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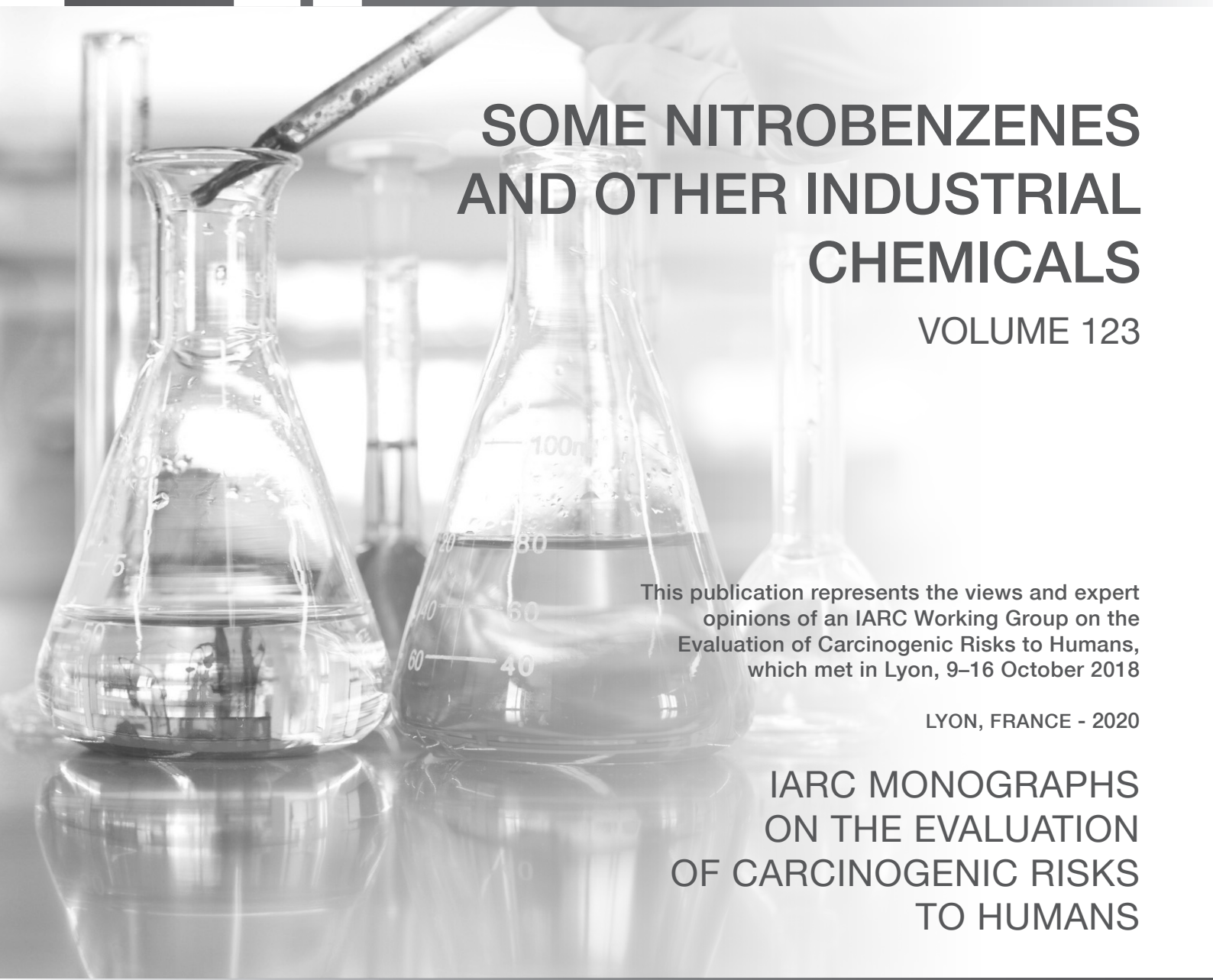

SOME NITROBENZENES AND OTHER INDUSTRIAL CHEMICALS

VOLUME 123

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International Agency for Research on Cancer

World Health Organization

2-CHLORONITROBENZENE

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

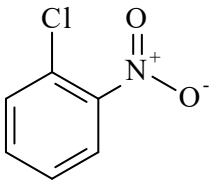
Chem. Abstr. Serv. Reg. No.: 88-73-3

Chem. Abstr. Serv. name:
1-chloro-2-nitrobenzene

IUPAC systematic name:
1-chloro-2-nitrobenzene

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

Description: yellow crystals

Boiling point: 246 °C

Melting point: 32–33 °C

Solubility: slightly soluble in water (441 mg/L at 25 °C); soluble in acetone, benzene, toluene, ethanol, and other organic solvents

Volatility: vapour pressure, 0.018 mm Hg [at 25 °C]

Relative vapour density (air = 1): 5.4

Octanol/water partition coefficient (P): log K_{ow} = 2.52 (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 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 (Booth, 2012).

1.2.2 Production volume

2-Chloronitrobenzene is included in the 2007 Organisation for Economic Co-operation and Development list of chemicals with a high production volume (OECD, 2009). For the year 1995, the worldwide (excluding eastern Europe) production of 2-chloronitrobenzene was reported as 111 800 tonnes from approximately 30 producers in western Europe (27 000 tonnes), China (39 000 tonnes), India (15 500 tonnes), Japan (9000 tonnes), the Republic of Korea (2300 tonnes), and the USA (19 000 tonnes) (OECD-SIDS, 2001).

The non-confidential production volumes for 2-chloronitrobenzene for the USA, as submitted to the United States Environmental Protection Agency (EPA) by companies for chemicals under the 1986–2002 Inventory Update Rule and for 2012–2015 under the Chemical Data Reporting Rule, are presented in Table 1.1. Over this period, annual production in the USA decreased from a peak of 23–45 thousand tonnes to less than 11 tonnes.

The European Chemicals Agency reported that 1–10 tonnes of 2-chloronitrobenzene per year are currently manufactured in and/or imported into the European Economic Area (ECHA, 2018).

Table 1.1 Production volumes for 2-chloronitrobenzene, USA[a]

Year	Production volume range, pounds [tonnes]
1986	(> 10–50) × 10^6 [4536–22 680]
1990	(> 50–100) × 10^6 [22 680–45 359]
1994	(> 50–100) × 10^6 [22 680–45 359]
1998	(> 50–100) × 10^6 [22 680–45 359]
2002	(> 10–50) × 10^6 [4536–22 680]
2012	(10–50) × 10^4 [45–227]
2013	(25–100) × 10^3 [11–45]
2014	< 25 × 10^3 [< 11]
2015	< 25 × 10^3 [< 11]

[a] Non-confidential information on production volumes submitted to the United States Environmental Protection Agency by companies for chemicals under the 1986–2002 Inventory Update Rule and for 2012–2015 under the Chemical Data Reporting Rule (HSDB, 2008; EPA, 2018a)

1.2.3 Use

2-Chloronitrobenzene is an important intermediate in the synthesis of colorants and specialty chemicals (Booth, 2012). The products obtained from 2-chloronitrobenzene – 2-chloroaniline and 3,3′-dichlorobenzidine – are important diazo components. 2-Chloronitrobenzene is also used to synthesize 2-nitrophenol, 2-nitroanisole, 2-ethoxynitrobenzene, and 2-nitroaniline. All of these chemicals are used as precursors of the corresponding amines and many other products, including *ortho*-anisidine, *ortho*-phenetidine, 3-amino-4-hydroxybenzenesulfonamide, picric acid, lumber preservatives, diaminophenol hydrochloride (a photographic developer), corrosion inhibitors, pigments, and agricultural chemicals. Sulfonation and chlorosulfonation also give important sulfonic acid and sulfonyl chloride derivatives (Booth, 2012).

1.3 Methods of measurement and analysis

1.3.1 Air

No specific methods have been published for the measurement of 2-chloronitrobenzene in air, although researchers have adapted the published method for 4-chloronitrobenzene (NIOSH, 2005) to measure concentrations of 2-chloronitrobenzene (Jones et al., 2006). The United States National Institute for Occupational Safety and Health (NIOSH) method is based on collecting samples in silica gel tubes, and analysis by gas chromatography combined with mass spectrometry or flame ionization detection.

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 contamination in water, soil, and wastewater to be measured (EPA, 1996). Method 8091 can detect nitroaromatics in water and soil at concentrations of parts per billion, and in wastewater samples at concentrations of parts per million.

1.3.3 Biomonitoring

There are no published biological monitoring methods for 2-chloronitrobenzene. [The Working Group noted that published methods for monitoring 4-chloronitrobenzene could be adapted for 2-chloronitrobenzene.]

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

2-Chloronitrobenzene is not known to occur naturally. The major source of environmental release of 2-chloronitrobenzene is the chemical plants at which it is produced and/or used as an intermediate. Minor sources of release into the environment may occur during transport, storage, or disposal to landfills; 2-chloronitrobenzene may also form in the environment through the oxidation of non-natural aromatic amines or the reaction of nitrogen oxides in highly polluted air with chlorinated aromatic hydrocarbons (Howard et al., 1976).

