(+)	50	US National Toxicology Program (1993)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	–[c]	2.5	Suzuki *et al.* (1987)

Table 3 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
2-Chloronitrobenzene (contd)				
SAS, *Salmonella typhimurium* TA1532, TA1950, TA1975, TA1978 or G46, reverse mutation	–	–	NR	Gilbert *et al.* (1980)
SAS, *Salmonella typhimurium* TA98NR, reverse mutation	0	–[c]	5	Suzuki *et al.* (1987)
SAS, *Salmonella typhimurium* TA98NR/1,8-DNP$_6$, reverse mutation	0	–[c]	2.5	Suzuki *et al.* (1987)
DMX, *Drosophila melanogaster,* sex-linked recessive lethal mutation	–		125 (adult feed)	Zimmering *et al.* (1985)
DMX, *Drosophila melanogaster,* sex-linked recessive lethal mutation	–		10 000 (adult inj.)	Zimmering *et al.* (1985)
DMX, *Drosophila melanogaster,* sex-linked recessive lethal mutation	–		60 (larval feed)	Zimmering *et al.* (1989)
SIC, Sister chromatic exchange, Chinese hamster cells *in vitro*	(+)[d]	(+)[d]	50	US National Toxicology Program (1993)
CIC, Chromosomal aberrations, Chinese hamster cells *in vitro*	?	(+)[d]	465	US National Toxicology Program (1993)
DVA, DNA strand breaks, cross-links or related damage in Swiss mouse brain cells *in vivo*	+		60	Cesarone *et al.* (1980)
DVA, DNA strand breaks, cross-links or related damage in Swiss mouse liver and kidney cells *in vivo*	+		60	Cesarone *et al.* (1982)
3-Chloronitrobenzene				
PRB, Prophage induction, SOS repair test, DNA strand breaks, cross links or related damage	–	–	NR	von der Hude *et al.* (1988)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	128	Haworth *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	50	Suzuki *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	630	Shimizu *et al.* (1983)

Table 3 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
3-Chloronitrobenzene (contd)				
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	128	Haworth *et al.* (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	630	Shimizu *et al.* (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	128	Haworth *et al.* (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	630	Shimizu *et al.* (1983)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	630	Shimizu *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	128	Haworth *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–[c]	50	Suzuki *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	630	Shimizu *et al.* (1983)
SIC, Sister chromatid exchange, Chinese hamster cells *in vitro*	–	–	160	Galloway *et al.* (1987)
CIC, Chromosomal aberrations, Chinese hamster cells *in vitro*	–	–	500	Galloway *et al.* (1987)
4-Chloronitrobenzene				
PRB, Prophage induction, SOS repair test, DNA strand breaks, cross links or related damage	–	–	NR	von der Hude *et al.* (1988)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	NR	Gilbert *et al.* (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	128	Haworth *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	50	Suzuki *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	0	630	Shimizu *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	192	US National Toxicology Program (1993)
SA3, *Salmonella typhimurium* TA1530, reverse mutation	–	–	NR	Gilbert *et al.* (1980)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	NR	Gilbert *et al.* (1980)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	(+)	256	Haworth *et al.* (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	0	315	Shimizu *et al.* (1983)

Table 3 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
4-Chloronitrobenzene (contd)				
SA5, *Salmonella typhimurium* TA1535, reverse mutation	(+)	+	256	US National Toxicology Program (1993)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	NR	Gilbert *et al.* (1980)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	384	Haworth *et al.* (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	0	630	Shimizu *et al.* (1983)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	0	630	Shimizu *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	NR	Gilbert *et al.* (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	384	Haworth *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–[c]	50	Suzuki *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	0	630	Shimizu *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	–[c]	50	Suzuki *et al.* (1987)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	385	US National Toxicology Program (1993)
SAS, *Salmonella typhimurium* TA1532, TA1950, TA1975, TA1978 or G46, reverse mutation	–	–	NR	Gilbert *et al.* (1980)
SAS, *Salmonella typhimurium* TA98NR, reverse mutation	0	–[c]	50	Suzuki *et al.* (1987)
SAS, *Salmonella typhimurium* TA98NR/1,8-DNP$_6$, reverse mutation	0	–[c]	50	Suzuki *et al.* (1987)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutation	–		100 ppm (adult feed)	Zimmering *et al.* (1985)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutation	–		100 (adult inj.)	Zimmering *et al.* (1985)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutation	–		80 (larval feed)	Zimmering *et al.* (1989)

