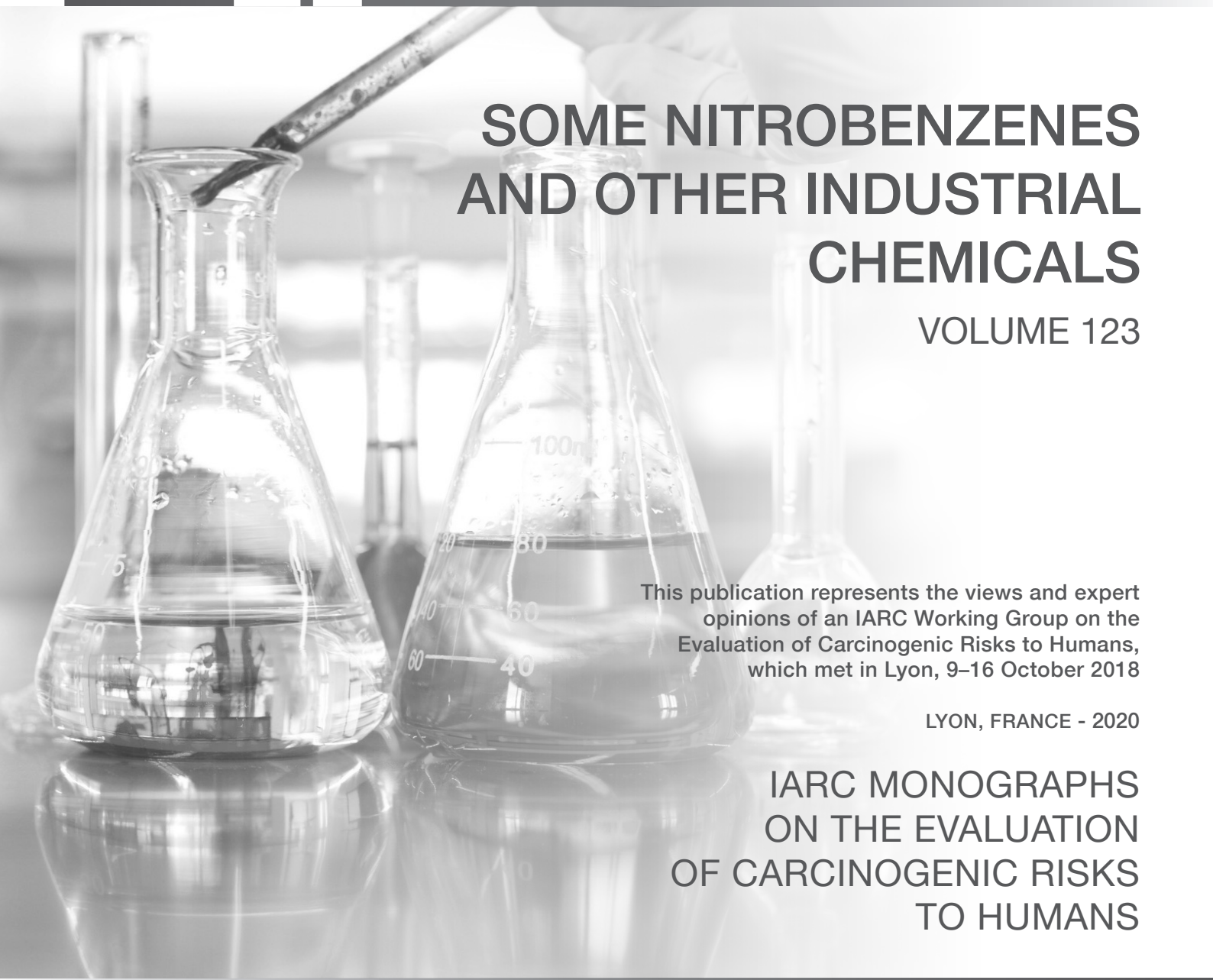
IARC MONOGRAPHS



SOME NITROBENZENES AND OTHER INDUSTRIAL CHEMICALS

VOLUME 123

This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 9–16 October 2018

LYON, FRANCE - 2020

IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer

World Health Organization

4-CHLORONITROBENZENE

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

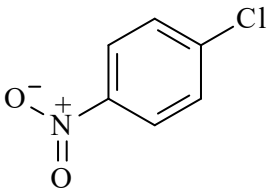
Chem. Abstr. Serv. Reg. No.: 100-00-5

Chem. Abstr. Serv. name: 1-chloro-4-nitrobenzene

IUPAC systematic name: 1-chloro-4-nitrobenzene

Synonyms: 4-chloronitrobenzene; *para*-chloronitrobenzene; 4-chloro-1-nitrobenzene; 4-CNB; 4-nitrochlorobenzene; *para*-nitrochlorobenzene; 1-nitro-4-chlorobenzene; 4-nitro-1-chlorobenzene.

1.1.2 Structural and molecular formulae, and relative molecular mass



Molecular formula: $C_6H_4ClNO_2$

Relative molecular mass: 157.55 (PubChem, 2018).

1.1.3 Chemical and physical properties of the pure substance

Description: yellow, crystalline solid with a sweet odour (PubChem, 2018)

Boiling point: 242 °C (PubChem, 2018)

Melting point: 82–84 °C (PubChem, 2018)

Solubility: slightly soluble in water (225 mg/L at 20 °C) (PubChem, 2018); soluble in acetone, boiling ethanol, diethyl ether, and carbon disulfide; sparingly soluble in cold ethanol (DECOS, 2002)

Volatility: vapour pressure, 0.09 mm Hg at 25 °C (PubChem, 2018)

Relative vapour density (air = 1): 5.44 (PubChem, 2018)

Octanol/water partition coefficient (P): log K_{ow} = 2.39 (PubChem, 2018)

Conversion factor: 1 ppm = 6.44 mg/m^3, at normal temperature (25 °C) and pressure (101 kPa)

Technical products and impurities: available commercially at purities of greater than 99% (Sigma-Aldrich, 2018).

1.2 Production and use

1.2.1 Production process

Continuous or batch nitration of chlorobenzene with mixed acids typically gives a 98% yield of an isomer mix comprising

2-chloronitrobenzene (34–36%), 4-chloronitrobenzene (63–65%), and 3-chloronitrobenzene (~1%). The isomers can be separated by a combination of fractional crystallization and distillation (Booth, 2012).

1.2.2 Production volume

4-Chloronitrobenzene is included in the 2007 Organisation for Economic Co-operation and Development list of chemicals with a high production volume (OECD, 2009).

The worldwide (excluding eastern Europe) production of 4-chloronitrobenzene amounted to 220 900 tonnes in 1995 by approximately 30 producers: about 54 000 tonnes in west Europe, 78 000 tonnes in China, 29 000 tonnes in India, 17 700 tonnes in Japan, 4700 tonnes in the Republic of Korea, and 37 500 tonnes in the USA (OECD-SIDS, 2002).

The production volumes for non-confidential chemicals reported under the 1986–2002 Inventory Update Rule submitted to the United States Environmental Protection Agency (EPA) for 4-chloronitrobenzene are presented in Table 1.1. Production volumes in the USA in more recent years are not publicly available.

About 250 000 tonnes per year of 4-chloronitrobenzene were produced in China in 2003 and 2004, which represented about 60% of the total annual global production (Shen et al., 2008). Production of 4-chloronitrobenzene in India has reportedly grown by 7.3% per year, and it was estimated that the combined production of 2- and 4-chloronitrobenzene would increase to 127 000 tonnes per year by 2010 (INERIS, 2010).

The European Chemicals Agency reports that 1–10 tonnes of 4-chloronitrobenzene per year are currently manufactured in and/or imported into the European Economic Area (ECHA, 2018). Production of 4-chloronitrobenzene ended in France in 2007 (INERIS, 2010).

Table 1.1 Production volumes for 4-chloronitrobenzene, USA[a]

Year	Production range in pounds [tonnes]
1986	$(50–100) \times 10^6$ [22 680–45 359]
1990	$(100–500) \times 10^6$ [45 359–226 796]
1994	$(50–100) \times 10^6$ [22 680–45 359]
1998	$(50–100) \times 10^6$ [22 680–45 359]
2002	$(50–100) \times 10^6$ [22 680–45 359]

[a] Non-confidential production volume information submitted to United States Environmental Protection Agency by companies for chemicals under the 1986–2002 Inventory Update Rule (HSDB, 2008)

1.2.3 Use

4-Chloronitrobenzene and its derivatives are used in many synthetic processes (Booth, 2012). Chemical intermediates produced from 4-chloronitrobenzene include: 4-chloroaniline, 4-nitrophenol, 4-nitroanisole, *para*-anisidine, 4-nitroaniline, 6-chloro-3-nitrobenzenesulfonic acid, 2,4-dinitrochlorobenzene, and 3,4-dichloronitrobenzene. 4-Chloronitrobenzene is a precursor for the synthesis of agricultural chemicals, including the herbicides nitrofen and fluoronitrofor, as well as antioxidants used in the rubber industry, for example, 4-isopropylamino-diphenylamine (Booth, 2012). The compound is also used in the pharmaceutical industry for the synthesis of several antibiotics, anxiolytics, and analgesics, including the widely used acetaminophen (i.e. paracetamol) (INERIS, 2010; Table 1.2).

The use of 4-chloronitrobenzene by activity sector in France was reported in 2010 as: chemistry, 86.49%; textile treatment, 5.33%; paint, pigments, colorants, and plastic production, 2.44%; leather treatments, 2.11%; paper industry, 1.43%; and others such as food processing, less than 1% (INERIS, 2010).

Table 1.2 Some pharmaceutical drugs synthesized from 4-chloronitrobenzene

Drug name	CAS No.	Function
Paracetamol (acetaminophen)	103-90-2	Analgesic
Phenacetin	62-44-2	Analgesic
Dapsone	80-08-0	Antibiotic (to treat leprosy)
Norfloxacin	70 458-96-7	Urinary antibiotic
Albendazole	54 965-21-8	Anti-helminthic
Alprazolam	28 981-97-7	Anxiolytic
Demoxepam	963-39-3	Anxiolytic
Estazolam	29 975-16-4	Sedative
Itraconazole	84 625-61-6	Antifungal
Diazepam	439-14-5	Anxiolytic

CAS, Chemical Abstracts Service
Compiled by the Working Group with data from INERIS (2010)

1.3 Methods of measurement and analysis

1.3.1 Air

The measurement of 4-chloronitrobenzene in air using the United States National Institute for Occupational Safety and Health method involves collecting the sample on silica gel tubes, followed by analysis by gas chromatography and either mass spectrometry or flame ionization detection (NIOSH, 2005). [The Working Group was not able to identify other published methods to measure 4-chloronitrobenzene in air.]

1.3.2 Other environmental media

EPA Method 8091 is a gas chromatography method that can be used to determine the concentration of nitroaromatics and cyclic ketones, allowing the measurement of contamination in water, soil, and waste matrices. Nitroaromatics can be detected in water and soil at concentrations of parts per billion and in waste matrices at concentrations of parts per million (EPA, 1996).

1.3.3 Biomonitoring

Dangwal & Jethani (1980) described a colorimetric method to measure 4-chloronitrobenzene in urine with a limit of detection of 0.6 mg/L, and Lewalter & Ellrich (1991) provided a method for the measurement of nitroaromatic compounds, including 4-chloronitrobenzene, in plasma using gas chromatography with electron capture detection (detection limit, 1 µg/L blood). The method of Lewalter & Ellrich (1991) was approved by the German Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK Commission).

A recent study compared several urinary biomarkers for 4-chloronitrobenzene exposure in a group of Chinese workers in a chemical factory in Tainjing City, China (Jones et al., 2006, 2007; Sabbioni et al., 2016). Urine samples of the workers and of the factory controls were analysed before and after acid hydrolysis to quantify the free and conjugated metabolites of 4-chloronitrobenzene using high-performance liquid chromatography and ultraviolet spectroscopy. The mercapturic acid *N*-acetyl-*S*-(4-nitrophenyl)-L-cysteine was the only metabolite detected in non-hydrolysed urine, accounting for approximately 51% of the total metabolites detected. The two remaining metabolites, 4-chloroaniline and 2-chloro-5-nitrophenol, were identified as cleavage products in hydrolysed urine and accounted for approximately 18% and 30% of the total metabolites detected, respectively. No 4-chloronitrobenzene metabolites were found in factory controls. There was a correlation between *N*-acetyl-*S*-(4-nitrophenyl)-L-cysteine and 4-chloroaniline, and between *N*-acetyl-*S*-(4-nitrophenyl)-L-cysteine and 2-chloro-5-nitrophenol ($r = 0.63$ and $r = 0.68$, respectively; $P < 0.01$, ranks). Creatinine-adjusted *N*-acetyl-*S*-(4-nitrophenyl)-L-cysteine was correlated with 4-chloronitrobenzene in air ($r = -0.30$; $P > 0.05$) (Jones et al., 2007).