2-Chloronitrobenzene is most likely to be found in air and water, with water being the most contaminated (accounting for 65.4% of 2-chloronitrobenzene found in the environment) (OECD-SIDS, 2001). Measurements of 2-chloronitrobenzene in water identified by the Working Group are summarized in Table 1.2. The compound was measured at concentrations of up to 37 µg/L in the Mississippi river, USA, in the 1960s (Middleton & Lichtenberg, 1960). In the USA, concentrations of up to 1800 mg/L were reported for 2-, 3-, and 4-chloronitrobenzenes in wastewater from a chloronitrobenzene-production plant in the 1970s (Howard et al., 1976). In 1976, an accidental release of chloronitrobenzenes from a plant producing chemicals and dyes was reported in France (Raguet et al., 2010); in the area of the accident, concentrations of 2-chloronitrobenzene of up to 1.8 mg/L in groundwater were measured (Duguet et al., 1988). Elsewhere in Europe, 2-chloronitrobenzene concentrations of up to 0.2 µg/L in German rivers (Feltes et al., 1990) and 0.8 µg/L in Italian rivers (Trova et al., 1991) were measured in the 1990s; more recent measurements in German rivers (2004) measured concentrations of less than 0.06 µg/L (Schäfer et al., 2011). Based on the available experimental data, 2-chloronitrobenzene is not readily biodegradable in water. [The Working Group noted that 4-chloronitrobenzene can be biodegraded by adapted microorganisms. Similar approaches could be successful with 2-chloronitrobenzene, but no confirmatory research was identified.]

1.4.2 Occurrence in food

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

1.4.3 Exposure in the general population

No information on the exposure of the general population to 2-chloronitrobenzene was available to the Working Group.

1.4.4 Occupational exposure

Occupational exposure to 2-chloronitrobenzene may occur through inhalation and skin contact at workplaces where this compound

Table 1.2 Environmental occurrence of 2-chloronitrobenzene

Location, collection date	Sampling matrix	Mean exposure concentration (range)	Comments	Reference
France, 1987	Groundwater	1500 (970–1828) 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	10 (maximum, 290) ng/L	0.2% positive measurements; ~500 sites throughout France	Lopez & Laurent (2013)
Mississippi, Cape Girardeau, MO, USA, 1957	River water	NR (4–37 µg/L)	Specific industrial discharge identified	Middleton & Lichtenberg (1960)
Mississippi, New Orleans, LA, USA, 1959	River water	NR (1–2 µg/L)		Middleton & Lichtenberg (1960)
Elbe, Germany, NR	River water	NR (0.04–0.20 µg/L)	Three samples	Feltes et al. (1990)
Bormida river, Italy, 1989–1990	River water	0.21 (0.02–0.84) µg/L	Monthly measurements at five sampling stations	Trova et al. (1991)
Germany, 1994–2004	River water	NR (maximum, 0.06 µg/L)	A total of 110 measurements from the four largest rivers of northern Germany; detection at > 20% of sites	Schäfer et al. (2011)
Netherlands, 1983–1984	Coastal water	11.0 (0.2–50) 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, 1.3 (0.5–2.1) ng/L	Heavy pollution due to large wastewater discharge	van Zoest and 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	71 (24–93) mg/L	Effluent from a chloronitrobenzene production plant	Swaminathan et al. (1987)

NR, not reported

[a] 3- and 4-Chloronitrobenzene also collected

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 2900 employees in the USA were potentially exposed to 2-chloronitrobenzene. The estimates were based on a survey of companies and did not involve measurements of exposure (NOES, 1995).

The exposure of workers at a chemical factory in China was reported in 2006. The median concentration of 2-chloronitrobenzene determined from the personal air samples of 19 workers was 0.37 mg/m^3 (Jones et al., 2006). In another study by the same authors in the same factory, the mean 8-hour average exposure level of 2-chloronitrobenzene of 19 workers was 0.49 mg/m^3 (Jones et al., 2007).

1.5 Regulations and guidelines

There are no international occupational exposure limit values available for 2-chloronitrobenzene.

The EPA has set a chronic oral reference dose of 0.003 mg/kg per day for 2-chloronitrobenzene (EPA, 2009). The EPA regional screening levels for 2-chloronitrobenzene are 1.8 mg/kg in resident soil, 1.0 ng/m^3 in resident air, and 0.24 µg/L in tap water (EPA, 2018b).

The French *Agence française de securité sanitaire de l'environnement et du travail* (AFSSET, 2009) proposed two toxicological reference values for ingestion of 2-chloronitrobenzene: a chronic reference toxicological value with a threshold based on the hepatotoxic effects of 0.08 mg/kg per day; and a non-threshold reference toxicological value based on potential carcinogenic effects of 6×10^{-8} mg/kg per day.

For all methaemoglobin inducers such as 2-chloronitrobenzene, a biological exposure index was set by the American Conference of Governmental Industrial Hygienists at 1.5% methaemoglobin in blood (ACGIH, 2008).

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

Evidence for the carcinogenic activity of 2-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 2-chloronitrobenzene in male and female rats and mice has since become available for evaluation (Matsumoto et al., 2006b).

See Table 3.1

3.1 Mouse

Oral administration

In the 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 fed diets containing 1-chloro-2-nitrobenzene [2-chloronitrobenzene] (21 chemicals were tested in the study; purity of most, 97–99%) at a concentration of 0 (control), 3000, or 6000 ppm for 8 months. After 8 months, the groups at 3000 and 6000 ppm had their dietary concentrations reduced to 1500 and 3000 ppm, respectively, for a further 10 months. After this 18-month dosing period, all groups were held for an additional 3 months on the control diet before being killed at 21 months. There was also a pooled control group of 99 males and 102 females [no additional details were provided]. Mice that died within the first 6 months of the study were discarded

Table 3.1 Studies of carcinogenicity with 2-chloronitrobenzene in experimental animals

Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Mouse, CD-1 derived from HaM/ICR mice (Charles River) (M) 6–8 wk 21 mo Weisburger et al. (1978)	Oral Of the 21 tested chemicals in the study, most were of purity 97–99% Diet 0 (control), lower dose (3000 ppm for 8 mo, 1500 ppm for 10 mo, then held for an additional 3 mo without dosing), higher dose (6000 ppm for 8 mo, 3000 ppm for 10 mo, then held for an additional 3 mo without dosing), 0 (pooled control) 25, 25, 25, 99 NR	*Liver*: hepatoma [hepatocellular carcinoma] 3/18, 7/17*, 3/16, 7/99	*$P < 0.025$ (vs pooled control)	Principal strengths: males and females used Principal limitations: limited number of dose groups; limited experimental details provided; 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 Of the 21 tested chemicals in the study, most were of purity 97–99% Diet 0 (control), lower dose (3000 ppm for 8 mo, 1500 ppm for 10 mo, then held for an additional 3 mo without dosing), higher dose (6000 ppm for 8 mo, 3000 ppm for 10 mo, then held for an additional 3 mo without dosing), 0 (pooled control) 25, 25, 25, 102 NR	*Liver*: hepatoma [hepatocellular carcinoma] 0/20, 5/22*, 5/19*, 1/102	*$P < 0.025$ (vs concurrent control and pooled control)	Principal strengths: males and females used Principal limitations: limited number of dose groups; limited experimental details provided; 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. (2006b)	Oral Purity, > 99% Diet 0, 100, 500, 2500 ppm, continuous dosing 50, 50, 50, 50 35, 35, 17, 8	*Liver* Hepatocellular adenoma 19/50, 29/50*, 30/50*, 34/50**	$P < 0.01$, Peto trend test; *$P < 0.05$, Fisher exact test; **$P < 0.01$, Fisher exact test	Principal strengths: males and females used; well-conducted GLP study The incidence of hepatoblastoma in all groups of treated male mice exceeded the maximum incidence in the JBRC historical control data for this tumour (3/348; maximum incidence, 2%). Incidence and statistics for combination of hepatocellular tumours not given, but reported in Japan Bioassay Research Center (2006a)

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Mouse, Crj:BDF$_1$ (M) 6 wk 2 yr Matsumoto et al. (2006b) (cont.)		Hepatocellular carcinoma 15/50, 14/50, 20/50, 35/50*	$P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	
		Hepatoblastoma 1/50, 6/50, 35/50*, 44/50*	$P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	
		Hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) 30/50, 36/50, 49/50*, 49/50*	$P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	
Mouse, Crj:BDF$_1$ (F) 6 wk 2 yr Matsumoto et al. (2006b)	Oral Purity, > 99% Diet 0, 100, 500, 2500 ppm, continuous dosing 50, 50, 50, 50 29, 34, 26, 5	*Liver* Hepatocellular adenoma 8/50, 22/50*, 48/50*, 38/50*	$P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	Principal strengths: males and females used; well-conducted GLP study Incidence and statistics for combination of hepatocellular tumours not given, but reported in Japan Bioassay Research Center (2006a)
		Hepatocellular carcinoma 0/50, 3/50, 14/50*, 48/50*	$P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	
		Hepatoblastoma 0/50, 0/50, 9/50*, 28/50*	$P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	
		Hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) 8/50, 24/50, 50/50*, 50/50*	$P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Charles River CD (M) 6–8 wk 24 mo Weisburger et al. (1978)	Oral Of the 21 tested chemicals in the study, most were of purity 97–99% Diet 0 (control), lower dose (1000 ppm for 6 mo, 500 ppm for 12 mo, then 6 mo without dosing), higher dose (2000 ppm for 6 mo, 1000 ppm for 12 mo, then 6 mo without dosing), 0 (pooled control) 25, 25, 25, 111 NR	*Multiple sites*: multiple tumours 1/22, 7/22*, 1/19, 14/111	*$P < 0.025$ (vs concurrent control and pooled control)	Principal limitations: limited number of dose groups; limited experimental details provided; limited macroscopic and microscopic evaluation; no study in female rats; small number of rats Histopathology conducted only on rats surviving after 6 mo
Full carcinogenicity Rat, F344/DuCrj (M) 6 wk 2 yr Matsumoto et al. (2006b)	Oral Purity, > 99% Diet 0, 80, 400, 2000 ppm, continuous dosing 50, 50, 50, 50 40, 40, 39, 0	*Liver* Hepatocellular adenoma 2/50, 3/50 (6%), 7/50, 1/50 Hepatocellular carcinoma 0/50, 0/50, 3/50 (6%), 1/50 Hepatocellular adenoma or carcinoma (combined) 2/50, 3/50, 10/50*, 2/50 *Kidney* Renal cell adenoma 0/50, 1/50, 0/50, 1/50 Renal cell carcinoma 0/50, 0/50, 0/50, 4/50 (8%)	$P < 0.05$, Peto trend test $P < 0.01$, Peto trend test $P < 0.01$, Peto trend test; *$P < 0.05$, Fisher exact test [NS] [$P < 0.015$, Cochran–Armitage trend test]	Principal strengths: males and females used; well-conducted GLP study The 2000 ppm exposure in male rats exceeded the maximum tolerated dose; the data for this dose level were not included for statistical analysis The incidence of hepatocellullar adenoma in male rats at 400 ppm (7/50, 14%) exceeded the maximum incidence for this tumour in the JBRC historical control data (7/400; maximum incidence, 6%) The incidence of hepatocellullar carcinoma in male rats at 400 ppm (3/50, 6%) exceeded the incidence for this tumour in the JBRC historical control data (0/400) Incidence and statistics for a combination of hepatocellular tumours not given, but reported in Japan Bioassay Research Center (2006b) The incidence of renal cell carcinoma in the group at 2000 ppm (4/50, 8%) exceeded the incidence in the JBRC historical control data (0/400)