Table 3 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
4-Chloronitrobenzene (contd)				
DIA, DNA strand breaks, cross-links or related damage, animal cells *in vitro*	+	0	5	Cesarone *et al.* (1984)
RIA, DNA repair exclusive of unscheduled DNA synthesis, animal cells *in vitro*	+	0	5	Cesarone *et al.* (1984)
SIC, Sister chromatid exchange, Chinese hamster cells *in vitro*	–	+	250	Galloway *et al.* (1987)
CIC, Chromosomal aberrations, Chinese hamster cells *in vitro*	(+)	+	600	Galloway *et al.* (1987)
CIC, Chromosomal aberrations, Chinese hamster cells *in vitro*	(+)	+	600	US National Toxicology Program (1993)
DVA, DNA strand breaks, cross-links or related damage, animal cells *in vivo*	+		30	Cesarone *et al.* (1983)

[a] +, positive; (+), weak positive; –, negative; 0 not tested; ?, inconclusive

[b] LED, lowest effective dose; HID, highest ineffective dose. In-vitro tests, μg/ml; in-vivo tests, mg/kg bw; NR, dose not reported

[c] Positive in the presence of 200 μg/plate norharman (CAS 244-63-3)

[d] Weak positive in one laboratory, negative in another

5. Summary of Data Reported and Evaluation

5.1 Exposure data

2-, 3- and 4-Chloronitrobenzenes are produced as a mixture by nitration of chlorobenzene. After separation, the three isomers are used as important chemical intermediates in the production of colourants, pharmaceuticals and a variety of other products. Human exposure to chloronitrobenzenes may occur during the production and use of these intermediates.

5.2 Human carcinogenicity data

No data on the carcinogenicity of 2-, 3- or 4-chloronitrobenzene to humans were available to the Working Group.

5.3 Animal carcinogenicity data

2-Chloronitrobenzene was tested for carcinogenicity by oral administration in the diet in one study in mice and in one study in rats. The studies were inadequate for an evaluation.

4-Chloronitrobenzene was tested for carcinogenicity by oral administration in the diet in one study in mice and in one study in rats. Although the study in mice reported an increased incidence of vascular tumours in exposed males and females, neither study was considered adequate for an evaluation.

3-Chloronitrobenzene has not been tested for carcinogenicity in experimental animals.

5.4 Other relevant data

4-Chloronitrobenzene is absorbed through inhalation and/or via the skin upon human exposure after which urinary metabolites of 4-chloronitrobenzene appear, which are the results of *N*-acetylation, nitro-group reduction and — to a lesser extent — ring-hydroxylation. Metabolism is slow, with elimination of metabolites occurring over many days. Considerable interindividual variation occurs in this metabolism.

The urinary metabolites of 4-chloronitrobenzene are qualitatively similar in humans and rats.

No data concerning the absorption, distribution, metabolism and excretion or toxic effects of 2- or 3-chloronitrobenzene in humans were available to the Working Group.

The disposition of 2-chloronitrobenzene in rats is similar to that of 4-chloronitrobenzene.

In humans, exposure to 4-chloronitrobenzene is associated with such symptoms as headache, palpitation, dizziness, nausea, vomiting and poor appetite. Cyanosis, methae-

moglobinaemia and anaemia also occur. Methaemoglobinaemia and anaemia occur in rats exposed to 4-chloronitrobenzene, 3- chloronitrobenzene or 2- chloronitrobenzene.

In a single study in rats, a maternally toxic dose of 4-chloronitrobenzene increased the resorption rate and the frequency of skeletal malformations. In female rats and mice, inhalation exposure to 4-chloronitrobenzene increased the oestrus cycle length. In rats, but not mice, inhalation exposure to the compound decreased spermatogenesis with atrophy of the seminiferous tubules. In a continuous breeding study, a progressive decrease in fertility was noted in mice receiving 4-chloronitrobenzene.

In rats and mice exposed to 2-chloronitrobenzene by inhalation, decreased spermatogenesis was observed. No significant change was observed in exposed females. In a continuous breeding study, fertility was not affected in mice receiving 2-chloronitrobenzene.

2-Chloronitrobenzene induced reverse mutations but not primary DNA damage in bacteria. It was not mutagenic to insects. In mammalian cells *in vitro*, it induced sister chromatid exchange and chromosomal aberrations. Intraperitoneal injection into mice resulted in DNA damage in the liver, kidney and brain.

3-Chloronitrobenzene gave negative results in bacterial mutagenicity assays and in cultured mammalian cell chromosomal assays.

4-Chloronitrobenzene induced reverse mutations but not primary DNA damage in bacteria. It was not mutagenic to insects. At toxic doses, it induced chromosomal aberrations, sister chromatid exchange and repairable DNA breaks in cultured mammalian cells. Intraperitoneal injection into mice induced DNA damage in the liver, kidney and brain.

5.5 Evaluation[1]

There is *inadequate evidence* in humans for the carcinogenicity of chloronitrobenzenes.

There is *inadequate evidence* in experimental animals for the carcinogenicity of chloronitrobenzenes.

Overall evaluation

Chloronitrobenzenes are *not classifiable as to their carcinogenicity to humans (Group 3).*

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[1] For definition of the italicized terms, see Preamble, pp. 24–27.

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