N-acetyl-*S*-(4-nitrophenyl)-L-cysteine would be an appropriate biomarker of exposure in urine

for a recent absorbed dose of 4-chloronitrobenzene; it is the major urinary metabolite and was detected in all exposed workers. Its concentration also correlated well with the concentration of the two other main metabolites (4-chloroaniline and 2-chloro-5-nitrophenol) and with the concentration of 4-chloronitrobenzene in air (Jones et al., 2007). The limit of detection of the method ranged over 0.10–1.0 μmol/L, depending on the analyte.

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

4-Chloronitrobenzene is not known to occur naturally.

The major sources of environmental release of 4-chloronitrobenzene are from chemical plants where it is produced or used as an intermediate. Minor sources of release into the environment may occur during transport, storage, or land burial, and the compound may form in the environment through the oxidation of synthetic aromatic amines or the reaction of nitrogen oxides in highly polluted air with chlorinated aromatic hydrocarbons (Howard et al., 1976). 4-Chloronitrobenzene is most likely to be found in air (65%) and water (OECD-SIDS, 2002).

Measurements of 4-chloronitrobenzene in water identified by the Working Group are summarized in Table 1.3.

In the 1970s, concentrations of up to 1800 mg/L were reported for 2-, 3-, and 4-chloronitrobenzenes in wastewater from a chloronitrobenzene production plant in the USA (Howard et al., 1976).

In 1976, an accidental release of chloronitrobenzenes was reported in France (Raguet et al., 2010); in the area of this accident, concentrations of 4-chloronitrobenzene of up to 0.12 mg/L were measured in the groundwater (Duguet et al., 1988).

Elsewhere in Europe, concentrations of 4-chloronitrobenzene of up to 0.3 μg/L in German rivers (Feltes et al., 1990) and of 0.37 μg/L in Italian rivers (Trova et al., 1991) were measured in the 1990s. Concentrations in German rivers decreased to less than 0.06 μg/L in 2004 (Schäfer et al., 2011).

High concentrations of 4-chloronitrobenzene of up to 16.3 mg/L were measured in river water in China in 1990 (Lang et al., 1993). In India, concentrations of the compound of up to 227 mg/L were measured in the wastewater from a chlorobenzene production plant (Swaminathan et al., 1987).

Based on the available experimental data, 4-chloronitrobenzene is not readily biodegradable in water. However, it can be biodegraded by adapted microorganisms; many studies on wastewater treatment and soil remediation have been published (e.g. Xia et al., 2011; Arora et al., 2012; Zhu et al., 2013; Xu et al., 2016).

Recent measurements of 4-chloronitrobenzene from 16 source water reservoirs in the Haihe river basin, China, were on average 0.017 μg/L, with a maximum value of 0.050 μg/L (less than the standard limit in China by a factor of 1000) (Gao et al., 2012).

1.4.2 Occurrence in food

4-Chloronitrobenzene has been reported at low levels in edible portions of various fish species from the Mississippi river in the USA (Yurawecz & Puma, 1983), and in fish from the river Main in Germany (Steinwandter, 1987).

1.4.3 Exposure of the general population

It is possible that the general population could be exposed to 4-chloronitrobenzene from the use of mouth washes containing chlorhexidine gluconate (Below et al., 2004, 2017). Forty-three patients who had orofacial operations were randomized to use a 0.2% chlorhexidine

Table 1.3 Environmental occurrence of 4-chloronitrobenzene

Location, collection date	Sampling matrix	Mean (range) exposure concentration	Comments	Reference
France, 1987	Groundwater	NR (5–123 µg/L)	Accidental pollution from a dye production plant; 2-chloronitrobenzene was the primary pollutant, accounting for 70% of the pollution	Duguet et al. (1988)
France, 2011	Groundwater	26 (maximum, 400) ng/L	0.2% positive measurements; ~500 sites throughout France	Lopez & Laurent (2013)
Elbe, Germany, NR	River water	NR (0.04–0.30 µg/L)		Feltes et al. (1990)
Bormida river, Italy, 1989–1990	River water	0.08 (0.002–0.37) µg/L	Monthly measurements at five sampling stations	Trova et al. (1991)
Songhua river, China, early 1990s	River water	NR (0.17–16.30 mg/L)	Reference in Chinese reported in Men et al. (2011)	Lang et al. (1993)
Daliao river, China, 2006	River water	NR (maximum, 0.896 mg/L)	28 sites in the dry season	Men et al. (2011)
Germany, 1994–2004	River water	NR (maximum, 0.06 µg/L)	110 measurements from the four largest rivers of northern Germany; detection at more than 20% of all sites sampled	Schäfer et al. (2011)
Haihe river, China, NR	River water	16.9 (< 10.5–50.0) ng/L	Detection in 62.5% of the 16 reservoirs of the Haihe river basin	Gao et al. (2012)
Netherlands, 1983–1984	Costal water	6.9 (0.1–31.0) ng/L	108 measurements throughout the year at nine locations	van de Meent et al. (1986)
Scheldt estuary, Netherlands and/or Belgium, 1986	Estuary water	Median, 1.4 (0.5–2.5) ng/L	Heavy pollution due to large wastewater discharges	van Zoest & van Eck (1991)
USA, early 1970s[a]	Wastewater	NR (1500–1800 mg/L)	Effluent from a 3-chloronitrobenzene production plant	Howard et al. (1976)
India, 1980s	Wastewater	166 (112–227) mg/L	Effluent from a chloronitrobenzene production plant	Swaminathan et al. (1987)
France, NR	Industrial wastewater	3.19 µg/L	31 industrial sites were positive out of the 2876 measured	INERIS (2010)
France, NR	Urban wastewater	0.55 µg/L	Three urban wastewater sites were positive out of the 167 measured	INERIS (2010)

NR, not reported

[a] 2-, 3-, and 4-Chloronitrobenzene collected

gluconate (n = 23) or an octenidine-based chlorhexidine-free (n = 20, controls) mouthwash once preoperatively and 3 times per day for 5 days postoperatively (Below et al., 2017). 4-Chloroaniline, which may metabolically transform to 4-chloronitrobenzene, was detectable in saliva at higher concentrations in the chlorhexidine group (0.55 mg/L) than the octenidine group (0.21 mg/L). However, 4-chloronitrobenzene in saliva was not significantly increased in the chlorhexidine group compared with the controls. [The Working Group noted that there is no experimental evidence that 4-chloronitrobenzene exposure arises from using chlorhexidine gluconate mouthwashes.]

1.4.4 Occupational exposure

Occupational exposure to 4-chloronitrobenzene may occur through inhalation and dermal contact at workplaces where this compound is produced or used. Exposure may also occur through inadvertent ingestion (CDC, 2016).

The National Occupational Exposure Survey conducted between 1981 and 1983 indicated that 2950 employees in the USA were potentially exposed to 4-chloronitrobenzene. The estimates were based on a survey of companies and did not involve measurements of exposure (NOES, 1995).

Long-term occupational exposure to aromatic nitro and amino compounds, including 4-chloronitrobenzene, was studied in 35 male Japanese workers involved in the production of dyes or pharmaceuticals (Yoshida et al., 1989). The workers were routinely exposed to aromatic nitro-amino compounds at concentrations of greater than 0.3 mg/m^3 (reported as 4-chloronitrobenzene), and exposure was described as occurring from skin contact and inhalation (Yoshida et al., 1993).

The exposure of workers from a chemical factory in China was reported by Jones et al. (2006). The median concentration of 4-chloronitrobenzene in air determined from four personal samples was 0.87 mg/m^3. In another study in the same factory by the same authors, the mean 8-hour average 4-chloronitrobenzene exposure of 19 workers was 1.17 mg/m^3. Urine samples from the workers and the factory controls were analysed before and after acid hydrolysis to quantify the free and conjugated metabolites of 4-chloronitrobenzene. The *N*-acetyl-*S*-(4-nitrophenyl)-L-cysteine metabolite in post-shift urine samples from the exposed workers (n = 38) was 0.30–34.3 µmol/L, 4-chloroaniline ranged from not detected to 10.5 µmol/L, and 2-chloro-5-nitrophenol ranged from not detected to 11.8 µmol/L (Jones et al., 2007). *N*-acetyl-*S*-(4-nitrophenyl)-L-cysteine, 4-chloroaniline, and 2-chloro-5-nitrophenol were not detected in the post-shift urine taken from unexposed control workers, or in other unexposed volunteers. *N*-acetyl-*S*-(4-nitrophenyl)-L-cysteine, 4-chloroaniline, and 2-chloro-5-nitrophenol were not detected in pre-shift urine samples (n = 5) from exposed workers (Jones et al., 2007).

1.5 Regulations and guidelines

The international occupational exposure limit values for 4-chloronitrobenzene, as published by the German *Institut für Arbeitsschutz* (IFA), are presented in Table 1.4.

The EPA has set a chronic oral reference dose of 0.0007 mg/kg bw per day for 4-chloronitrobenzene (EPA, 2015), and regional screening levels of 4.4 mg/kg for resident soil, 210 ng/m^3 for resident air, and 1.2 µg/L for tap water (EPA, 2018).

The French *Agence française de securité sanitaire de l'environnement et du travail* (AFSSET, 2009) proposed two toxicological reference values (TRVs) for ingestion of 4-chloronitrobenzene: a chronic TRV with a threshold based on haematotoxic effects of 0.034 mg/kg bw per day; and a non-threshold TRV based on potential carcinogenic effects of 5×10^{-8} mg/kg bw per day.

Table 1.4 International limit values for 4-chloronitrobenzene

Country or region	8-hour limit		Short-term limit	
	(ppm)	(mg/m³)	(ppm)	(mg/m³)
Australia	0.1	0.64		
Austria	0.075	0.5	0.3	2.0
Belgium	0.1	0.65		
Canada, Ontario	0.1			
Canada, Québec (Province)	0.1	0.64		
China		0.6		
Denmark	0.1	0.64	0.2	1.28
Finland		1.0		3.0[a]
Hungary		0.5		2.0
Ireland		1.0		2.0[b]
Japan (JSOH)	0.1	0.64		
New Zealand	0.1	0.64		
Poland		0.6		
Republic of Korea	0.1	0.6		
Romania			0.16[a]	1.0[a]
Singapore	0.1	0.64		
Spain[c]	0.1	0.65		
Switzerland	0.075			
UK		1		2
USA (OSHA)		1		

JSOH, Japan Society for Occupational Health; OSHA, United States Occupational Safety and Health Administration

[a] 15-minute average value

[b] 15-minute reference period

[c] Skin exposure

Reproduced from IFA (2018)

For all methaemoglobin inducers such as 4-chloronitrobenzene, a biological exposure index was set by the American Conference of Governmental Industrial Hygienists at 1.5% methaemoglobin in blood (ACGIH, 2008).

In China, the maximum concentration of 4-chloronitrobenzene is regulated at 500 µg/L in surface water and at 50 µg/L in drinking-water (Shen et al., 2008).

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

The evidence for the carcinogenic activity of 4-chloronitrobenzene was previously reviewed by the Working Group in *IARC Monographs* Volume 65 (IARC, 1996). On the basis of one study in male mice, one study in female mice, and one study in male rats (Weisburger et al., 1978), the Working Group concluded that there was *inadequate evidence* in experimental animals for the carcinogenicity of chloronitrobenzenes [2-, 3-, and 4-chloronitrobenzene]. An additional study with 4-chloronitrobenzene in male and female rats and mice has since become available for evaluation (Matsumoto et al., 2006).