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, F344/DuCrj (F) 6 wk 2 yr Matsumoto et al. (2006b)	Oral Purity, > 99% Diet 0, 80, 400, 2000 ppm, continuous dosing 50, 50, 50, 50 41, 42, 45, 39	*Liver* Hepatocellular adenoma 0/50, 0/50, 2/50, 20/50*	$P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	Principal strengths: males and females used; well-conducted GLP study The incidence of hepatocellullar carcinoma in female rats at 2000 ppm (4/50, 8%) exceeded the incidence for this tumour in the JBRC historical control data (0/400) Incidence and statistics for a combination of hepatocellular tumours not given, but reported in Japan Bioassay Research Center (2006b) The incidence of renal cell adenoma in the group exposed at 2000 ppm (2/50, 4%) exceeded the maximum incidence in the JBRC historical control data (1/400; maximum incidence, 2%)
		Hepatocellular carcinoma 0/50, 0/50, 0/50, 4/50 (8%)	$P < 0.01$, Peto trend test	
		Hepatocellular adenoma or carcinoma (combined) 0/50, 0/50, 2/50, 23/50*	$P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test	
		Kidney: renal cell adenoma 0/50, 0/50, 0/50, 2/50 (4%)	[NS]	

F, female; GLP, good laboratory practice; JBRC, Japan Bioassay Research Centre; M, male; mo, month; NR, not reported; NS, not significant; vs, versus; wk, week; yr, year

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 in males was 3/18 (controls), 7/17 (lower dose), and 3/16 (higher dose); the incidence in the group exposed at the lower dose was significantly increased ($P < 0.025$) compared with the pooled control group (7/99), but was not significant when compared with the concurrent control group (3/18). The incidence of hepatocellular carcinoma in females was significantly increased at the lower and higher doses (control, 0/20; lower dose, 5/22; and higher dose, 5/19; $P < 0.025$) compared with the concurrent control and pooled control (incidence of 1/102) groups (Weisburger et al., 1978). [The Working Group noted that the limitations of the study included the small number of mice at the start and the small number necropsied, the use of only two dose groups, and the limited histopathological examination and reporting.]

In a study of carcinogenicity with *ortho*-chloronitrobenzene [2-chloronitrobenzene] (Matsumoto et al., 2006b), groups of 50 male and 50 female Crj:BDF_1 mice (age, 6 weeks) were fed diets containing 2-chloronitrobenzene (purity, > 99%) at a concentration of 0 (control), 100, 500, or 2500 ppm for 2 years. Based on feed consumption, the estimated dose given to male mice was 0, 11, 54, or 329 mg/kg body weight (bw) per day, and to female mice 0, 14, 69, or 396 mg/kg bw per day, for the groups exposed at 0, 100, 500, or 2500 ppm, respectively. The survival of the male and female mice at 500 and 2500 ppm was reduced, attributed to the development of malignant tumours of the liver. The number surviving until being killed at the termination of the experiment was 35, 35, 17, and 8 (males), and 29, 34, 26, and 5 (females) at 0, 100, 500, and 2500 ppm, respectively. The body weights of male and female mice were significantly reduced for the groups at the two higher doses. 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 was a dose-related increase in the incidence of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma in male and female mice. In males at 0, 100, 500, and 2500 ppm, the incidence of hepatocellular tumours was: hepatocellular adenoma, 19/50, 29/50 ($P < 0.05$), 30/50 ($P < 0.05$), and 34/50 ($P < 0.01$) ($P < 0.01$, Peto trend test); hepatocellular carcinoma, 15/50, 14/50, 20/50, and 35/50 ($P < 0.01$) ($P < 0.01$, Peto trend test); and hepatoblastoma, 1/50, 6/50, 35/50 ($P < 0.01$), and 44/50 ($P < 0.01$) ($P < 0.01$, Peto trend test). The combined incidence of hepatocellular tumours was not reported. In females at 0, 100, 500, and 2500 ppm, the incidence of hepatocellular tumours was: hepatocellular adenoma, 8/50, 22/50 ($P < 0.01$), 48/50 ($P < 0.01$), and 38/50 ($P < 0.01$) ($P < 0.01$, Peto trend test); hepatocellular carcinoma, 0/50, 3/50, 14/50 ($P < 0.01$), and 48/50 ($P < 0.01$) ($P < 0.01$, Peto trend test); and hepatoblastoma, 0/50, 0/50, 9/50 ($P < 0.01$), and 28/50 ($P < 0.01$) ($P < 0.01$, Peto trend test). In the original study report (Japan Bioassay Research Center, 2006a), the incidence of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) was reported as 30/50, 36/50, 49/50 ($P < 0.01$), and 49/50 ($P < 0.01$) ($P < 0.01$, Peto trend test) for males, and 8/50, 24/50 ($P < 0.01$), 50/50 ($P < 0.01$), and 50/50 ($P < 0.01$) ($P < 0.01$, Peto trend test) for females. [The Working Group noted that this was a well-conducted study that complied with good laboratory practice (GLP) and used males and females.]

3.2 Rat

Oral administration

In a 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 2-chloronitrobenzene (21 chemicals were tested in the study; purity of most, 97–99%) at a concentration of 0 (control), 1000, or 2000 ppm for 6 months. The dietary concentrations for the groups at 1000 and 2000 ppm were then reduced to 500 and 1000 ppm, respectively, for another 12 months. All rats were then held for a further 6 months on the control diet, until being killed at the termination of the experiment 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 rats. 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. The number of rats with multiple tumours was increased in male rats at the lowest dose, with an incidence of 1/22 (control), 7/22 (lower dose), and 1/19 (higher dose), respectively. The tumour incidence in the group exposed at the lower dose was significantly increased ($P < 0.025$) compared with that in the concurrent control group (1/22) and the pooled control group (14/111) (Weisburger et al., 1978). [The Working Group noted that the limitations of the study included the small number of rats at the start of the experiment, the small number necropsied, the use of only two dose groups and one sex, and the limited histopathological examination and reporting.]

In a study of carcinogenicity with 2-chloronitrobenzene (Matsumoto et al., 2006b), groups of 50 male and 50 female Fischer 344/DuCrj rats (age, 6 weeks) were fed diets containing 2-chloronitrobenzene (purity, > 99%) at a concentration of 0 (control), 80, 400, or 2000 ppm for 2 years. Based on feed consumption, the estimated dose given was 0, 4, 19, or 99 mg/kg bw per day (males) and 0, 4, 22, or 117 mg/kg bw per day (females) at 0, 80, 400, or 2000 ppm, respectively. The survival of the male rats at 2000 ppm was significantly lower after 76 weeks and all died before the end of the 2-year dosing period, this being attributed to chronic progressive nephropathy. The exposure of male rats at 2000 ppm exceeded the maximum tolerated dose. [The data for the males at 2000 ppm were not included for statistical analysis.] No significant difference in survival was found in male rats at 80 or 400 ppm compared with controls. The final number of rats surviving until being killed at the termination of the experiment was 40, 40, 39, and 0 for males and 41, 42, 45, and 39 for females for the groups at 0, 80, 400, and 2000 ppm, respectively. The body weight of males at 2000 ppm was decreased after 20 weeks. The body weight of females at 2000 ppm was decreased by 10% at 78 weeks, and by 18% at 2 years. The males at 400 ppm showed significantly decreased terminal body weight (10% decrease), although the females at 400 ppm did not. 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 hepatocellular adenoma and hepatocellular carcinoma in male and female rats. In males at 0, 80, and 400 ppm, the incidence of hepatocellular adenoma was 2/50, 3/50, and 7/50 ($P < 0.05$, Peto trend test), and the incidence of hepatocellular carcinoma was 0/50, 0/50, and 3/50 ($P < 0.01$, Peto trend test). The incidence of hepatocellular adenoma and hepatocellular carcinoma in males at 400 ppm exceeded the maximum tumour incidence (0/400) in the Japan Bioassay Research Center

(JBRC) historical control database. In females at 0, 80, 400, and 2000 ppm, the incidence of hepatocellular adenoma was 0/50, 0/50, 2/50, and 20/50 ($P < 0.01$) ($P < 0.01$, Peto trend test) and the incidence of hepatocellular carcinoma was 0/50, 0/50, 0/50, and 4/50 ($P < 0.01$, Peto trend test). The incidence of hepatocellular carcinoma in females at 2000 ppm exceeded the maximum tumour incidence (0/400) in the JBRC historical control database. An increase in the incidence of non-neoplastic liver lesions was seen in exposed rats, including liver cell foci in male and female rats, and spongiosis hepatis in male rats. In the original study report (Japan Bioassay Research Center, 2006b), the incidence of hepatocellular adenoma or carcinoma (combined) for male rats was 2/50, 3/50, 10/50 ($P < 0.05$), and 2/50 (exposure at 2000 ppm, which exceeded the maximum tolerated dose) ($P < 0.01$, Peto trend test) and for female rats was 0/50, 0/50, 2/50, and 23/50 ($P < 0.01$) ($P < 0.01$, Peto trend test).

Spontaneous, age-related chronic progressive nephropathy was exacerbated in a dose-related manner in male and female rats. Nephropathy was more severe in males than in females, and caused the early death of males in the group at 2000 ppm. The incidence of renal cell adenoma in males was 0/50 (control), 1/50 (80 ppm), 0/50 (400 ppm), and 1/50 (2000 ppm). There was a significant positive trend [$P < 0.015$, Cochran–Armitage trend test] in the incidence of renal cell carcinoma in males: 0/50 (control), 0/50, 0/50, and 4/50. The incidence of renal cell carcinoma in males in historical controls was 0/400. In females, the corresponding incidence of renal cell adenoma was 0/50, 0/50, 0/50, and 2/50. The incidence of renal cell adenoma in females at 2000 ppm (4%) exceeded the maximum incidence in the JBRC historical control database (1/400; maximum incidence, 2%), although it was not statistically significant. There were no renal cell carcinomas in any of the groups of female rats. The renal cell tumours were larger than tumours associated with chronic progressive nephropathy, and the renal cell carcinomas had a histopathologically pleomorphic appearance. In addition, urothelial hyperplasia of the kidney pelvis and the deposit of brown pigment of the proximal tubule were seen in male rats at 400 ppm. Urothelial hyperplasia of the pelvis was seen in females at 2000 ppm, and the deposit of brown pigment of the proximal tubule was observed in females at 400 and 2000 ppm (Matsumoto et al., 2006b). [The Working Group noted that this was a well-conducted study that complied with GLP and used males and females.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

Other than the data on haemoglobin adducts discussed in the following paragraph, no data on metabolism in humans were available to the Working Group. [The metabolic profile of 2-chloronitrobenzene resembles that of 4-chloronitrobenzene in rats (Bucher, 1993), and the major metabolites of 4-chloronitrobenzene in rats have been found to occur in humans exposed to 4-chloronitrobenzene (Yoshida, 1994). The Working Group therefore expected the metabolic pathways of 2-chloronitrobenzene in humans to be similar to those of 4-chloronitrobenzene (see Fig. 4.1 in the monograph on 4-chloronitrobenzene in the present volume).]

Haemoglobin adducts were studied in workers exposed to 2-chloronitrobenzene in a chemical factory in China (Jones et al., 2006). Blood samples were obtained from 39 exposed workers, 15 factory controls, and 6 non-factory controls. The mean duration of work-years was about 6 years in the exposed workers. Air exposure concentrations of 2-chloronitrobenzene were

measured for a subset of 19 exposed workers with personal samplers, yielding a mean 8-hour time-weighted average of 0.49 mg/m^3. Hydrolysable haemoglobin adducts of 2-chloroaniline were detected in 38 of the 39 blood samples from workers exposed to 2-chloronitrobenzene (mean, 92.4 pg/mg; median, 82.9 pg/mg), indicating the availability of the 2-chloronitrobenzene metabolite, *N*-hydroxy-2-chloroaniline, a highly reactive intermediate metabolite. The haemoglobin adducts of 2-chloroaniline were also detected (at lower concentrations) in the blood samples from 13 of the 15 factory controls, but not in any of the non-factory control samples. Haemoglobin adduct concentrations did not correlate with 2-chloronitrobenzene concentrations in air, possibly indicating the importance of other routes of exposure (Jones et al., 2006).

4.1.2 Experimental systems

Dermal absorption studies were conducted in three groups of three male Fischer 344 rats exposed to 2-chloronitro[^{14}C]benzene as a single dermal application at a dose of 0.65, 6.5, or 65 mg/kg bw [0.0325, 0.325, or 3.25 mg/cm^2] (Bucher, 1993). Urine and faeces were collected for up to 72 hours. Based upon measurements of eliminated radiolabel, 33–40% of the administered dose of 2-chloronitrobenzene was absorbed from the skin within 72 hours, with absorption increasing nonlinearly with increasing dose. Urinary excretion of radiolabel over 72 hours accounted for 21–28% of the administered dose; faecal excretion accounted for 11–15%.

Bucher (1993) exposed groups of eight male Fischer 344 rats to 2-chloronitro[^{14}C]benzene at a single dose of 2, 20, or 200 mg/kg bw by gavage, and excretions were collected for up to 72 hours. Minimum absorption (determined by the percentage of the administered dose recovered in the urine or tissues) was 61–77%. A comparison of this finding with the Bucher (1993) dermal results discussed above shows greater absorption by oral exposure than by dermal exposure. The 2-chloronitrobenzene was then rapidly metabolized and excreted primarily in the urine. At the lower doses, about 6% and 3% of the total administered radiolabel was found in tissues after 24 and 72 hours, respectively. The highest dose had the greatest absorption (at least 77%) and was eliminated more slowly, with about 20% and 4% retention after 24 and 72 hours, respectively. At 24 hours, the greatest percentage of radiolabel at the lower doses was 4% in the liver, and the highest concentrations were in the liver and kidney. At 24 hours after administration of the highest dose, the greatest percentage of radiolabel was 13% in fat; the highest concentrations occurred in fat, followed by kidney and liver. At 72 hours after administration of the lower doses, the greatest percentage of radiolabel (2%) and the highest concentrations occurred in the liver. At 72 hours after administration of the highest dose, the greatest percentage of radiolabel was also in the liver (1.6%), although the highest concentrations occurred in the kidney. High-performance liquid chromatography analysis of urine revealed the presence of up to 23 metabolites [metabolites unspecified] (Bucher, 1993).

Groups of four young adult (age, 10–12 weeks) or geriatric (age, 19–20 months) male Fischer 344 rats were given repeated gavage doses of 2-chloronitro[^{14}C]benzene at 65 mg/kg bw on days 1, 5, and 9 and unlabelled parent compound on days 2, 3, 4, 6, 7, 8, 10, and 11 (Bucher, 1993). In young adult rats, urinary excretion accounted for 71–74% of the administered dose and faecal excretion 20–27%. Approximately 5% of the administered radiolabel was found in the tissues 72 hours after the day 9 dose, primarily in the liver (3.4%); the highest concentrations of radiolabel occurred in the liver and kidney. In geriatric rats, faecal excretion was similar to that in young adults; however, urinary excretion was greater than in the young adults (85% versus 71%). At later time points in geriatric rats, there

was no change in faecal excretion but urinary excretion decreased to the levels observed in the young adults. Further, the percentage of radiolabel retained in the geriatric rats 72 hours after the day 9 dose (8.2%) was greater than that in the young adults (4.7%). Most of the retention was in the liver (6%). The highest concentrations of radiolabel were found in the liver and kidney (Bucher, 1993).

Rickert & Held (1990) studied the metabolism of radiolabelled 2-chloronitrobenzene by isolated male Fischer 344 rat hepatocytes and hepatic microsomes. Incubation of 2-chloronitro[^{14}C]benzene with rat hepatocytes yielded 2-chloroaniline (19.2% of total radiolabel), 2-chloroaniline-*N*-glucuronide (14.2% of total radioactivity), and *S*-(2-nitrophenyl)glutathione (13.3% of total radiolabel). Incubation of the radiolabelled parent with microsomes demonstrated that the reduction to 2-chloroaniline was mediated by metabolism dependent upon cytochrome P450; formation was inhibited by SKF 525-A, metyrapone, and carbon monoxide.

Bray et al. (1956) examined the metabolism of 2-chloronitrobenzene in female rabbits given 2-chloronitrobenzene [route unspecified] at 0.1 g/kg [whether diet or body weight was 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 administered dose was excreted in the urine as either 2-chloroaniline (9% of the administered dose) or derivatives of phenolic metabolites. The phenols formed were excreted mainly as conjugates with sulfuric and glucuronic acids (66% of the administered dose). The formation of mercapturic acid from 2-chloronitrobenzene metabolism appears to be a minor metabolic pathway in rabbits.

4.2 Mechanisms of carcinogenesis

This section summarizes the available evidence for the key characteristics of carcinogens (Smith et al., 2016), on whether 2-chloronitrobenzene: is genotoxic; induces oxidative stress; induces chronic inflammation; and alters cell proliferation, cell death, or nutrient supply.