See Table 3.1

Table 3.1 Studies of carcinogenicity with 4-chloronitrobenzene in experimental animals

Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Mouse, CD-1 derived from HaM/ICR mice (Charles River) (M) 6–8 wk 21 mo Weisburger et al. (1978)	Oral 4-Chloronitrobenzene; among the 21 tested chemicals in the study, most were 97–99% pure Diet 0 (control), 3000, 6000, 0 (pooled control) ppm for 18 mo 25, 25, 25, 99 NR	*Liver*: hepatoma [hepatocellular carcinoma] 1/14, 4/14*, 0/14, 7/99 *Vascular*: tumours [histology and sites unspecified] 0/14, 2/14, 4/14*, 5/99	*$P < 0.025$ vs pooled controls *$P < 0.025$ vs concurrent and pooled controls	Principal strengths: males and females used Principal limitations: limited number of dose groups; limited experimental details; limited macroscopic and microscopic evaluation; small number of mice Histopathology conducted only on mice surviving after 6 mo
Mouse, CD-1 derived from HaM/ICR mice (Charles River) (F) 6–8 wk 21 mo Weisburger et al. (1978)	Oral 4-Chloronitrobenzene; among the 21 tested chemicals in the study, most were 97–99% pure Diet 0 (control), 3000, 6000, 0 (pooled control) ppm for 18 mo 24, 25, 25, 102 NR	*Vascular*: tumours [histology and sites unspecified] 0/15, 2/20, 7/18*, 9/102	*$P < 0.025$ vs concurrent and pooled controls	Principal strengths: males and females used Principal limitations: limited number of dose groups; limited experimental details; limited macroscopic and microscopic evaluation; small number of mice Histopathology conducted only on mice surviving after 6 mo
Mouse, Crj:BDF_1 (M) 6 wk 2 yr Matsumoto et al. (2006)	Oral 4-Chloronitrobenzene, > 99.9% Diet 0, 125, 500, 2000 ppm 50, 50, 50, 50 47, 49, 42, 38	*Liver* Hepatocellular adenoma 3/50, 2/50, 4/50, 2/50 Hepatocellular carcinoma 1/50, 3/50, 1/50, 6/50 Haemangiosarcoma 2/50, 2/50, 1/50, 1/50 *Lymph node*: malignant lymphoma 2/50, 2/50, 1/50, 8/50	NS $P < 0.01$, Peto trend test NS $P < 0.01$, Peto trend test	Principal strengths: males and females used; well-conducted GLP study Terminal body weights of males exposed at 2000 ppm were decreased by 5% compared with controls; incidence for combination of hepatocellular tumours not given; except for males exposed at 2000 ppm, there was no significant difference in survival in treated groups (no survival statistics given)

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Mouse, Crj:BDF_1 (F) 6 wk 2 yr Matsumoto et al. (2006)	Oral 4-chloronitrobenzene, > 99.9% Diet 0, 125, 500, 2000 ppm 50, 50, 50, 50 32, 35, 35, 29	*Liver* Hepatocellular adenoma 4/50, 1/50, 3/50, 3/50 Hepatocellular carcinoma 2/50, 0/50, 2/50, 5/50 Haemangiosarcoma 0/50, 1/50, 0/50, 5/50* *Lymph node*: malignant lymphoma 11/50, 11/50, 7/50, 12/50	 NS $P < 0.05$, Peto trend test $P < 0.01$, Peto trend test; *$P < 0.05$, Fisher exact test NS	Principal strengths: well-conducted GLP study; males and females used Incidence for combination of hepatocellular tumours not given
Rat, Charles River CD (M) 6–8 wk 24 mo Weisburger et al. (1978)	Oral 4-Chloronitrobenzene; among the 21 tested chemicals in the study, most were 97–99% pure Diet 0 (control), low dose (2000 ppm for 3 mo, 250 ppm for 2 mo, 500 ppm for 13 mo, 0 for 6 mo), high dose (4000 ppm for 3 mo, 500 ppm for 2 mo, 1000 ppm for 13 mo, 0 for 6 mo), 0 ppm (pooled control) 25, 25, 25, 111 NR	Any tumour type: no significant increase in incidence		Principal limitations: limited number of dose groups; limited experimental details; limited macroscopic and microscopic evaluation Only male rats were studied; histopathology conducted only on rats surviving after 6 mo

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Rat, F344/DuCrj (M) 6 wk 2 yr Matsumoto et al. (2006)	Oral 4-Chloronitrobenzene, > 99.9% Diet 0, 40, 200, 1000 ppm 50, 50, 50, 50 43, 46, 42, 12	*Spleen* Fibroma 0/50, 0/50, 1/50, 15/50*	$P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	Principal strengths: males and females used; well-conducted GLP study Incidence for combination of splenic tumours not given; survival was reduced in the group exposed at 1000 ppm; terminal body weight was significantly decreased in the group exposed at 1000 ppm
		Fibrosarcoma 0/50, 1/50, 0/50, 29/50*	$P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	
		Osteosarcoma 0/50, 0/50, 0/50, 11/50*	$P < 0.01$, Peto trend test; *$P < 0.01$ Fisher exact test	
		Sarcoma [NOS] 0/50, 0/50, 1/50, 6/50*	$P < 0.01$, Peto trend test; *$P < 0.05$, Fisher exact test	
		Haemangiosarcoma 0/50, 0/50, 5/50*, 7/50*	$P < 0.01$, Peto trend test; *$P < 0.05$, Fisher exact test	
		Adrenal gland: pheochromocytoma 7/50, 7/50, 6/50, 16/50	$P < 0.01$, Peto trend test	

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Rat, F344/DuCrj (F) 6 wk 2 yr Matsumoto et al. (2006)	Oral 4-Chloronitrobenzene, > 99.9% Diet 0, 40, 200, 1000 ppm 50, 50, 50, 50 36, 41, 38, 28	*Spleen* Fibroma 0/50, 0/50, 1/50, 3/50 Fibrosarcoma 0/50, 0/50, 0/50, 17/50* Osteosarcoma 0/50, 0/50, 0/50, 3/50 Sarcoma [NOS] 0/50, 0/50, 0/50, 1/50 Haemangiosarcoma 0/50, 0/50, 2/50, 4/50 *Adrenal gland*: pheochromocytoma 3/50, 6/50, 4/50, 16/50*	 $P < 0.05$, Peto trend test $P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test $P < 0.01$, Peto trend test NS $P < 0.01$, Peto trend test $P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	Principal strengths: males and females used; well-conducted GLP study Incidence for combination of splenic tumours not given; terminal body weight was significantly decreased in groups exposed at 200 and 1000 ppm

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

3.1 Mouse

Oral administration

In a study by Weisburger et al. (1978), groups of 25 male and 25 female CD-1 mice (derived from HaM/ICR mice) (age, 6–8 weeks) were given diets containing 1-chloro-4-nitrobenzene [4-chloronitrobenzene] at a concentration of 0 (control), 3000, or 6000 ppm (21 chemicals were tested in the study; purity of most was 97–99%) for 18 months. The mice were then held for 3 months on the control diet before being killed at 21 months. There was a pooled control group of 99 males and 102 females [no additional details provided]. Mice that died within the first 6 months of the study were discarded without necropsy. Complete gross necropsy was carried out on all other mice. Tissues examined histopathologically included all grossly abnormal organs, tumour masses, lung, liver, spleen, kidney, adrenal gland, heart, urinary bladder, stomach, intestines, and reproductive organs. Information on survival, body weight, or non-neoplastic lesions was not reported.

The incidence of hepatocellular carcinoma was significantly increased in males at the lower dose: 1/14 (control), 4/14 (lower dose; $P < 0.025$ versus pooled controls, 7/99), and 0/14 (higher dose). The incidence of vascular tumours [histology and sites unspecified] was significantly increased in males at the higher dose: 0/14 (control), 2/14 (lower dose), and 4/14 (higher dose; $P < 0.025$ versus concurrent (0/14) and pooled controls (5/99). The incidence of vascular tumours [histology and sites unspecified] was significantly increased in female mice at the higher dose: 0/15 (control), 2/20 (lower dose), and 7/18 (higher dose); $P < 0.025$ versus concurrent (0/15) and pooled controls (9/102) (Weisburger et al., 1978). [The Working Group noted that the limitations of the study included the small number of mice at the start, the small number of mice necropsied, the use of only two dose groups, and the limited histopathological examination and reporting.]

In a study of carcinogenicity that complied with good laboratory practice (GLP) (Matsumoto et al., 2006), groups of 50 male and 50 female Crj:BDF_1 mice (age, 6 weeks) were given diets containing 4-chloronitrobenzene (purity, > 99.9%) at a concentration of 0 (control), 125, 500, or 2000 ppm for 2 years. Based on feed consumption, the estimated amount of chemical given was 0, 15, 60, and 240 mg/kg body weight (bw) per day (males) and 0, 18, 73, and 275 mg/kg bw per day (females) for the groups at 0, 125, 500, and 2000 ppm, respectively. The survival in treated groups of males and females was similar to that of controls except for male mice at 2000 ppm, for which survival was reduced. The final number of mice surviving until termination of the experiment was 47, 49, 42, and 38 for males and 32, 35, 35, and 29 for females. The terminal body weights in treated groups of males and females were similar those of controls, with the terminal body weight of males at 2000 ppm decreased by 5%. All mice, including those found dead or in a moribund state, as well as those surviving to the end of the 2-year exposure period, underwent complete necropsy.

There were dose-related increases in the incidence of different tumour types in male and female mice. In males, the incidence of liver tumours for groups at 0 (control), 125, 500, and 2000 ppm was: hepatocellular adenoma, 3/50, 2/50, 4/50, and 2/50; hepatocellular carcinoma, 1/50, 3/50, 1/50, and 6/50 ($P < 0.01$, Peto trend test); and haemangiosarcoma, 2/50, 2/50, 1/50, and 1/50. The incidence of malignant lymphoma in males was 2/50, 2/50, 1/50, and 8/50 ($P < 0.01$, Peto trend test).

In females, the incidence of liver tumours was: hepatocellular adenoma, 4/50, 1/50, 3/50, and 3/50; hepatocellular carcinoma, 2/50, 0/50, 2/50, and 5/50 ($P < 0.05$, Peto trend test); and haemangiosarcoma, 0/50, 1/50, 0/50, and 5/50 ($P < 0.05$, Fisher exact test) ($P < 0.01$, Peto trend

test). [The Working Group noted that the incidence of the combination of hepatocellular tumours was not reported in the study.]

In male mice, there were increases in the incidence of splenic non-neoplastic lesions, including congestion and extramedullary haematopoiesis. In female mice, there were increases in the incidence of splenic non-neoplastic lesions, including congestion, deposit of haemosiderin, and ossification (Matsumoto et al., 2006). [The Working Group noted that this was a well-conducted GLP study in males and females.]

3.2 Rat

Oral administration

In the study by Weisburger et al. (1978), groups of 25 male Charles River CD rats (derived from Sprague-Dawley rats) (age, 6–8 weeks) were fed diets containing 4-chloronitrobenzene (21 chemicals were tested in the study; purity for most, 97–99%) at a concentration of 0 (control), 2000, or 4000 ppm for 3 months. Dietary concentrations were then lowered to 0, 250, and 500 ppm for 2 months and then increased to 0, 500, and 1000 ppm for 13 months; rats were held for a further 6 months on the control diet before being killed at 24 months. There was a pooled control group of 111 male rats [no additional details provided]. Rats that died within the first 6 months of the study were discarded without necropsy. Complete gross necropsy was carried out on all other animals. Tissues examined histopathologically included all grossly abnormal organs, tumour masses, lung, liver, spleen, kidney, adrenal gland, heart, urinary bladder, stomach, intestines, reproductive organs, and pituitaries. Information on survival, body weight, or non-neoplastic lesions was not reported. No significant increase in tumour incidence in male rats was reported (Weisburger et al., 1978). [The Working Group noted that the limitations of the study included the small number of rats at the start, the small number of rats necropsied, the use of only two dose groups and one sex, and the limited histopathological examination and reporting.]

In a GLP study of carcinogenicity with 4-chloronitrobenzene (Matsumoto et al., 2006), groups of 50 male and 50 female Fischer 344/DuCrj rats (age, 6 weeks) were fed diets containing 4-chloronitrobenzene (purity, > 99.9%) at a concentration of 0 (control), 40, 200, or 1000 ppm for 2 years. Based on feed consumption, the estimated amount of chemical given was 0, 1.5, 7.7, and 41.2 mg/kg bw per day (males) and 0, 1.9, 9.8, and 53.8 mg/kg bw per day (females) for the groups at 0, 40, 200, and 1000 ppm, respectively. The number of surviving rats was decreased for males at 1000 ppm; the numbers surviving were 43, 46, 42, and 12 (males) and 36, 41, 38, and 28 (females) for the groups at 0, 40, 200, and 1000 ppm, respectively. Terminal body weight was decreased in the male rats at 1000 ppm and in the female rats at 200 and 1000 ppm. All rats, including those found dead or in a moribund state, as well as those surviving to the end of the 2-year exposure period, underwent complete necropsy.

There was a dose-related increase in the incidence of splenic tumours in male rats, including spleen fibroma – 0/50, 0/50, 1/50, and 15/50 ($P < 0.01$); $P < 0.01$, Peto trend test – and spleen fibrosarcoma – 0/50, 1/50, 0/50, and 29/50 ($P < 0.01$); $P < 0.01$, Peto trend test. The incidence of splenic osteosarcoma – 0/50, 0/50, 0/50, and 11/50 ($P < 0.01$); $P < 0.01$, Peto trend test – splenic sarcoma (not otherwise specified) – 0/50, 0/50, 1/50, and 6/50 ($P < 0.05$); $P < 0.01$, Peto trend test – and splenic haemangiosarcoma – 0/50, 0/50, 5/50 ($P < 0.05$), and 7/50 ($P < 0.05$); $P < 0.01$, Peto trend test – was also significantly increased.