4.2.1 Genetic and related effects

See Table 4.1, Table 4.2, and Table 4.3

(a) Humans

Sabbioni et al. (2016) analysed lymphocytes from a subset of the same workers studied by Jones et al. (2006), who were exposed to 2-chloronitrobenzene, to assess the formation of chromosomal aberrations. Samples from 24 exposed workers and 13 factory controls were analysed. These workers were also exposed to other chloronitrobenzenes, and the major isomer was 4-chloronitrobenzene (70%). There was a non-statistically significant increase in the frequency of chromosomal aberrations in the half of the exposed subset with the highest level of 2-chloroaniline–haemoglobin adducts compared with the half with the lowest adduct levels, but not in the group exposed to chloronitrobenzene compared with 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 observation in the larger group of workers studied by Jones et al. (2006) that the unexposed were of a notably higher average age. Further, the higher exposures to 4-chloronitrobenzene (and other chloronitrobenzenes) in the workers studied meant that effects could not be specifically attributed to 2-chloronitrobenzene.]

Table 4.1 Genetic and related effects of 2-chloronitrobenzene in exposed humans and in non-human mammals in vivo

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments on study quality	Reference
Chromosomal aberrations	Exposed humans	Lymphocytes	(+)	Mean (± standard deviation) 0.49 ± 0.32 mg/m³ (8-h TWA)	Mean number of years working in the factory, 5.9 ± 3.5	Effects could not be attributed to 2-chloronitrobenzene specifically, because of concurrent exposures to other chloronitrobenzenes	Sabbioni et al. (2016)
DNA adducts	Rat, Wistar (F)	Liver	–	NR	Gavage, 1×, 0.5 M solutions (0.1 mL per 100 g bw)		Jones & Sabbioni (2003)
DNA strand breaks	Mouse, Swiss (M)	Brain	+	60 mg/kg bw	Intraperitoneal injection, 1×, 60 or 180 mg/kg bw		Cesarone et al. (1980)
DNA strand breaks	Mouse, Swiss (M)	Liver and Kidney	+	60 mg/kg bw	Intraperitoneal injection, NR		Cesarone et al. (1982)

bw, body weight; F, female; HID, highest ineffective dose; LED, lowest effective dose; M, male; NR, not reported; TWA, time-weighted average.

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

Table 4.2 Genetic and related effects of 2-chloronitrobenzene in non-human mammalian cells in vitro

End-point	Species, cell line	Results[a]		Concentration (LEC or HIC)	Comments on study quality	Reference
		Without metabolic activation	With metabolic activation			
Sister-chromatid exchange	Chinese hamster ovary	+	–	50 µg/mL	Positive in two trials	Bucher (1993)
Sister-chromatid exchange	Chinese hamster ovary	–	+	500 µg/mL	This is a test in a separate laboratory reported by Bucher (1993); positive results in two trials	Bucher (1993)
Chromosomal aberrations	Chinese hamster ovary	–	+	500 µg/mL	Positive results in two trials	Bucher (1993)
Chromosomal aberrations	Chinese hamster ovary	(+)	–	160 µg/mL	This is a test in a separate laboratory reported by Bucher (1993); only one trial was conducted	Bucher (1993)
Chromosomal aberrations	Chinese hamster lung, CHL/IU	+	NT	NR	Positive for numerical, but not structural, chromosomal aberrations with 24 or 48 h treatments, but not shorter times	JETOC (1996)

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

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

Table 4.3 Genetic and related effects of 2-chloronitrobenzene in non-mammalian experimental systems

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments on study quality	Reference
		Without metabolic activation	With metabolic activation			
Drosophila melanogaster (adult)	Sex-linked recessive lethal mutations	–	NA	125 mg/kg (feed); 10 000 mg/kg (injection)		Zimmering et al. (1985)
Drosophila melanogaster (larvae)	Sex-linked recessive lethal mutations	–	NA	60 mg/kg (feed)		Zimmering et al. (1989)
Escherichia coli PQ37	DNA strand breaks	–	–	NR	Prophage induction, SOS repair test, cross-links, or related damage were tested with the same result	von der Hude et al. (1988)
Salmonella typhimurium TA100	Reverse mutation	–	+	38 µg/mL		Bucher (1993)
Salmonella typhimurium TA100	Reverse mutation	–	+	38 µg/mL		Haworth et al. (1983)
Salmonella typhimurium TA98, TA100	Reverse mutation	–	–	50 µg/mL		Suzuki et al. (1983)
Salmonella typhimurium TA100, TA1535, TA1537	Reverse mutation	–	NT	630 µg/mL		Shimizu et al. (1983)
Salmonella typhimurium TA98	Reverse mutation	–	(+)	50 µg/mL		Bucher (1993)
Salmonella typhimurium TA98	Reverse mutation	–	(+)	77 µg/mL		Haworth et al. (1983)
Salmonella typhimurium TA98, TA1538	Reverse mutation	+	NT	80 µg/mL		Shimizu et al. (1983)
Salmonella typhimurium TA1535, TA1537	Reverse mutation	–	–	128 µg/mL		Haworth et al. (1983)
Salmonella typhimurium TA98NR	Reverse mutation	NT	–	5 µg/mL		Suzuki et al. (1987)
Salmonella typhimurium TA98, TA98NR/1,8-DNP6	Reverse mutation	NT	–	2.5 µg/mL		Suzuki et al. (1987)
Salmonella typhimurium TA98, TA100, TA1530, TA1532, TA1535, TA1537, TA1538, TA1950, TA1975, TA1978, or G46	Reverse mutation	–	–	NR		Gilbert et al. (1980)

Table 4.3 (continued)

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments on study quality	Reference
		Without metabolic activation	With metabolic activation			
Salmonella typhimurium TA98, TA100	Reverse mutation	–	+	NR	TA98 gave positive results with hamster but not rat S9; TA98 and TA100 gave positive results in both trials	JETOC (1996)
Salmonella typhimurium TA1535, TA1537	Reverse mutation	–	–	NR	Negative results in both trials	JETOC (1996)
Escherichia coli WP2 *uvrA*	Reverse mutation	–	+	NR	WP2 *uvrA* gave positive results with hamster but not rat S9; and positive results in one of two trials	JETOC (1996)
Calf thymus DNA	DNA adducts	+	NA	250 µg		Jones & Sabbioni (2003)

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

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

(b) Experimental systems

Jones & Sabbioni (2003) did not observe DNA adducts in hepatic DNA from female Wistar rats exposed to 2-chloronitrobenzene or 2-chloroaniline by gavage, despite the formation of haemoglobin adducts by both compounds. Adducts in calf thymus DNA were seen after exposure to the 2-chloronitrobenzene metabolite, 2-chloroaniline.

In Swiss CD-1 mice exposed to 2-chloronitrobenzene by intraperitoneal injection, DNA single-strand breaks were observed in the liver, kidney, and brain (Cesarone et al., 1980, 1982).

2-Chloronitrobenzene induced sister-chromatid exchange and chromosomal aberrations in Chinese hamster ovary cells (Bucher, 1993). In Chinese hamster lung cells, 2-chloronitrobenzene gave positive results for numerical but not structural chromosomal aberrations after 24 or 48 hours (but not less) of treatment (JETOC, 1996).

2-Chloronitrobenzene did not induce sex-linked recessive lethal mutations in the 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).

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

The 2-chloroaniline metabolite of 2-chloronitrobenzene has also been tested for genotoxicity in a variety of assays, and the results are inconsistent. In vivo, 2-chloroaniline induced micronucleus formation in rat bone marrow, but not in mouse bone marrow or peripheral blood cells; in vitro, the metabolite gave positive results for mutagenicity in a mouse lymphoma cell assay, but not in *S. typhimurium* (NTP, 1998).

4.2.2 Oxidative stress

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

In rats and mice, methaemoglobin formation is a well-established effect of exposure to 2-chloronitrobenzene from multiple routes (Bucher, 1993; Matsumoto et al., 2006a). Methaemoglobin formation is attributed to the *N*-hydroxy-2-chloroaniline metabolite. In erythrocytes, such *N*-hydroxyarylamines can engage in Kiese redox cycling, yielding methaemoglobin and increasing cellular oxidative stress (Sabbioni, 2017). The oxidative damage to the erythrocytes is thought to be the underlying cause of many of the toxic effects observed in response to 2-chloronitrobenzene exposure (Bucher, 1993).

Paranich et al. (1993) studied the effects of 2-chloronitrobenzene in the spleen and liver of rats and reported that short-term (5 days), but not longer-term (30 days), exposure caused some changes in lipid peroxidation, antioxidative activity, and vitamin E concentration that were variable across markers and tissues.

4.2.3 Chronic inflammation

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

In a 2-week study with 2-chloronitrobenzene administered by inhalation (Bucher, 1993), granulomatous inflammation of the liver was observed in mice. In a 13-week study, inflammation characterized by fibrosis and accumulations of mononuclear inflammatory cells was observed in the livers of mice (Bucher, 1993; Travlos et al., 1996).

4.2.4 Altered 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. In a 4-week inhalation study in rats, extramedullary haematopoiesis in the spleen was reported (Nair et al., 1986). Extramedullary haematopoiesis, primarily erythropoiesis, was observed in the spleens of mice but not rats exposed for 13 weeks by inhalation (Bucher, 1993; Travlos et al., 1996). In a 13-week dietary study (Matsumoto et al., 2006a), extramedullary haematopoiesis in the spleen was found in mice and rats; erythropoiesis in the bone marrow was observed for rats only. In a 2-year dietary study (Matsumoto et al., 2006b), extramedullary haematopoiesis in the spleen was observed in mice and rats.