In female rats, there were also dose-related increases in the incidence of splenic tumours, including splenic fibroma – 0/50, 0/50, 1/50, and 3/50; $P < 0.05$, Peto trend test – and splenic fibrosarcoma – 0/50, 0/50, 0/50, and 17/50 ($P < 0.01$);

$P < 0.01$, Peto trend test. The incidence of splenic osteosarcoma (0/50, 0/50, 0/50, and 3/50; $P < 0.01$, Peto trend test) and of splenic haemangiosarcoma (0/50, 0/50, 2/50, and 4/50; $P < 0.01$, Peto trend test) was also increased.

There were increases in the incidence of splenic non-neoplastic lesions, including capsule hyperplasia, fibrosis, fatty metamorphosis, and extramedullary haematopoiesis, in male and female rats in the groups at 200 and 1000 ppm.

In male rats, there was a significant positive trend in the incidence of adrenal gland pheochromocytoma (7/50, 7/50, 6/50, and 16/50; $P < 0.01$, Peto trend test). In female rats, there was a significant increase in the incidence of adrenal gland pheochromocytoma – 3/50, 6/50, 4/50, and 16/50 ($P < 0.01$); $P < 0.01$, Peto trend test. The incidence of adrenal gland medullary hyperplasia was increased in male rats at 40 ppm and in female rats at 1000 ppm (Matsumoto et al., 2006). [The Working Group noted that this was a well-conducted GLP study in males and females.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

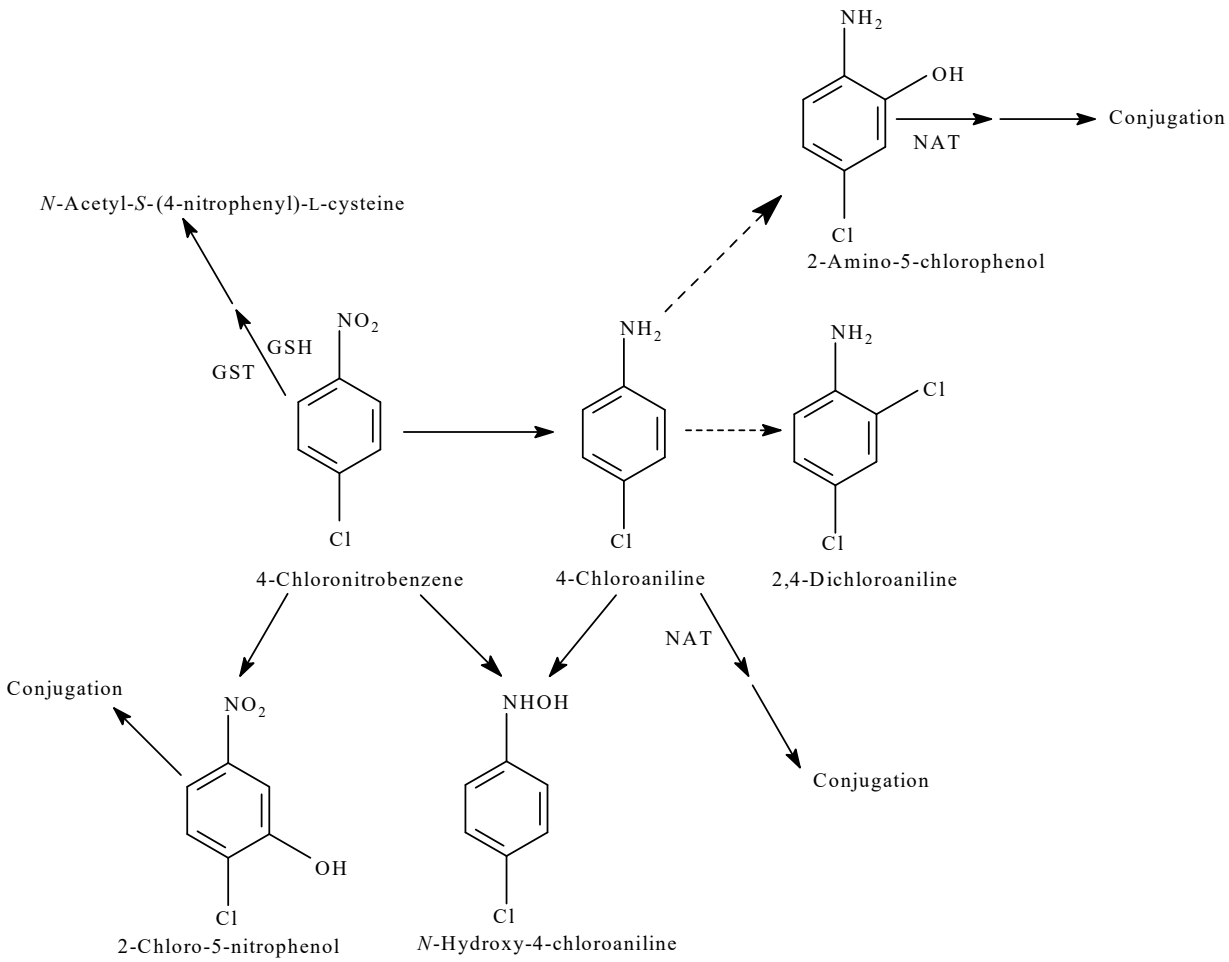
4.1.1 Humans

The excretion of urinary metabolites of 4-chloronitrobenzene has been studied in connection with an episode of accidental poisoning by 4-chloronitrobenzene (Yoshida et al., 1992, 1993). Five major urinary metabolites were identified (Yoshida et al., 1992). In the more extensive study of the poisoning, Yoshida et al. (1993) analysed urinary elimination of the five metabolites for 14–29 days in six exposed workers. The parent compound was not excreted; however, metabolites were eliminated for the total observation period (total metabolites, 179–1076 mg). The urinary elimination of each metabolite appeared to fit into a one-compartment model.

There was a complicated metabolic pattern with considerable inter-individual variation, which had three major pathways (see Fig. 4.1). The most important pathway 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, on average). In the second pathway, there was also a slow reduction of the nitro group, resulting in the formation of 4-chloroaniline which was rapidly metabolized further by: (a) fast *N*-acetylation to a variety of *N*-conjugated metabolites (detected in the urine after hydrolysis as 4-chloroaniline, 29.9%); (b) ring-hydroxylation to 2-amino-5-chlorophenol (8.7%), which was also further *N*-acetylated; and (c) chlorination to 2,4-dichloroaniline, which was readily excreted (1.2%). Chlorination was a novel metabolic pathway in humans, hypothesized by Yoshida et al. (1992) to be catalysed by myeloperoxidases from activated leukocytes associated with toxicity induced by 4-chloronitrobenzene. Finally, in the third pathway, there was a slow ring hydroxylation of the parent compound to give 2-chloro-5-nitrophenol (12.2%).

The excretion of urinary metabolites of 4-chloronitrobenzene has also been studied in workers chronically exposed to 4-chloronitrobenzene (Jones et al., 2007). Post-shift urine samples were obtained from 38 exposed workers (36 men, 2 women) in a Chinese chemical factory; five pre-shift samples were also taken from a subset of the workers. Air exposure levels of 4-chloronitrobenzene were measured for 19 workers with personal samplers, yielding a mean 8-hour time-weighted average (TWA) of 1.17 mg/m^3. Three urinary metabolites were detected in the post-shift urine samples of the chronically exposed workers, reflecting each of the three major pathways discussed in the paragraph above: the mercapturic acid *N*-acetyl-*S*-(4-nitrophenyl)-L-cysteine (51%); conjugated 4-chloroaniline (18%);

Fig. 4.1 Proposed metabolic pathway of 4-chloronitrobenzene in humans



GSH, glutathione; GST, glutathione *S*-transferase; NAT, *N*-acetyltransferase

and conjugated 2-chloro-5-nitrophenol (30%). *N*-acetyl-*S*-(4-nitrophenyl)-L-cysteine was detected in all 38 post-shift samples, 4-chloroaniline was detected in 29 of the 38 samples, and 2-chloro-5-nitrophenol was detected in 36 of the 38 samples. As with the accidently exposed workers studied by Yoshida et al. (1992, 1993), no parent compound was observed. In contrast to the accidently exposed workers, 2,4-dichloroaniline was not observed within the limits of quantitation of the assay, and there was no evidence of the *N*-acetylated metabolite of 2-amino-5-chlorophenol. No metabolites were detected in control samples from unexposed workers or in the pre-shift samples. Contrasting with the observation of metabolites during the 14–29 days after exposure of the accidently exposed workers, the absence of detection of two of the urinary metabolites and the absence of detectable metabolites in the pre-shift samples might be a reflection of the different amount of 4-chloronitrobenzene absorbed, as indicated by the 1000-fold higher levels of 4-chloronitrobenzene metabolites reported for the accidently exposed compared with the chronically exposed workers (Jones et al., 2007).

A study of haemoglobin adducts in the same group of Chinese chemical factory workers

exposed to 4-chloronitrobenzene has also been conducted (Jones et al., 2006). In the haemoglobin adduct study, blood samples were obtained from 39 exposed workers. The mean 8-hour TWA from a subset of 19 workers was 1.17 mg/m^3, as reported by Jones et al. (2007), and the median was 0.87 mg/m^3. Hydrolysable haemoglobin adducts of 4-chloroaniline were detected in all 39 blood samples from workers exposed to 4-chloronitrobenzene (mean, 1037 pg/mg; median, 1013 pg/mg), indicating the availability of the reactive intermediate metabolite *N*-hydroxy-4-chloroaniline that was not apparent in the studies of urinary metabolites. The haemoglobin adducts of 4-chloroaniline were also detected, at lower concentrations, in all of the blood samples from factory controls (n = 15) and in 1 of the 6 non-factory control samples. As with the urinary metabolites, haemoglobin adduct concentrations were not correlated with air levels, possibly indicating the importance of other routes of exposure.

4.1.2 Experimental systems

(a) Absorption, distribution, and excretion

Dermal absorption studies of groups of three male Fischer 344 rats exposed to 4-chloronitro[^{14}C]benzene by single dermal application at 0.65, 6.5, and 65 mg/kg bw [0.0325, 0.325, and 3.25 mg/cm^2] were conducted (Bucher, 1993). Urine and faeces were collected for up to 72 hours. Based upon measurements of eliminated radiolabel, 51–62% of 4-chloronitrobenzene was absorbed from the skin within 72 hours, with absorption increasing non-significantly with increasing dose. Urinary excretion of radiolabel over 72 hours accounted for 43–45% of the administered dose; faecal excretion accounted for 5–12%, and increased with increasing dose.

Bucher (1993) exposed groups of eight male Fischer 344 rats to 4-chloronitro[^{14}C]benzene as a single gavage dose at 2, 20, or 200 mg/kg bw, and urine and faeces were collected for up to 72 hours. Minimum absorption (determined by the percentage of the administered dose recovered in the urine or tissues) of the 4-chloronitrobenzene was 73–78%. A comparison of this finding with the results for dermal application in Bucher (1993), discussed in the previous paragraph, demonstrates greater absorption by oral exposure than by dermal exposure. 4-Chloronitrobenzene was rapidly metabolized and excreted, primarily in the urine. At the lower doses, about 23% and 5% of the administered radiolabel was found in tissues after 24 and 72 hours, respectively. The highest dose was eliminated more slowly, with about 75% greater tissue retention at both time points. At 24 hours the greatest percentage of radiolabel was in fat, increasing from 15% to 28% across the dose levels. For all dose levels, the highest concentrations of radiolabel at 24 hours were found in fat, followed by blood cells, kidney, liver, and spleen. With the exception of blood cells and spleen, tissue levels of radiolabel declined between 24 and 72 hours. At 72 hours, the greatest percentage of radioactivity at the lower doses was 3% in blood cells; at the highest dose, it was 4% in fat. The highest concentrations at 72 hours occurred in blood cells, followed by fat and spleen. High-performance liquid chromatography analysis of urine revealed the presence of up to 25 metabolites from 4-chloronitrobenzene [metabolites unspecified].