Other proliferative effects observed in the subchronic and chronic studies with 2-chloronitrobenzene include: hyperplasia of the nasal cavity in rats, considered to be due to irritation, and regeneration of the proximal convoluted tubules in rat kidneys in a 13-week study of exposure by inhalation (Bucher, 1993); capsule hyperplasia in the spleens of rats in a 13-week dietary study (Matsumoto et al., 2006a); and kidney tubule hyperplasia in male rats and capsule hyperplasia in the spleens of rats in a 2-year dietary study (Matsumoto et al., 2006b).

4.3 Other adverse effects

4.3.1 Humans

No data were available to the Working Group.

4.3.2 Experimental systems

In rodents fed diets containing 2-chloronitrobenzene at a concentration of 0, 80, 400, or 2000 ppm w/w for Fischer 344 rats and at 0, 100, 500, or 2500 ppm for BDF_1 mice for 2 years, the target tissue for carcinogenicity was the liver (Matsumoto et al., 2006b). The non-neoplastic toxic effects observed in the target tissue in the 2-year study included: hepatotoxicity in mice, as shown by increases in blood markers of liver damage (starting in the intermediate dose group); hepatotoxicity in rats, as indicated by increases in blood markers in females (males were not analysed); and histopathological changes, such as necrosis and hydropic degeneration, in male and female rats at the highest doses (Matsumoto et al., 2006b). In addition, exacerbated chronic progressive nephropathy occurred in male and female rats and caused significant early mortality in the males at the highest dose from week 73. The other main non-neoplastic toxic effects observed in the 2-year study included: haematotoxicity, as indicated by increased reticulocyte counts in mice (starting in the intermediate dose group); a variety of blood parameter perturbations in rats, including decreased erythrocyte counts and haemoglobin levels, and increased reticulocyte counts and methaemoglobin for the intermediate and highest dose; and haemosiderin deposits and extramedullary haematopoiesis in the spleens of mice and rats starting from the lowest or the intermediate dose depending on species (Matsumoto et al., 2006b).

The non-neoplastic toxic effects of 2-chloronitrobenzene exposure were also observed in several subchronic studies. In a small 2-week inhalation study, Bucher (1993) observed early evidence of liver toxicity and haematotoxicity in male and female Fischer 344 rats and $B6C3F_1$ mice. In a 4-week study in Sprague-Dawley rats treated by inhalation, Nair et al. (1986) reported haematotoxicity in both males and females. In a 13-week inhalation study, Bucher (1993) observed

hepatotoxicity in male and female Fischer 344 rats and B6C3F$_1$ mice, with mice being the most sensitive. Rats, particularly males, exhibited some kidney lesions. In a 13-week dietary study, Matsumoto et al. (2006a) reported signs of hepatotoxicity in male and female Fischer 344 rats and BDF$_1$ mice, particularly male mice; however, no kidney effects were reported. Haematotoxicity occurred in male and female rats and mice in both 13-week studies, with rats being more sensitive than mice.

Several assessments of the reproductive effects of exposure to 2-chloronitrobenzene have also been conducted. In the 13-week inhalation study by Bucher (1993), decreased spermatogenesis was observed in male rats in the group at the highest dose. In a continuous breeding study in breeding pairs of CD-1 Swiss mice, Bucher (1993) observed no effects on fertility after exposure to 2-chloronitrobenzene by gavage at doses of up to 160 mg/kg bw.

In a 13-week study of exposure by gavage to the 2-chloronitrobenzene metabolite, 2-chloroaniline, increased levels of methaemoglobin and related haematotoxic effects were observed in rats and mice (NTP, 1998).

4.4 Data relevant to comparisons across agents and end-points

4.4.1 High-throughput screening programmes

Data from standardized biochemical and cell-based assays can contribute to the understanding of carcinogenicity potential based on comparisons of activity in these assays across several end-points linked to characteristics common to carcinogens. Previous volumes of the *IARC Monographs* (IARC 2017a, b) and other publications (Chiu et al., 2018; Guyton et al., 2018) have described the key characteristics of carcinogens and their link to high-throughput screening (HTS) assays. Currently, 7 of the 10 key characteristics have some coverage in HTS assays used by the EPA and the United States National Institutes of Health (Chiu et al., 2018).

HTS assay results for the eight compounds and key metabolites assessed in the present volume of the *IARC Monographs* are evaluated here; all except *para*-nitroanisole (Chemical Abstracts Service registry number, CAS No. 100-17-4) have been evaluated in at least some of the HTS assays used by the EPA and the United States National Institutes of Health (EPA, 2018c). There are also HTS assay results available for several key metabolites of compounds considered in the present volume. The specific assays tested for each chemical and the mapping to the key characteristics can be found in Annex 1 (http://publications.iarc.fr/584). Findings for assays mapped to key characteristics for the eight compounds and the four metabolites evaluated are summarized in Table 4.4 and Table 4.5, respectively.

It is important to note two caveats that are relevant to the eight compounds evaluated in *IARC Monographs* Volume 123: (i) the assays used either fully lacked or had uncharacterized and generally low xenobiotic metabolism, limiting observations primarily to effects elicited by the parent compounds; and (ii) it may be difficult to reach the concentrations required for bioactivity for compounds of lower molecular mass and/or high vapour pressure in these assays. In particular, *N*,*N*-dimethylacetamide, *para*-nitroanisole, *ortho*-phenylenediamine, and 2-amino-4-chlorophenol have a relative molecular mass of less than 150, and others (2-chloronitrobenzene, 4-chloronitrobenzene, 1,4-dichloro-2-nitrobenzene, and 2,4-dichloro-1-nitrobenzene) have a relative molecular mass of just over 150. The assay end-points mapped to key characteristics considered in the present volume are described briefly below. Instances in which a quality concern was raised concerning compound purity are noted in Section 4.4.2.

Table 4.4 Summary of activity of agents reviewed in *IARC Monographs* Volume 123 and tested in ToxCast and/or Tox21 high-throughput screening assays[a]

Key characteristic (total no. of assays mapped to characteristic)[b]	Number of positive results out of the number of assays tested							
	2-Chloronitrobenzene (CAS No. 88-73-3)	4-Chloronitrobenzene (CAS No. 100-00-5)	1,4-Dichloro-2-nitrobenzene (CAS No. 89-61-2)	2,4-Dichloro-1-nitrobenzene (CAS No. 611-06-3)	2-Amino-4-chlorophenol (CAS No. 95-85-2)	*ortho*-Phenylenediamine dihydrochloride (CAS No. 615-28-1)	*ortho*-Phenylenediamine (CAS No. 95-54-5)	*N*,*N*-Dimethylacetamide (CAS No. 127-19-5)
1. Is electrophilic or can be metabolically activated (2)	0 out of 1	0 out of 2	NT	0 out of 1	0 out of 1	0 out of 1	0 out of 2	0 out of 2
2. Is genotoxic (12)	0 out of 7	0 out of 7	0 out of 1	0 out of 6	5 out of 6	2 out of 6	4 out of 9	0 out of 7
4. Induces epigenetic alterations (5)	0 out of 4	0 out of 4	0 out of 4	NT	NT	NT	3 out of 4	0 out of 4
5. Induces oxidative stress (16)	0 out of 7	0 out of 12	0 out of 4	1 out of 3	1 out of 3	1 out of 3	5 out of 15	1 out of 7
6. Induces chronic inflammation (47)	0 out of 2	0 out of 47	0 out of 1	0 out of 1	0 out of 1	0 out of 1	1 out of 47	0 out of 2
8. Modulates receptor-mediated effects (98)	1 out of 58	0 out of 79	3 out of 36	0 out of 22	4 out of 22	4 out of 22	8 out of 79	1 out of 78
10. Alters cell proliferation, cell death, or nutrient supply (119)	0 out of 29	1 out of 52	0 out of 8	1 out of 21	1 out of 21	1 out of 21	9 out of 69	1 out of 29
Total hits out of total number of assays evaluated	1 out of 108	1 out of 203	3 out of 54	2 out of 54	11 out of 54	8 out of 54	31 out of 225	3 out of 152

CAS No., Chemical Abstracts Service number; NT, not tested; Tox21, Toxicology in the 21st Century programme; ToxCast, Toxicity Forecaster programme

[a] No data on high-throughput screening were available for *para*-nitroanisole

[b] Seven of the 10 key characteristics have mapped high-throughput screening assay end-points as described by Chiu et al. (2018). The mapping file with findings for agents considered in Volume 123 is available in the supplementary material (Annex 1). No assay end-points in ToxCast or Tox21 were determined to be applicable to the evaluation of three key characteristics, namely: alters DNA repair or causes genomic instability, is immunosuppressive, and causes immortalization

Table 4.5 Summary of activity of key metabolites of agents reviewed in *IARC Monographs* Volume 123 and tested in ToxCast and/or Tox21 high-throughput screening assays