In repeat-dose studies, groups of four young adult (age, 10–12 weeks) or geriatric (age, 19–20 months) male Fischer 344 rats were exposed to 4-chloronitro[^{14}C]benzene at a dose of 65 mg/kg bw by gavage on days 1, 5, and 9, and unlabelled compound on days 2, 3, 4, 6, 7, 8, 10, and 11 (Bucher, 1993). In young adult rats, urinary and faecal excretion accounted for 71–80% and 13–15% of the administered dose, respectively. Approximately 2% of the administered radiolabel was found in the tissues, primarily in blood cells and fat, with the highest concentrations in blood cells and spleen. Urinary excretion in geriatric rats was similar to that of young adults; however,

faecal excretion in geriatric rats was about half of that in the young adults. Furthermore, the percentage of radiolabel retained in the geriatric rats 72 hours after the dose on day 9 (17%) was greater than that in the young adults (2%). Most of the retention was in fat (11%), followed by skeletal muscle and blood cells. The highest concentration of radiolabel was in fat, followed by blood cells, testes, and spleen.

(b) Metabolism

Bray et al. (1956) examined the metabolism of 4-chloronitrobenzene in female rabbits given 4-chloronitrobenzene [exposure route unspecified] at 0.2 g/kg [whether diet or body weight not specified]. Urine was collected over 24-hour periods until metabolites were no longer excreted (usually after 48 hours). The main metabolic processes were reduction and hydroxylation. Nearly the entire dose was excreted in the urine as 4-chloroaniline (9% of the administered dose as free chloroaniline) or derivatives of phenolic metabolites. The phenols formed were excreted mainly as conjugates with sulfuric and glucuronic acids (40% of the administered dose). The formation of mercapturic acid from 4-chloronitrobenzene appears to be a minor metabolic pathway in rabbits.

Yoshida et al. (1991) identified the urinary metabolites of 4-chloronitrobenzene in rats by gas chromatography and mass spectrometry. Male Sprague-Dawley rats were given a single intraperitoneal injection of 4-chloronitrobenzene at 100 mg/kg bw, and urine was collected 8–24 hours after dosing. 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. In a later study, 4-chloro-oxanilic acid was also identified as a metabolite in the urine of rats exposed to 4-chloronitrobenzene (Yoshida, 1994).

Rickert & Held (1990) studied the metabolism of radiolabelled 4-chloronitrobenzene in isolated hepatocytes and hepatic microsomes from male Fischer 344 rats. Incubation of 4-chloronitro[^{14}C]benzene with rat hepatocytes yielded 4-chloroaniline (15.4% of total radioactivity), *S*-(4-nitrophenyl)glutathione (10.4%), and 4-chloroacetaniline (16.3%). Incubation of the radiolabelled parent with microsomes demonstrated that the reduction to 4-chloroaniline was mediated by metabolism dependent on cytochrome P450, as this reduction was inhibited by SKF 525-A, metyrapone, and carbon monoxide.

4.2 Mechanisms of carcinogenesis

This section summarizes the available evidence for the key characteristics of carcinogens (Smith et al., 2016), on whether 4-chloronitrobenzene: is genotoxic; alters DNA repair; induces oxidative stress; alters cell proliferation, cell death, or nutrient supply; induces chronic inflammation; or is immunosuppressive.

4.2.1 Genetic and related effects

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

(a) Humans

Sabbioni (2017) assessed the formation of chromosomal aberrations in lymphocytes from a subset of the same workers exposed to 4-chloronitrobenzene studied by Jones et al. (2006). Samples from 24 exposed workers and 13 factory controls were analysed. These workers were also exposed to other chloronitrobenzenes; however, the major isomer was 4-chloronitrobenzene (70%). There was a statistically significant increase in chromosomal aberrations in the half of the exposed subset with the highest levels of 4-chloroaniline–haemoglobin adducts compared with the half

Table 4.1 Genetic and related effects of 4-chloronitrobenzene and its metabolite 4-chloroaniline in exposed humans and in human cells in vitro

End-point	Tissue, cell type (if specified)	Results[a]		Agent, concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Exposed humans						
Chromosomal aberrations	Lymphocytes	+	NT	4-Chloronitrobenzene, 0.87 mg/m^3 (TWA 8 h)	Effects could not be attributed to 4-chloronitrobenzene specifically, because of concurrent exposures to other chloronitrobenzenes	Sabbioni (2017)
Human cells in vitro						
DNA strand breaks	Exfoliated cells from breast milk (including mammary epithelial and various immune cells)	+	NT	4-Chloroaniline, 0. 71 mM		Martin et al. (2000)
DNA strand breaks	Fibroblast cell line	+	NT	4-Chloroaniline, 0.5 mM in the presence of H_2O_2 (40 µM)		Lueken et al. (2004)

HIC, highest ineffective concentration; LEC, lowest effective concentration; NT, not tested; TWA, time-weighted average

[a] +, positive

Table 4.2 Genetic and related effects of 4-chloronitrobenzene and its metabolite 4-chloroaniline in non-human mammals in vivo

End-point	Species, strain, (sex)	Tissue	Results[a]	Agent, dose (LED or HID)	Route, duration, dosing regimen	Reference
DNA adducts	Rat, Wistar (F)	Liver	–	4-Chloronitrobenzene or 4-chloroaniline, 0.5 mmol/kg bw	Gavage 1×, 24 h after dosing	Jones & Sabbioni (2003)
DNA strand breaks	Mouse, Swiss CD-1 (M)	Brain, liver, kidney	+	4-Chloronitrobenzene, 60 mg/kg bw	Intraperitoneal injection 1×, 16 h after dosing	Cesarone et al. (1983)
DNA strand breaks	Rat, Sprague-Dawley (M)	Liver, stomach	+	4-Chloroaniline, 75 and 150 mg/kg bw per day	Gavage 1×/day for 3 days	Barfield & Burlinson (2015)

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

[a] +, positive; –, negative

Table 4.3 Genetic and related effects of 4-chloronitrobenzene and its metabolite 4-chloroaniline in non-human mammalian cells in vitro

End-point	Species, tissue, cell line	Results[a]		Agent, concentration (LEC or HIC)	Comments on study quality	Reference
		Without metabolic activation	With metabolic activation			
DNA strand breaks	Rat, hepatocytes	+	–	4-Chloronitrobenzene, 50 µM		Cesarone et al. (1984)
Chromosomal aberrations	Chinese hamster lung	–	(+)	4-Chloronitrobenzene, NR	Structural chromosomal aberrations; cytotoxicity, NR	JETOC (1996)
Chromosomal aberrations	Chinese hamster ovary	(+)	(+)	4-Chloronitrobenzene, 600 µg/mL	Without activation, one trial gave negative results (HIC, 500 µg/mL) and two gave positive results (LEC, 700 and 900 µg/mL); with activation, one trial gave negative results (HIC, 5000 µg/mL) and the other gave positive results; cytotoxicity was seen with positive results	Bucher (1993)
Sister-chromatid exchange	Chinese hamster ovary	–	+	4-Chloronitrobenzene, 250 µg/mL	Positive with activation in two trials	Bucher (1993)
Mutation/*Tk*	Mouse, L5178 lymphoma cells	+	+	4-Chloroaniline, NR		NTP (1989)
Chromosomal aberrations	Chinese hamster ovary	–	+	4-Chloroaniline, NR		NTP (1989)
Sister-chromatid exchange	Chinese hamster ovary	+	+	4-Chloroaniline, NR		NTP (1989)

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

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

Table 4.4 Genetic and related effects of 4-chloronitrobenzene and its metabolite 4-chloroaniline in non-mammalian experimental systems

Test system (species, strain)	End-point	Results[a]		Agent, concentration (LEC or HIC)	Reference
		Without metabolic activation	With metabolic activation		
Drosophila melanogaster (adult)	Sex-linked recessive lethal mutations	–	NA	4-Chloronitrobenzene, 100 ppm either by feeding or injection	Zimmering et al. (1985)
Drosophila melanogaster (larvae)	Sex-linked recessive lethal mutations	–	NT	4-Chloronitrobenzene, 80 ppm (feed)	Zimmering et al. (1989)
Salmonella typhimurium TA100, TA1530, TA1535, TA1537, TA98, TA1532, TA1950, TA1975, TA1978, G46	Reverse mutation	–	–	4-Chloronitrobenzene, NR	Gilbert et al. (1980)
Salmonella typhimurium TA100	Reverse mutation	–	+	4-Chloronitrobenzene, 128 µg/mL	Haworth et al. (1983)
Salmonella typhimurium TA1535	Reverse mutation	+/–	–	4-Chloronitrobenzene, 256 µg/mL	Haworth et al. (1983)
Salmonella typhimurium TA98, TA1537	Reverse mutation	–	–	4-Chloronitrobenzene, 384 µg/mL	Haworth et al. (1983)
Salmonella typhimurium TA100, TA98	Reverse mutation	–	–	4-Chloronitrobenzene, 50 µg/mL	Suzuki et al. (1983)
Salmonella typhimurium TA98, TA98NR, TA98NR/1,8-DNP6	Reverse mutation	NT	–	4-Chloronitrobenzene, 50 µg/mL	Suzuki et al. (1987)
Salmonella typhimurium TA100	Reverse mutation	+/–	NT	4-Chloronitrobenzene, 630 µg/mL	Shimizu et al. (1983)
Salmonella typhimurium TA1535	Reverse mutation	+	NT	4-Chloronitrobenzene, 315 µg/mL	Shimizu et al. (1983)
Salmonella typhimurium TA1537, TA1538	Reverse mutation	–	NT	4-Chloronitrobenzene, 630 µg/mL	Shimizu et al. (1983)
Salmonella typhimurium TA100	Reverse mutation	–	+	4-Chloronitrobenzene, 192 µg/mL	Bucher (1993)
Salmonella typhimurium TA98	Reverse mutation	–	–	4-Chloronitrobenzene, 385 µg/mL	Bucher (1993)
Salmonella typhimurium TA1535	Reverse mutation	–	(+)	4-Chloronitrobenzene, 256 µg/mL	Bucher (1993)
Escherichia coli PQ37	DNA strand breaks	–	–	4-Chloronitrobenzene, NR	von der Hude et al. (1988)
Hens' eggs	Micronucleus formation	+	NT	4-Chloroaniline, 0.5 mg per egg	Greywe et al. (2012)

Table 4.4 (continued)

Test system (species, strain)	End-point	Results[a]		Agent, concentration (LEC or HIC)	Reference
		Without metabolic activation	With metabolic activation		
Salmonella typhimurium TA98, TA100	Reverse mutation	–	+	4-Chloroaniline, NR	NTP (1989)
Salmonella typhimurium TA97, TA1535, TA1537	Reverse mutation	–	–	4-Chloroaniline, NR	NTP (1989)
Calf thymus DNA	DNA adducts	+	NT	4-Chloroaniline, 60 µmol	Jones & Sabbioni (2003)

HIC, highest ineffective concentration; LEC, lowest effective concentration; NA, not applicable; NR, not reported; NT, not tested; ppm, parts per million

[a] +, positive; –, negative; (+), positive in a study of limited quality; +/–, equivocal (variable response in several experiments within an adequate study)

with the lowest adduct levels, but not in the group exposed to chloronitrobenzene versus the unexposed group. [The Working Group noted that the latter comparison could be limited by the small number of workers evaluated, as well as by the fact that the unexposed workers were of notably higher average age and had some exposure to 2-chloronitrobenzene, as evidenced by the haemoglobin adducts also measured in the unexposed workers.]

The 4-chloroaniline metabolite of 4-chloronitrobenzene caused single-strand DNA breaks in exfoliated cells isolated from human breast milk, which included mammary epithelial cells and various immune cells (Martin et al., 2000). 4-Chloroaniline also acted synergistically with non-cytotoxic doses of H_2O_2 to induce DNA strand breaks in a human fibroblast cell line (Lueken et al., 2004).

(b) Experimental systems

Jones & Sabbioni (2003) did not observe DNA adducts in the liver in female Wistar rats exposed in vivo to 4-chloronitrobenzene or 4-chloroaniline by gavage, despite the formation of haemoglobin adducts by both compounds. Adducts in calf thymus DNA were seen after exposure to 4-chloroaniline (Jones & Sabbioni, 2003).

4-Chloronitrobenzene injected intraperitoneally into male Swiss CD-1 mice induced DNA single-strand breaks in liver, kidney, and brain (Cesarone et al., 1983). 4-Chloronitrobenzene also induced DNA single-strand breaks in rat hepatocytes in vitro (Cesarone et al., 1984).

In Chinese hamster ovary cells, 4-chloronitrobenzene induced chromosomal aberrations with and without S9, and sister-chromatid exchange in the presence of S9, but the positive response for chromosomal aberrations occurred only at doses that were toxic (Bucher, 1993). 4-Chloronitrobenzene induced structural chromosomal aberrations in Chinese hamster lung cells, but information on cytotoxicity was not reported (JETOC, 1996).

4-Chloronitrobenzene did not induce sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* when given to adults either by feeding or by injection, or to larvae by feeding (Zimmering et al., 1985, 1989).

Results of mutagenic testing in multiple strains of *Salmonella typhimurium* were largely but not entirely negative with or without metabolic activation across several studies (e.g. Gilbert et al., 1980; Haworth et al. 1983).