Key characteristic (total no. of assays mapped to characteristic)[a]	Number of positive results out of the number of assays tested			
	2-Chloroaniline (CAS No. 95-51-2)	4-Chloroaniline (CAS No. 106-47-8)	*para*-Nitrophenol (CAS No. 100-02-7)	Acetamide (CAS No. 60-35-5)
1. Is electrophilic or can be metabolically activated (2)	0 out of 1	0 out of 2	0 out of 2	0 out of 2
2. Is genotoxic (12)	0 out of 6	0 out of 9	1 out of 9	0 out of 9
4. Induces epigenetic alterations (5)	NT	0 out of 4	0 out of 4	0 out of 4
5. Induces oxidative stress (16)	0 out of 3	0 out of 15	0 out of 15	0 out of 15
6. Induces chronic inflammation (47)	0 out of 1	0 out of 47	0 out of 47	0 out of 47
8. Modulates receptor-mediated effects (98)	0 out of 22	2 out of 76	2 out of 79	1 out of 79
10. Alters cell proliferation, cell death, or nutrient supply (119)	0 out of 21	3 out of 69	1 out of 61	2 out of 68
Total hits out of total number of assays evaluated	0 out of 54	5 out of 222	4 out of 217	3 out of 224

CAS No., Chemical Abstracts Service number; NT, not tested; Tox21, Toxicology in the 21st Century programme; ToxCast, Toxicity Forecaster programme

[a] Seven of the 10 key characteristics have mapped high-throughput screening assay end-points as described by Chiu et al. (2018). The mapping file with findings for agents considered in Volume 123 is available in the supplementary material (Annex 1). No assay end-points in ToxCast or Tox21 were determined to be applicable to the evaluation of three key characteristics, namely: alters DNA repair or causes genomic instability, is immunosuppressive, and causes immortalization

1. *Is electrophilic or can be metabolically activated*: 2 assay end-points consisting of cytochrome P450 biochemical activity assays, including aromatase.
2. *Is genotoxic*: 12 assay end-points consisting of assays for induction of cellular TP53 protein and DNA repair-sensitive cellular assays. These assay end-points do not directly assess genotoxicity, but rather the downstream effects of DNA damage.
3. *Alters DNA repair or causes genomic instability*: 0 assay end-points.
4. *Induces epigenetic alterations*: 5 assay end-points including biochemical assays targeting histone deacetylases and other enzymes modifying chromatin, as well as cellular transcription factor assays involved in epigenetic regulation.
5. *Induces oxidative stress*: 16 assay end-points, all cellular assays targeting nuclear factor-like 2 and/or the antioxidant response element (ARE), other stress-related transcription factors, and protein upregulation in response to reactive oxygen species.
6. *Induces chronic inflammation*: 47 assay end-points measuring protein expression levels in primary human cells in complex environments.
7. *Is immunosuppressive*: 0 assay end-points.
8. *Modulates receptor-mediated effects*: 98 assay end-points targeting nuclear receptors (including aryl hydrocarbon receptor) in cellular assays for transactivation, receptor dimerization, and nuclear translocation, as well as biochemical radioligand binding assays and coregulatory recruitment assays.
9. *Causes immortalization*: 0 assay end-points.
10. *Alters cell proliferation, cell death, or nutrient supply*: 119 assay end-points measuring cytotoxicity by a wide variety of assay formats in cell lines, primary human cells, and developing zebrafish larvae.

4.4.2 Outcomes for chemicals tested

(a) 2-Chloronitrobenzene

For 2-chloronitrobenzene (CAS No. 88-73-3), results were available for 108 assay end-points of the 299 assay end-points mapped to key characteristics. 2-Chloronitrobenzene was considered active in only 1 of the 108 assays, an assay that assessed binding to the human retinoid X receptor β transcription factor in the HepG2 human liver cell line. In addition, a key metabolite of 2-chloronitrobenzene, 2-chloroaniline (CAS No. 95-51-2), was not positive in any of the 54 assay end-points evaluated.

(b) 4-Chloronitrobenzene

4-Chloronitrobenzene (CAS No. 100-00-5) was evaluated in 203 of the assay end-points mapped to key characteristics, and was only considered active in 1 assay end-point mapped to the "Alters cell proliferation, cell death, or nutrient supply" key characteristic. This assay is cell based and measures viability based on adenosine triphosphate (ATP) content in a human intestinal cell line (HCT116). 4-Chloroaniline (CAS No. 106-47-8), a key metabolite of 4-chloronitrobenzene, was active in 5 of the 222 assay end-points evaluated. Three assays of proliferative effects mapped to "Alters cell proliferation, cell death, or nutrient supply" and 2 evaluating aryl hydrocarbon receptor (AhR) agonist activity and estrogen receptor α antagonism activity mapped to "Modulates receptor-mediated effects".

(c) 1,4-Dichloro-2-nitrobenzene

1,4-Dichloro-2-nitrobenzene (CAS No. 89-61-2) was considered active in 3 of the 54 assay end-points evaluated. Concerning "Modulates receptor-mediated effects", the assay end-points assessed induction of reporter transcripts for the human aryl hydrocarbon receptor response element (AhRE, responsive to AhR), human nuclear receptor subfamily 1, group I, member 2 response element (PXRE, responsive to NR1I2);

and human peroxisome proliferator-activated receptor gamma (PPARγ) transcription factors in the HepG2 human liver cell line. Induction of PPARγ activity was the most sensitive target for 1,4-dichloro-2-nitrobenzene.

(d) 2,4-Dichloro-1-nitrobenzene

2,4-Dichloro-1-nitrobenzene (CAS No. 611-06-3) has been assessed in 54 of the assay end-points mapped to the key characteristics of carcinogens, and was considered active in 2 of those assay end-points: Tox21_ARE_BLA agonist assay end-point mapped to the "Induces oxidative stress" key characteristic; and TP53 viability assay end-point mapped to the "Alters cell proliferation, cell death, or nutrient supply" key characteristic. The former assay is a cell-based transcription response element reporter assay regulated by the human nuclear factor erythroid 2-like factor 2 (NFE2L2) changes in the human liver HepG2 cell line, and the latter assay is cell-based and measures viability based on ATP content in a human intestinal cell line (HCT116).

(e) 2-Amino-4-chlorophenol

2-Amino-4-chlorophenol (CAS No. 95-85-2) was considered active in 11 of the 54 assay end-points assessed and mapped to the key characteristics of carcinogens. It was considered active in 5 of the 6 assay end-points evaluated, and mapped to the "Is genotoxic" key characteristic. These 5 assay end-points are cell based using the human intestinal cell line HCT116, and are designed to measure changes in transcriptional gene expression as a result of modulation of TP53. Activity was seen at non-cytotoxic concentrations. 2-Amino-4-chlorophenol was considered active in the Tox21_ARE_BLA agonist assay end-point mapped to the "Induces oxidative stress" key characteristic. 2-Amino-4-chlorophenol was also considered active in 4 assay end-points mapped to the "Modulates receptor-mediated effects" key characteristic and 1 assay end-point mapped to the "Alters cell proliferation, cell death, or nutrient supply" key characteristic. The 4 receptor-mediated assay end-points include: (i) transcription as a result of antagonist activity regulated by the human androgen receptor (AR) in the human kidney cell line HEK293T; (ii) transcription as a result of antagonist activity regulated by the human AR in the human breast cell line MDAKB2; (iii) transcription as a result of antagonist activity regulated by the human thyroid hormone receptor α and β (THRA and THRB) in the rat pituitary gland cell line GH3; and (iv) transcription as a result of agonist activity regulated by the human AhR in the human liver cell line HepG2. The Toxicity Testing in the 21st Century (Tox21) matrix metalloproteinases (MMP) ratio assay end-point is mapped to the "Alters cell proliferation, cell death, and nutrient supply", and assesses mitochondrial toxicity by measuring mitochondrial membrane potential in the human liver cell line HepG2.

(f) ortho-Phenylenediamine dihydrochloride and ortho-phenylenediamine

ortho-Phenylenediamine dihydrochloride (CAS No. 615-28-1) was considered active in 8 of the 54 assay end-points evaluated, and *ortho*-phenylenediamine (CAS No. 95-54-5) was active in 31 of the 225 assay end-points evaluated and mapped to key characteristics. Activity was seen in the absence of cytotoxicity for both *ortho*-phenylenediamine dihydrochloride and *ortho*-phenylenediamine in all mapped assays. For both compounds, there was a quality control concern identified by the testing laboratory.

ortho-Phenylenediamine was considered active in 4 out of the 9 assay end-points under "Is genotoxic", and *ortho*-phenylenediamine dihydrochloride was considered active in 2 assay end-points mapped to the "Is genotoxic" key characteristic. End-points that were active measure transcription regulated by the human ATPase family AAA domain containing 5

(ATAD5) agonist activity in human kidney cell line (HEK293T), and activation and transcription regulated by TP53. *ortho*-Phenylenediamine was considered active in 3 of the 4 assay end-points mapped to "Induces epigenetic alterations". These assay end-points evaluate transcription regulated by Pax6, Sp1, and Sox transcription factors. Consistent with three other nitrobenzenes evaluated, *ortho*-phenylenediamine and *ortho*-phenylenediamine hydrochloride were considered active in the ARE agonist assay end-point in the "Induces oxidative stress" key characteristic, measuring transcription regulated by the human nuclear factor erythroid 2-like factor 2 (NFE2L2) in the human liver cell line (HepG2). Further, *ortho*-phenylenediamine was active in 5 additional assay end-points mapped to "Induces oxidative stress", including assays evaluating oxidative stress and kinase activation in HepG2 cells, and transcription regulated via HIF1α, MRE, and NRF2. *ortho*-Phenylenediamine was considered active in 1 out of 47 assay end-points mapped to the "Induces chronic inflammation" key characteristic. This assay quantifies the level of chemokine (C-C motif) ligand 2 protein (CCL2) as indicative of proinflammation.

ortho-Phenylenediamine dihydrochloride and *ortho*-phenylenediamine were also considered active in 4 and 8 assay end-points, respectively, under the "Modulates receptor-mediated effects" key characteristic. These assays considered transcription regulated by agonist activity of the human AR in the human breast cell line MDAKB2, transcription regulated by human estrogen receptor 1 antagonist activity (ESR1) in the human kidney cell line HEK293T, transcription regulated by human AHR agonist activity in human liver cell line HepG2, and transcription regulated by antagonist activity of the human nuclear receptor subfamily 1, group H, member 4 (NR1H4) in a human kidney cell line (HEK293T). Transcription regulated via additional nuclear receptors, including retinoic acid receptor, vitamin D receptor, and PPAR, were also modulated by *ortho*-phenylenediamine. *ortho*-Phenylenediamine was also active in 9 additional assay end-points, including those that measure mitochondrial membrane potential, cell loss, and mitotic arrest in HepG2 and fibroblast cells, and transcriptional regulation via transcription factors related to cell death and proliferation.