4-Chloronitrobenzene gave negative results in the *Escherichia coli* SOS-chromotest (von der Hude et al., 1988).

The metabolite 4-chloroaniline induced DNA damage in vivo in a comet assay in the liver and stomach of male Sprague-Dawley rats (Barfield & Burlinson, 2015), although Jones & Sabbioni (2003) did not observe adducts in hepatic DNA of female Wistar rats. 4-Chloroaniline also exhibited some genotoxic activity in multiple assays in vitro reported by NTP (1989), including mutagenicity in *S. typhimurium* strains TA98 and TA100 with S9, but not in TA97, TA1535, or TA1537. 4-Chloroaniline also tested positive for mutagenicity in mouse lymphoma cells with and without S9, for induction of sister-chromatid exchange with and without S9, and for chromosomal aberrations with S9 in Chinese hamster ovary cells. 4-Chloroaniline gave positive results in a hen's egg test in vitro for micronucleus formation (Greywe et al., 2012).

4.2.2 DNA repair

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

Cesarone et al. (1984) measured the repair of single-strand DNA breaks in freshly isolated rat hepatocytes after 3 hours of exposure to

4-chloronitrobenzene in vitro, and observed incomplete DNA repair at 48 hours.

4.2.3 Oxidative stress

Methaemoglobin formation is a well-established effect of exposure to 4-chloronitrobenzene by multiple routes in humans, mice, and rats (Bucher, 1993; Yoshida et al., 1993; Matsumoto et al., 2006a). Methaemoglobin formation is attributed to the *N*-hydroxy-4-chloroaniline metabolite. In erythrocytes, such *N*-hydroxyarylamines can engage in Kiese redox cycling, yielding methaemoglobin and increasing cellular oxidative stress (Sabbioni, 2017).

Paranich et al. (1993) studied the effects of 4-chloronitrobenzene in the spleen and liver in rats and reported that short-term (5-day) but not longer-term (30-day) exposure caused increased lipid peroxidation and decreased vitamin E concentration in the spleen, but not liver. No effect was seen on antioxidative activity.

4.2.4 Alters cell proliferation, cell death, or nutrient supply

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

Haematopoietic proliferation arising from the need to replace damaged erythrocytes was observed in several subchronic and chronic studies with 4-chloronitrobenzene. In a 4-week study in rats treated by inhalation, extramedullary haematopoiesis in the spleen was reported (Nair et al., 1986). In both the Bucher (1993) 13-week study of inhalation exposure and the Matsumoto et al. (2006a) 13-week study of dietary exposure, erythropoiesis in the bone marrow and extramedullary haematopoiesis in the spleen were observed in rats and mice. In the latter study, extramedullary haematopoiesis in the liver of mice and rats was also reported. In a 2-year study of dietary exposure, extramedullary haematopoiesis in the spleen of rats and male mice was observed (Matsumoto et al., 2006b).

Other proliferative effects observed in the subchronic and chronic studies included: squamous cell hyperplasia of the forestomach epithelium in female mice in the Bucher (1993) 13-week study of inhalation exposure, possibly from grooming (Travlos et al., 1996); capsular hyperplasia in the spleens of rats in the Matsumoto et al. (2006a) 13-week study of oral exposure by diet; and fibroblast hyperplasia in the spleens of rats and adrenal gland hyperplasia in rats in the Matsumoto et al. (2006b) 2-year study of oral exposure by diet.

4.2.5 Chronic inflammation

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

In the Bucher (1993) 13-week study of inhalation exposure to 4-chloronitrobenzene, chronic inflammation of the Harderian gland and capsular fibrosis of the spleen, accompanied by mononuclear inflammatory cell infiltrates, was observed in rats (Bucher, 1993; Travlos et al., 1996).

4.2.6 Immunosuppression

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

Li et al. (1998) investigated immunotoxicity in BDF_1 male mouse splenocytes after a single intraperitoneal injection of 300 mg/kg (acute treatment), or intraperitoneal injections of 30 mg/kg bw three times per week for 4 weeks (subchronic treatment). Compared with controls, natural killer cell activity was decreased after both treatments, cytotoxic T-cell activity was

decreased primarily after subchronic treatment, and lipopolysaccharide-stimulated B-cell proliferation was decreased primarily after the acute treatment. In a second study in BDF_1 male mouse splenocytes after a single intraperitoneal injection of 300 mg/kg bw, Li et al. (1999) reported that, compared with controls, B-cells, CD4 and CD8 T-cells, and natural killer cells were significantly decreased, although macrophages and nucleated erythrocytes were increased.

In a study in sheep peripheral blood lymphocytes in vitro, Kačmár et al. (1995) observed that the 4-chloronitrobenzene metabolite, 4-chloroaniline, decreased the mitogenic stimulation of the lymphocytes.

4.3 Other adverse effects

4.3.1 Humans

Eleven longshoremen were poisoned with 4-chloronitrobenzene as a result of the accidental tearing of bags during loading. It was assumed that both inhalation and skin absorption occurred. Symptoms reported included cyanosis, and laboratory tests revealed methaemoglobinaemia, anaemia, reticulocytosis, and Heinz bodies (Yoshida et al., 1992, 1993).

4.3.2 Experimental systems

A 2-year study of Fischer 344 rats and BDF_1 mice exposed orally to 4-chloronitrobenzene by diet indicated that the major target tissue for carcinogenicity in male and female rats is the spleen; in mice, cancers of the liver (in males and females) and lymphoma (in males) were also observed (see Section 3). The non-neoplastic toxic effects observed in the 2-year study included haematotoxicity in the rats exposed at the two higher doses (200 and 1000 ppm) and mice exposed at the two higher doses (500 and 2000 ppm), as indicated by haematology findings and non-neoplastic lesions in the spleen in both rats and mice, and increased incidence of adrenal gland hyperplasia and pheochromocytoma in rats (Matsumoto et al., 2006b).

The non-neoplastic toxic effects of exposure to 4-chloronitrobenzene were also observed in several subchronic studies. In a small 2-week inhalation study, Bucher (1993) observed early evidence of haematotoxicity and spleen effects in Fischer 344 rats and $B6C3F_1$ mice and kidney lesions in rats. In a 4-week inhalation study in Sprague-Dawley rats, Nair et al. (1986) reported haematotoxicity in males and females, including effects associated with methaemoglobinaemia, effects on haematological parameters, and histopathological changes in the spleen. In a 13-week inhalation study, Bucher (1993) observed haematotoxicity and spleen lesions in male and female Fischer 344 rats and $B6C3F_1$ mice, with rats being the most sensitive. In rats, effects on the liver, kidney, and Harderian gland were also observed, as well as testicular atrophy in males. In mice, liver effects were reported, as well as hyperplasia of the forestomach in females. In a 13-week study of dietary exposure, Matsumoto et al. (2006a) also reported haematotoxicity and spleen lesions in male and female Fischer 344 rats and BDF_1 mice, with rats being the most sensitive. Signs of hepatotoxicity were also apparent, particularly in mice.

The United States National Toxicology Program (NTP, 1989) conducted a 2-year bioassay with the 4-chloronitrobenzene metabolite, 4-chloroaniline. Non-neoplastic effects in Fischer 344 rats included an increased incidence of fibrosis of the spleen and dose-related increases in methaemoglobin and other signs of haematotoxicity, such as bone marrow hyperplasia and hepatic haemosiderosis, in males and females, and adrenal medullary hyperplasia in females. Effects in $B6C3F_1$ mice included increased haemosiderin deposits in males and females as well as increased extramedullary haematopoiesis in the liver of females.

4.4 Data relevant to comparisons across agents and end-points

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

5. Summary of Data Reported

5.1 Exposure data

4-Chloronitrobenzene is a high production volume chemical that is currently produced primarily in China and India. Between 1995 and 2004, production volumes in China increased 3-fold from 78 000 to 250 000 tonnes per annum; the 2004 production volume represented 60% of total annual global production.

4-Chloronitrobenzene is used as an intermediate in the synthesis of various chemicals, including agricultural chemicals and antioxidants used in the rubber industry. It is also used: in the pharmaceutical industry for the synthesis of certain drugs; in the treatment of textiles and leather; in the production of paint, pigments, colorants, and plastics; and in the paper industry.

The compound is not known to occur naturally, but it can be released to the environment as a by-product of production or manufacture; release may also occur during transport, storage, or disposal, or accidentally. It has been detected in various water sources in Asia, Europe, and North America, and is considered moderately persistent in the environment. 4-Chloronitrobenzene has also been detected at low concentrations in edible fish.

Occupational exposure is expected to occur primarily through inhalation in workplaces where 4-chloronitrobenzene is produced or used as an intermediate in the manufacture of other products; exposure may also occur through skin contact or inadvertent ingestion. Detectable levels of 4-chloronitrobenzene have been measured in workplace air in chemical factories in China and Japan, and its metabolites have been detected in the urine of Chinese chemical factory workers.

No quantitative data on exposure to 4-chloronitrobenzene in the general population were available to the Working Group.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

4-Chloronitrobenzene was tested for carcinogenicity in well-conducted good laboratory practice (GLP) studies of oral exposure by diet, including one study in male and female mice and one study in male and female rats, conducted in the same laboratory. In limited studies of oral exposure by diet in another laboratory, 4-chloronitrobenzene was tested in one study in male and female mice and one study in male rats.

In the GLP study of oral exposure by diet in male mice, 4-chloronitrobenzene induced a significant positive trend in the incidence of hepatocellular carcinoma and malignant lymphoma. In the limited study of oral exposure by diet in male mice, 4-chloronitrobenzene induced a significant increase in the incidence of hepatocellular carcinoma and vascular tumours.

In the GLP study of oral exposure by diet in female mice, 4-chloronitrobenzene induced a significant positive trend in the incidence of hepatocellular carcinoma and liver haemangiosarcoma; 4-chloronitrobenzene also induced a significant increase in the incidence of liver haemangiosarcoma. In the limited study of oral exposure by diet in female mice, 4-chloronitrobenzene induced a significant increase in the incidence of vascular tumours.

In the GLP study of oral exposure by diet in male rats, 4-chloronitrobenzene induced a significant positive trend in the incidence and a significant increase in the incidence of

splenic tumours (fibroma, fibrosarcoma, osteosarcoma, sarcoma (not otherwise specified), and haemangiosarcoma); 4-chloronitrobenzene also induced a significant positive trend in the incidence of adrenal gland pheochromocytoma. In the limited study of oral exposure by diet in male rats, 4-chloronitrobenzene did not induce a significant increase in the incidence of any tumours.

In the GLP study of oral exposure by diet in female rats, 4-chloronitrobenzene induced a significant positive trend in the incidence of splenic tumours (fibroma, fibrosarcoma, osteosarcoma, and haemangiosarcoma) and adrenal gland pheochromocytoma; 4-chloronitrobenzene also induced a significant increase in the incidence of splenic fibrosarcoma and adrenal gland pheochromocytoma.

5.4 Mechanistic and other relevant data

Data on absorption, distribution, metabolism and excretion in humans are available from occupational exposures, including an accidental high-exposure episode. The major metabolic pathways include glutathione conjugation and reduction to 4-chloroaniline, which is further metabolized including to the reactive intermediate *N*-hydroxy-4-chloroaniline. In rats exposed dermally, orally, or by inhalation, 4-chloronitrobenzene is absorbed, widely distributed to tissues, and excreted as metabolites in the urine and faeces.

Concerning the key characteristics of carcinogens, there is *moderate* evidence that 4-chloronitrobenzene is genotoxic. An increased frequency of chromosomal aberrations was observed in workers exposed to various chloronitrobenzenes including 4-chloronitrobenzene. DNA strand breaks were observed in: two studies of human cells in vitro; in mouse liver, kidney, and brain after exposure by intraperitoneal injection; and in rat hepatocytes exposed in vitro. In Chinese hamster cells, 4-chloronitrobenzene increased the frequency of sister-chromatid exchanges, but results on chromosomal aberrations were inconclusive. Mutagenicity tests in multiple strains of *Salmonella typhimurium* gave largely negative results with or without metabolic activation. Genotoxicity results for the 4-chloroaniline metabolite were generally positive in a variety of assays.

There is *moderate* evidence that 4-chloronitrobenzene induces oxidative stress. Oxidative damage to erythrocytes was observed after exposure to 4-chloronitrobenzene. Methaemoglobinaemia was observed in humans and rodents, and toxic effects to erythrocytes were seen in mice and rats in subchronic and chronic exposure studies. In orally exposed rats, 4-chloronitrobenzene elevated lipid peroxidation in the spleen and liver.

There is *moderate* evidence that 4-chloronitrobenzene alters cell proliferation, cell death, or nutrient supply in rodents. No data in humans were available. In subchronic and chronic studies in rodents, erythropoiesis increased in bone marrow, spleen, and liver, and hyperplasia occurred in female mouse forestomach, rat spleen, and rat adrenal gland.

There is *weak* evidence (scarcity of data) that 4-chloronitrobenzene induces chronic inflammation (in the rat spleen) and is immunosuppressive.

Haematotoxicity was seen in highly exposed humans and in rodents, and rodent kidney and liver toxicity was also observed.

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of 4-chloronitrobenzene.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 4-chloronitrobenzene.

6.3 Overall evaluation

4-Chloronitrobenzene is *possibly carcinogenic to humans* (*Group 2B*).

References

ACGIH (2008). TLVs and BEIs. Threshold limit values for chemical substances and physical agents and biological exposure indices. Cincinnati (OH), USA: American Conference of Governmental Industrial Hygienists.

AFSSET (2009). Valeurs toxicologiques de référence (VTR). Élaboration de VTR chroniques par voie orale pour les isomères ortho, méta et para du chloronitrobenzène. Avis relatif à l'élaboration de VTR chroniques par voie orale du chloronitrobenzène (isomères ortho, méta et para)." Avis de l'Agence française de sécurité sanitaire de l'environnement et du travail (AFSSET). Saisine AFSSET No. 070057. Available from: https://www.anses.fr/fr/system/files/CHIM2007et0057Ra.pdf [French], accessed December 2019.

Arora PK, Sasikala Ch, Ramana ChV (2012). Degradation of chlorinated nitroaromatic compounds. *Appl Microbiol Biotechnol*, 93(6):2265–77. doi:10.1007/s00253-012-3927-1 PMID:22331236

Barfield W, Burlinson B (2015). *p*-Chloroaniline, *t*-butylhydroquinone, and methyl carbamate: Rat in vivo comet test, JaCVAM trial phase 4.2. *Mutat Res Genet Toxicol Environ Mutagen*, 786–788:98–103. doi:10.1016/j.mrgentox.2015.05.007 PMID:26212298

Below H, Assadian O, Baguhl R, Hildebrandt U, Jäger B, Meissner K, et al. (2017). Measurements of chlorhexidine, *p*-chloroaniline, and *p*-chloronitrobenzene in saliva after mouth wash before and after operation with 0.2% chlorhexidine digluconate in maxillofacial surgery: a randomised controlled trial. *Br J Oral Maxillofac Surg*, 55(2):150–5. doi:10.1016/j.bjoms.2016.10.007 PMID:27789177

Below H, Lehan N, Kramer A (2004). HPLC determination of the antiseptic agent chlorhexidine and its degradation products 4-chloroaniline and 1-chloro-4-nitrobenzene in serum and urine. *Mikrochim Acta*, 146(2):129–35. doi:10.1007/s00604-004-0194-6

Booth G (2012). Nitro compounds, aromatic. Ullmann's encyclopedia of industrial chemistry. Weinheim, Germany: Wiley-VCH Verlag GmbH & Co. KGaA.

Bray HG, James SP, Thorpe WV (1956). The metabolism of the monochloronitrobenzenes in the rabbit. *Biochem J*, 64(1):38–44. doi:10.1042/bj0640038 PMID:13363803

Bucher J (1993). NTP technical report on the toxicity studies of 2-chloronitrobenzene (CAS No. 88-73-3) and 4-chloronitrobenzene (CAS No. 100-00-5) administered by inhalation to F344/N rats and B6C3F$_1$ mice. *Toxic Rep Ser*, 33:1–F25. PMID:12209191

CDC (2016). NIOSH pocket guide to chemical hazards: *p*-nitrochlorobenzene. Atlanta (GA), USA: Centers for Disease Control and Prevention. Available from: https://www.cdc.gov/niosh/npg/npgd0452.html, accessed December 2019.

Cesarone CF, Bolognesi C, Santi L (1983). DNA damage induced in vivo in various tissues by nitrochlorobenzene derivatives. *Mutat Res*, 116(3–4):239–46. doi:10.1016/0165-1218(83)90061-7 PMID:6835248

Cesarone CF, Fugassa E, Gallo G, Voci A, Orunesu M (1984). Influence of the culture time on DNA damage and repair in isolated rat hepatocytes exposed to nitrochlorobenzene derivatives. *Mutat Res*, 131(5–6):215–22. PMID:6738566

Dangwal SK, Jethani BM (1980). A simple method of determination of nitrobenzene and nitrochlorobenzene in air and urine. *Am Ind Hyg Assoc J*, 41(11):847–50. doi:10.1080/15298668091425752 PMID:7457378

DECOS (2002). *p*-Chloronitrobenzene: evaluation of the carcinogenicity and genotoxicity. Nr 2002/03OSH, Den Haag : Dutch Expert Committee on Occupational Standards, a committee of the Health Council of the Netherlands. Available from: https://www.healthcouncil.nl/, accessed December 2019.

Duguet JP, Anselme C, Mazounie P, Mallevialle J (1988). Application of the ozone-hydrogen peroxide combination for the removal of toxic compounds from a groundwater. In: Angeletti G, Bjørseth A, editors. Organic micropollutants in the aquatic environment, Proceedings of the Fifth European Symposium, Rome, 20–22 October 1987. Dordrecht, Netherlands: Kluwer Academic Publishers; pp. 299–309.

ECHA (2018). 1-Chloro-4-nitrobenzene. European Chemicals Agency Database. Helsinki, Finland: European Chemicals Agency. Available from: https://echa.europa.eu/fr/brief-profile/-/briefprofile/100.002.554, accessed December 2019.

EPA (1996). SW-846 Test Method 8091: nitroaromatics and cyclic ketones by gas chromatography. Washington (DC), USA: United States Environmental Protection Agency. Available from: https://www.epa.gov/sites/production/files/2015-12/documents/8091.pdf, accessed December 2019.

EPA (2015). Provisional peer reviewed toxicity values for p-chloronitrobenzene (CASRN 100-00-5), EPA/690/R-15/004F. Cincinnati (OH), USA: Office of Superfund Remediation and Technology Innovation, United States Environmental Protection Agency. Available from: https://cfpub.epa.gov/ncea/pprtv/documents/Chloronitrobenzenep.pdf, accessed December 2019.

EPA (2018). Regional screening levels (RSLs). Washington (DC), USA: United States Environmental Protection Agency. Available from: https://www.epa.gov/risk/regional-screening-levels-rsls, accessed December 2019.

Feltes J, Levsen K, Volmer D, Spiekermann M (1990). Gas chromatographic and mass spectrometric determination of nitroaromatics in water. *J Chromatogr A*, 518(1):21–40. doi:10.1016/S0021-9673(01)93159-0 PMID:2258406

Gao J, Liu L, Liu X, Lu J, Hao H, Yuan H, et al. (2012). The organic contamination survey and health risk assessment of 16 source water reservoirs in Haihe River basin. *Water Sci Technol*, 65(6):998–1006. doi:10.2166/wst.2012.072 PMID:22377994

Gilbert P, Saint-Ruf G, Poncelet F, Mercier M (1980). Genetic effects of chlorinated anilines and azobenzenes on *Salmonella typhimurium*. *Arch Environ Contam Toxicol*, 9(5):533–41. doi:10.1007/BF01056933 PMID:7002063

Greywe D, Kreutz J, Banduhn N, Krauledat M, Scheel J, Schroeder KR, et al. (2012). Applicability and robustness of the hen's egg test for analysis of micronucleus induction (HET-MN): results from an inter-laboratory trial. *Mutat Res*, 747(1):118–34. doi:10.1016/j.mrgentox.2012.04.012 PMID:22580102

Haworth S, Lawlor T, Mortelmans K, Speck W, Zeiger E (1983). Salmonella mutagenicity test results for 250 chemicals. *Environ Mutagen*, 5(Suppl 1):1–142. doi:10.1002/em.2860050703 PMID:6365529

Howard PH, Santodonato J, Saxena J, Malling J, Greninger D (1976). Investigation of selected environmental contaminants: nitroaromatics (EPA Report No. EPA-560/2-76-010; US NTIS PB-275078). Washington (DC), USA: United States Environmental Protection Agency, Office of Toxic Substances.

HSDB (2008). 1-Chloro-2-nitrobenzene. Hazardous Substances Data Bank. A Toxnet database. Bethesda (MD), USA: United States National Library of Medicine. Available from: https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+hsdb:@term+@rn+@rel+88-73-3, accessed December 2019.

IARC (1996). Printing processes and printing inks, carbon black and some nitro compounds. *IARC Monogr Eval Carcinog Risks Hum*, 65:1–578. Available from: http://publications.iarc.fr/83 PMID:9148039

IFA (2018). 1-Chloro-4-nitrobenzene. GESTIS International Limit Values database. Germany: Institut für Arbeitsschutz der Deutschen Gesetzlichen Unfallversicherung (Institute for Occupational Safety and Health of the German Social Accident Insurance). Available from: https://www.dguv.de/ifa/gestis/gestis-internationale-grenzwerte-fuer-chemische-substanzen-limit-values-for-chemical-agents/index-2.jsp, accessed December 2019.

INERIS (2010). Données technico-économiques sur les substances chimiques en France: 1-chloro-4-nitrobenzène, INERIS-DRC-10-102861-01255A; 34 pp. Verneuil en Halatte, France: Institut National de l'Environnement Industriel et des Risques (INERIS). Available from: https://substances.ineris.fr/fr/substance/getDocument/3023 [French], accessed December 2019.

JETOC (1996). *p*-Chloronitrobenzene. In: Mutagenicity test data of existing chemical substances. Based on toxicity investigation system of the industrial safety and health law. Tokyo, Japan: Japan Chemical Industry Ecology-Toxicology and Information Center (JETOC); pp. 56, 472–473. Available from: http://anzeninfo.mhlw.go.jp/user/anzen/kag/sokatutbl.htm [Japanese], accessed December 2019.

Jones CR, Liu YY, Sepai O, Yan H, Sabbioni G (2006). Internal exposure, health effects, and cancer risk of humans exposed to chloronitrobenzene. *Environ Sci Technol*, 40(1):387–94. doi:10.1021/es050693p PMID:16433376

Jones CR, Sabbioni G (2003). Identification of DNA adducts using HPLC/MS/MS following in vitro and in vivo experiments with arylamines and nitroarenes. *Chem Res Toxicol*, 16(10):1251–63. doi:10.1021/tx020064i PMID:14565767

Jones CR, Sepai O, Liu YY, Yan H, Sabbioni G (2007). Urinary metabolites and health effects in workers exposed chronically to chloronitrobenzene. *Biomarkers*, 12(1):1–20. doi:10.1080/13547500600799250 PMID:17438650

Kačmár P, Pistl J, Mikula I (1995). The effect of *p*-chloroaniline on leucocytes of sheep peripheral blood under the migration-inhibition test conditions. *Immunopharmacol Immunotoxicol*. 17(3):577–84. doi:10.3109/08923979509016388 PMID:8576547

Lang PZ, Long FS, Yuan X, Ding YZ, Wang MJ, Zhao YH (1993). The research on the toxic organic pollutants in water of middle stream of Songhua River (Shaokou-Songhua River Section). *Advances Environ Sci*, 1:47–55. [Chinese]

Lewalter J, Ellrich D (1991). Nitroaromatic compounds (nitrobenzene, *p*-nitrotoluene, *p*-nitrochlorobenzene, 2,6-dinitrotoluene, *o*-dinitrobenzene, 1-nitronaphthalene, 2-nitronaphthalene, 4-nitrobiphenyl) [Biomonitoring Methods, 1991]. The MAK Collection for Occupational Health and Safety.

Li Q, Minami M, Hanaoka T, Yamamura Y (1999). Acute immunotoxicity of *p*-chloronitrobenzene in mice: II. Effect of *p*-chloronitrobenzene on the immunophenotype of murine splenocytes determined by flow

cytometry. *Toxicology*, 137(1):35–45. doi:10.1016/S0300-483X(99)00065-7 PMID:10513998

Li Q, Minami M, Inagaki H (1998). Acute and subchronic immunotoxicity of *p*-chloronitrobenzene in mice. I. Effect on natural killer, cytotoxic T-lymphocyte activities and mitogen-stimulated lymphocyte proliferation. *Toxicology*, 127(1-3):223–32. doi:10.1016/S0300-483X(98)00027-4 PMID:9699808

Lopez B, Laurent A (2013). Campagne exceptionnelle d'analyse des substances présentes dans les eaux souterraines de métropole Exploitation des résultats à l'échelle de la métropole. Rapport final BRGM/RP-61853-FR. ONEMA, France. Available from: https://www.eaufrance.fr/IMG/pdf/campexESO_2011_201306.pdf [French], accessed December 2019.