(g) para-*Nitroanisole*

para-Nitroanisole (CAS No. 100-17-4) has not been evaluated in HTS assays; however, there are HTS data available for the key metabolite *para*-nitrophenol (CAS No. 100-02-07). *para*-Nitrophenol was active in 4 out of 217 assay end-points mapped to key characteristics: 1 in "Is genotoxic" assessing TP53-mediated transcription; 2 in "Modulates receptor-mediated effects" assessing androgen-receptor agonist activity; and 1 in "Alters cell proliferation, cell death, or nutrient supply" (MMP ratio assay).

(h) N,N-*Dimethylacetamide*

N,*N*-Dimethylacetamide (CAS No. 127-19-5) was considered active in 3 of the 152 assay end-points evaluated and mapped to key characteristics. Similar to three of the other nitrobenzenes evaluated in this report, it was considered active in the ARE agonist assay end-point under "Induces oxidative stress". *N*,*N*-Dimethylacetamide was active in the human estrogen receptor 1 responsive cell growth assay end-point in the human breast cell line T47DF. This assay end-point is mapped to the "Modulates receptor-mediated effects" key characteristic. Finally, *N*,*N*-dimethylacetamide was active in a viability assay in the human HepG2 liver cell line mapped to the "Alters cell proliferation, cell death, or nutrient supply" key characteristic. A key metabolite, acetamide (CAS No. 60-35-5), was considered active in 3 out of the 224 assay end-points evaluated: 1 assessing modulation of PPARγ (mapped to "Modulates receptor-mediated effects"); and 2 assay end-points assessing

cell loss in HepG2 cells and modulation of transforming growth factor-β family (TGFβ), which are mapped to "Alters cell proliferation, cell death, or nutrient supply".

4.4.3 Overall considerations

In summary, there are gaps in coverage in the HTS data with respect to the key characteristics of carcinogens. In addition, with the exception of chemicals relevant to the monographs on 2-amino-4-chlorophenol and on *ortho*-phenylenediamine and *ortho*-phenylenediamine dihydrochloride, all other compounds in the present volume and their key metabolites were active for few (< 10%) HTS assay end-points in the Toxicity Forecaster (ToxCast) and/or Toxicology in the 21st Century (Tox21) programmes mapped to key characteristics in which they had been tested. The HTS data are therefore generally consistent with the conclusions of *weak* or *moderate* evidence for these compounds. 2-Amino-4-chlorophenol was active in 11 of 54 assay end-points. *ortho*-Phenylenediamine and *ortho*-phenylenediamine dihydrochloride were active in 31 of 225 and 8 of 54 assay end-points, respectively; however, confidence in the HTS data available for *ortho*-phenylenediamine was decreased as a result of the quality control concern identified by the testing laboratory.

For the present volume of the *IARC Monographs*, the key characteristics mapping file is provided as supplementary material (see Annex 1, available from http://publications.iarc.fr/584). When comparing with the previous volumes of the *IARC Monographs* that include HTS data, it is important to note that the assays mapped to each key characteristic have been refined as additional assays have been added and as the relevance of individual assays to key characteristics has been evaluated. All ToxCast/Tox21 data were downloaded from the EPA Dashboard V2 (https://comptox.epa.gov/dashboard) during 4–16 June 2018 for primary compounds and 9–11 October 2018 for metabolites and *ortho*-phenylenediamine.

5. Summary of Data Reported

5.1 Exposure data

2-Chloronitrobenzene is a chemical with a high production volume that is manufactured worldwide. United States annual production has decreased significantly over time (from 23–45 thousand tonnes during 1986–2002 to < 11 tonnes during 2012–2015); similarly, recent European production volumes are low. Data on current production volumes for regions outside of Europe and the USA were not available.

2-Chloronitrobenzene is used as an intermediate in the synthesis of colorants and chemicals; downstream uses include lumber preservatives, corrosion inhibitors, pigments, and agricultural chemicals.

Although not known to occur naturally, 2-chloronitrobenzene can be released to the environment as a by-product of production or manufacturing; release may also occur during transport, storage, or disposal, or accidentally. It has been detected in water and is moderately persistent in the environment; 2-chloronitrobenzene has also been detected at low levels in edible fish.

Occupational exposure is expected to occur primarily through inhalation in workplaces where 2-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 2-chloronitrobenzene have been measured in workplace air in chemical factories in China and Japan.

Quantitative information on exposure in the general population was not available.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

2-Chloronitrobenzene was tested for carcinogenicity in well-conducted good laboratory practice (GLP) studies of oral exposure by diet in the same laboratory, one in male and female mice and one in male and female rats. In limited studies of oral exposure by diet in another laboratory, 2-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, 2-chloronitrobenzene induced a significant positive trend and a significant increase in the incidence of: hepatocellular adenoma; hepatocellular carcinoma; hepatoblastoma; and hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined). In the limited study of oral exposure by diet in male mice, 2-chloronitrobenzene induced a significant increase in the incidence of hepatocellular carcinoma.

In the GLP study of oral exposure by diet in female mice, 2-chloronitrobenzene induced a significant positive trend and a significant increase in the incidence of: hepatocellular adenoma; hepatocellular carcinoma; hepatoblastoma; and hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined). In the limited study of oral exposure by diet in female mice, 2-chloronitrobenzene induced a significant increase in the incidence of hepatocellular carcinoma.

In the GLP study of oral exposure by diet in male rats, 2-chloronitrobenzene induced a significant positive trend in the incidence of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or hepatocellular carcinoma (combined); there was also a significant increase in the incidence of hepatocellular adenoma or hepatocellular carcinoma (combined). In the same study, there was a significant positive trend in the incidence of renal cell carcinoma in male rats. In the limited study in male rats, 2-chloronitrobenzene induced a significant increase in the number of rats bearing multiple tumours (of unspecified type).

In the GLP study of oral exposure by diet in female rats, 2-chloronitrobenzene induced a significant positive trend in the incidence of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or hepatocellular carcinoma (combined). 2-Chloronitrobenene also induced a significant increase in the incidence of hepatocellular adenoma, and hepatocellular adenoma or carcinoma (combined); the incidence of hepatocellular carcinoma in the group exposed at the highest dose exceeded the historical control range.

5.4 Mechanistic and other relevant data

In one occupational study, adducts of haemoglobin and the reactive metabolite *N*-hydroxy-2-chloroaniline were detected, but no other human data on absorption, distribution, metabolism or excretion were available. In rats, dermally and orally administered 2-chloronitrobenzene is absorbed, widely distributed to tissues, and excreted as metabolites in urine and faeces. In one study in female rabbits, the primary metabolites excreted were 2-chloroaniline and phenolic derivatives.

Concerning the key characteristics of carcinogens, there is *moderate* evidence that 2-chloronitrobenzene induces oxidative stress. No data in humans were available. In mice and rats, 2-chloronitrobenzene increases methaemoglobin and causes oxidative damage to erythrocytes in subchronic and chronic exposure studies.

There is *moderate* evidence that 2-chloronitrobenzene alters cell proliferation, cell death, or nutrient supply in rodents. No data in humans were available. In multiple studies in mice and rats of different duration up to 2 years, regenerative haematopoietic proliferation, primarily erythropoiesis, occurs in multiple tissues, primarily the spleen. Rat renal and splenic hyperplasia was seen in subchronic and chronic studies.

There is *weak* evidence (scarcity of data) that 2-chloronitrobenzene induces chronic inflammation in the liver in mice.

There is *weak* evidence that 2-chloronitrobenzene is genotoxic. The only study in humans showed a non-statistically significant increase in chromosomal aberrations in workers exposed to various chloronitrobenzenes including 2-chloronitrobenzene. In Swiss mice, DNA single-strand breaks were seen in the liver, kidney, and brain. In Chinese hamster cells, 2-chloronitrobenzene increased sister-chromatid exchanges and chromosomal aberrations in some tests but not others. Several mutagenicity tests in multiple strains of *Salmonella typhimurium* gave largely but not entirely negative results, with or without metabolic activation. 2-Chloroaniline gave inconsistent results in a variety of genotoxicity assays.

Exposure of rodents to 2-chloronitrobenzene resulted in toxicity in the liver, kidney, haematopoietic system, and male reproductive tract.

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of 2-chloronitrobenzene.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 2-chloronitrobenzene.

6.3 Overall evaluation

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

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