Lueken A, Juhl-Strauss U, Krieger G, Witte I (2004). Synergistic DNA damage by oxidative stress (induced by H_2O_2) and nongenotoxic environmental chemicals in human fibroblasts. *Toxicol Lett*, 147(1):35–43. doi:10.1016/j.toxlet.2003.10.020 PMID:14700526

Martin FL, Cole KJ, Williams JA, Millar BC, Harvey D, Weaver G, et al. (2000). Activation of genotoxins to DNA-damaging species in exfoliated breast milk cells. *Mutat Res*, 470(2):115–24. doi:10.1016/S1383-5742(00)00046-6 PMID:11027965

Matsumoto M, Aiso S, Senoh H, Yamazaki K, Arito H, Nagano K, et al. (2006). Carcinogenicity and chronic toxicity of *para*-chloronitrobenzene in rats and mice by two-year feeding. *J Environ Pathol Toxicol Oncol*, 25(3):571–84. doi:10.1615/JEnvironPatholToxicolOncol.v25.i3.20 PMID:17073560

Matsumoto M, Aiso S, Senoh H, Yamazaki K, Arito H, Nagano K, et al. (2006b). Carcinogenicity and chronic toxicity of *para*-chloronitrobenzene in rats and mice by two-year feeding. *J Environ Pathol Toxicol Oncol*, 25(3):571–84. doi:10.1615/JEnvironPatholToxicolOncol.v25.i3.20 PMID:17073560

Matsumoto M, Aiso S, Umeda Y, Arito H, Nagano K, Yamamoto S, et al. (2006a). Thirteen-week oral toxicity of *para*- and *ortho*- chloronitrobenzene in rats and mice. *J Toxicol Sci*, 31(1):9–22. doi:10.2131/jts.31.9 PMID:16538040

Men B, Wang H, He M, Lin C, Quan X (2011). Distribution patterns of nitroaromatic compounds in the water, suspended particle and sediment of the river in a long-term industrial zone (China). *Environ Monit Assess*, 177(1-4):515–26. doi:10.1007/s10661-010-1652-8 PMID:20711858

Nair RS, Johannsen FR, Levinskas GJ, Terrill JB (1986). Subchronic inhalation toxicity of p-nitroaniline and p-nitrochlorobenzene in rats. *Fundam Appl Toxicol*, 6(4):618–27. doi:10.1016/0272-0590(86)90174-0 PMID:3710033

NTP (1989). NTP toxicology and carcinogenesis studies of *para*-chloroaniline hydrochloride (CAS No. 20265-96-7) in F344/N rats and B6C3F$_1$ mice (gavage studies). *Natl Toxicol Program Tech Rep Ser*, 351:1–256. PMID:12704433

NIOSH (2005). NIOSH manual of analytical methods. Nitroaromatic compounds: Method 2005. 4th ed. Cincinnati (OH), USA: National Institute for Occupational Safety and Health.

NOES (1995). National Occupational Exposure Survey 1981–1983, Unpublished data as of November 1997. Cincinnati (OH), USA: United States Department of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health.

OECD (2009). The 2007 OECD list of high production volume chemicals. Number 112. Organisation for Economic Co-operation and Development screening information dataset. Available from: http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm, accessed December 2019.

OECD-SIDS (2002). 1-Chloro-4-nitrobenzene.SIDS Initial assessment report for SIAM 15 (Boston, USA. 22-25 October 2002). Paris, France: Organisation for Economic Co-operation and Development screening information dataset. Available from: https://hpvchemicals.oecd.org/UI/handler.axd?id=ad5cafea-2e80-4360-9aba-9056532403f0, accessed December 2019.

Paranich LI, Paranich AV, Vasilenko NM, Bugaĭ EV (1993). [The action of nitrobenzene and its chlorine derivatives on the indices of antioxidative homeostasis in rat tissues]. *Biull Eksp Biol Med*, 116(10):402–5. PMID:8117965 [Russian]

PubChem (2018). *p*-Chloronitrobenzene. PubChem Open Chemistry Database. Bethesda (MD), USA: National Center for Biotechnology Information. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/7474, accessed December 2019.

Raguet S, Clinard F, Mouly D, Boyer S, Sauleau E, Buemi A (2010). Incidence des cancers dans la population exposée à la pollution de la nappe phréatique au Nord-Ouest de Mulhouse de 1988 à 2002. Available from: http://invs.santepubliquefrance.fr//Publications-et-outils/Rapports-et-syntheses/Environnement-et-sante/2010/Incidence-des-cancers-dans-la-population-exposee-a-la-pollution-de-la-nappe-phreatique-Nord-Ouest-de-Mulhouse-1988-a-2002 [French], accessed December 2019.

Rickert DE, Held SD (1990). Metabolism of chloronitrobenzenes by isolated rat hepatocytes. *Drug Metab Dispos*, 18(1):5–9. PMID:1970777

Sabbioni G (2017). Hemoglobin adducts and urinary metabolites of arylamines and nitroarenes. *Chem Res Toxicol*, 30(10):1733–66. doi:10.1021/acs.chemrestox.7b00111 PMID:28933159

Sabbioni G, Norppa H, Hirvonen A, Järventaus H, Jones CR, Yan H, et al. (2016). Biomarkers of exposure, effect, and susceptibility in workers exposed to chloronitrobenzenes. *Biomarkers*, 21(8):721–30. doi:10.3109/1354750X.2016.1172116 PMID:27121198

Schäfer RB, von der Ohe PC, Kühne R, Schuüuürmann G, Liess M (2011). Occurrence and toxicity of 331 organic pollutants in large rivers of north Germany over a decade (1994 to 2004). *Environ Sci Technol*, 45(14):6167–74. doi:10.1021/es2013006 PMID:21663313

Shen JM, Chen ZL, Xu ZZ, Li XY, Xu BB, Qi F (2008). Kinetics and mechanism of degradation of *p*-chloronitrobenzene in water by ozonation. *J Hazard Mater*, 152(3):1325–31. doi:10.1016/j.jhazmat.2007.08.009 PMID:17889996

Shimizu M, Yasui Y, Matsumoto N (1983). Structural specificity of aromatic compounds with special reference to mutagenic activity in *Salmonella typhimurium* – a series of chloro- or fluoro-nitrobenzene derivatives. *Mutat Res*, 116(3-4):217–38. doi:10.1016/0165-1218(83)90060-5 PMID:6339893

Sigma-Aldrich (2018). 1-Chloro-4-nitrobenzene. Available from: https://www.sigmaaldrich.com/catalog/product/aldrich/c59122?lang=en&region=GB&cm_sp=Insite-_-prodRecCold_xviews-_-prodRecCold5-1, accessed December 2019.

Smith MT, Guyton KZ, Gibbons CF, Fritz JM, Portier CJ, Rusyn I, et al. (2016). Key characteristics of carcinogens as a basis for organizing data on mechanisms of carcinogenesis. *Environ Health Perspect*, 124(6):713–21. doi:10.1289/ehp.1509912 PMID:26600562

Steinwandter H (1987). Research in environmental pollution. II. Determination of polychlorinated nitrobenzenes (PCNB's) in Main river fish. *Fresenius Z Anal Chem*, 326(2):139–41. doi:10.1007/BF00468498

Suzuki J, Koyama T, Suzuki S (1983). Mutagenicities of mono-nitrobenzene derivatives in the presence of norharman. *Mutat Res*, 120(2-3):105–10. doi:10.1016/0165-7992(83)90150-1 PMID:6341826

Suzuki J, Takahashi N, Kobayashi Y, Miyamae R, Ohsawa M, Suzuki S (1987). Dependence on *Salmonella typhimurium* enzymes of mutagenicities of nitrobenzene and its derivatives in the presence of rat-liver S9 and norharman. *Mutat Res*, 178(2):187–93. doi:10.1016/0027-5107(87)90268-5 PMID:3295532

Swaminathan K, Kondawar VK, Chakrabarti T, Subrahmanyam PVR (1987). Identification and quantification of organics in nitro aromatic manufacturing wastewaters. *Indian J Environ Health*, 1987(29):32–8.

Travlos GS, Mahler J, Ragan HA, Chou BJ, Bucher JR (1996). Thirteen-week inhalation toxicity of 2- and 4-chloronitrobenzene in F344/N rats and B6C3F$_1$ mice. *Fundam Appl Toxicol*, 30(1):75–92. doi:10.1006/faat.1996.0045 PMID:8812232

Trova C, Cossa G, Gandolfo G (1991). Behavior and fate of chloronitrobenzenes in a fluvial environment. *Bull Environ Contam Toxicol*, 47(4):580–5. doi:10.1007/BF01700949 PMID:1786442

van de Meent D, den Hollander HA, Pool WG, Vredenbregt MJ, van Oers HAM, de Greef E, et al. (1986). Organic micropollutants in Dutch coastal waters. *Water Sci Technol*, 18(4-5):73–81. doi:10.2166/wst.1986.0182

van Zoest R, van Eck GTM (1991). Occurrence and behaviour of several groups of organic micropollutants in the Scheldt estuary *Sci Total Environ*, 103(1):57–71. doi:10.1016/0048-9697(91)90353-G

von der Hude W, Behm C, Gürtler R, Basler A (1988). Evaluation of the SOS chromotest. *Mutat Res*, 203(2):81–94. doi:10.1016/0165-1161(88)90023-4 PMID:3280989

Weisburger EK, Russfield AB, Homburger F, Weisburger JH, Boger E, Van Dongen CG, et al. (1978). Testing of twenty-one environmental aromatic amines or derivatives for long-term toxicity or carcinogenicity. *J Environ Pathol Toxicol*, 2(2):325–56. PMID:84039

Xia S, Li H, Zhang Z, Zhang Y, Yang X, Jia R, et al. (2011). Bioreduction of *para*-chloronitrobenzene in drinking water using a continuous stirred hydrogen-based hollow fiber membrane biofilm reactor. *J Hazard Mater*, 192(2):593–8. doi:10.1016/j.jhazmat.2011.05.060 PMID:21715088

Xu X, Shao J, Li M, Gao K, Jin J, Zhu L (2016). Reductive Transformation of *p*-chloronitrobenzene in the upflow anaerobic sludge blanket reactor coupled with microbial electrolysis cell: performance and microbial community. *Bioresour Technol*, 218:1037–45. doi:10.1016/j.biortech.2016.07.037 PMID:27455127

Yoshida M, Sunaga M, Hara I, Katsumata M, Minami M (1989). Elevation of urinary *N*-acetyl-beta-D-glucosaminidase and beta-galactosidase activities in workers with long-term exposure to aromatic nitroamino compounds. *Bull Environ Contam Toxicol*, 43(1):1–8. doi:10.1007/BF01702229 PMID:2758123

Yoshida T (1994). Pharmacokinetic study of *p*-chloronitrobenzene in rat. *Drug Metab Dispos*, 22(2):275–80. PMID:8013283

Yoshida T, Andoh K, Tabuchi T (1991). Identification of urinary metabolites in rats treated with *p*-chloronitrobenzene. *Arch Toxicol*, 65(1):52–8. doi:10.1007/BF01973503 PMID:2043051

Yoshida T, Tabuchi T, Andoh K (1992). Identification of urinary metabolites of human subjects acutely poisoned by *p*-chloronitrobenzene. *Xenobiotica*, 22(12):1459–70. doi:10.3109/00498259209056696 PMID:1494891

Yoshida T, Tabuchi T, Andoh K (1993). Pharmacokinetic study of *p*-chloronitrobenzene in humans suffering from acute poisoning. *Drug Metab Dispos*, 21(6):1142–6. PMID:7905396

Yurawecz MP, Puma BJ (1983). Identification of chlorinated nitrobenzene residues in Mississippi River fish. *J Assoc Off Anal Chem*, 66(6):1345–52. PMID:6685729

Zhu L, Lin H, Qi J, Xu X (2013). Enhanced transformation and dechlorination of *p*-chloronitrobenzene in the combined ZVI-anaerobic sludge system. *Environ Sci Pollut Res Int*, 20(9):6119–27. doi:10.1007/s11356-013-1631-z PMID:23536276

Zimmering S, Mason JM, Valencia R (1989). Chemical mutagenesis testing in Drosophila. VII. Results of 22 coded compounds tested in larval feeding experiments. *Environ Mol Mutagen*, 14(4):245–51. doi:10.1002/em.2850140406 PMID:2583131

Zimmering S, Mason JM, Valencia R, Woodruff RC (1985). Chemical mutagenesis testing in Drosophila. II. Results of 20 coded compounds tested for the National Toxicology Program. *Environ Mutagen*, 7(1):87–100. doi:10.1002/em.2860070105 PMID:3917911