2-NITROTOLUENE

2-Nitrotoluene was considered by a previous IARC Working Group in 1995 (IARC, 1996). Since that time new data have become available, which have been incorporated in this *Monograph,* and taken into consideration in the present evaluation.

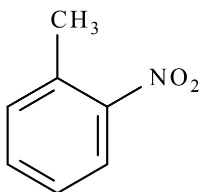
1. Exposure Data

1.1 Chemical and physical data

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 88-72-2
Chem. Abstr. Name:
1-Methyl-2-nitrobenzene
IUPAC Systematic Name:
1-methyl-2-nitrobenzene
Synonyms: 2-Methylnitrobenzene; 2-methyl-1-nitrobenzene; *ortho*-methyl-nitrobenzene; *ortho*-mononitrotoluene; *ortho*-nitrotoluene; 2-nitrotoluol; *ortho*-nitrotoluol
EINECS No.: 201-853-3

1.1.2 Structural and molecular formulae and relative molecular mass



$C_7H_7NO_2$
Relative molecular mass: 137.14

1.1.3 Chemical and physical properties of the pure substance

Description: Yellowish liquid at ambient temperatures (O'Neil *et al.*, 2006)
Boiling-point: 220.4 °C (O'Neil *et al.*, 2006)
Melting-point: -9.3 °C (O'Neil *et al.*, 2006)
Density: 1.162 at 15 °C (O'Neil *et al.*, 2006)
Spectroscopy data: Infrared (prism [4692], grating [437]), ultraviolet (UV) [1292], nuclear magnetic resonance (proton [676], C-13 [857]) and mass spectral data have been reported (Sadtler Research Laboratories, 1980)
Solubility: Slightly soluble in water (652 mg/L at 30 °C); soluble in benzene, diethyl ether, ethanol and petroleum ether (O'Neil *et al.*, 2006)
Volatility: Vapour pressure, 0.188 mm Hg at 20 °C; relative vapour density (air = 1), 4.72 (IARC, 1996; HSDB, 2004)
Stability: Combustible when exposed to heat or open flame; potentially reacts explosively with alkali (Sax & Lewis, 1989)
Octanol/water partition coefficient (P): log P, 2.30 (O'Neil *et al.*, 2006)
Conversion factor: $mg/m^3 = 5.6 \times ppm$ (calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming

a temperature of 25 °C and pressure of 101 kPa.

1.1.4 Technical products and impurities

2-Nitrotoluene is available commercially at a purity of ≥ 99.5%, and containing 3-nitrotoluene (0.2%) and 4-nitrotoluene (0.01%) as typical impurities (European Commission, 2008).

1.1.5 Analysis

Levels of 2-nitrotoluene can be determined in workplace air by drawing the air sample through a solid sorbent tube containing silica gel, desorbing with methanol and analysing with gas chromatography and flame ionization detection. The range of detection for this method is 1.97–9.86 mg/m^3 for a 30-L air sample (NIOSH, 1998).

2-Nitrotoluene can be measured in surface or groundwater by pre-concentration through salting-out extraction with acetonitrile and sodium chloride and analysis with high performance liquid chromatography with dual wavelength UV detection (US EPA, 2006). It can also be determined in water and wastewater by homogeneous liquid–liquid extraction or dispersive liquid–liquid micro-extraction and analysis with gas chromatography with flame ionization detection (Ebrahimzadeh *et al.*, 2007, 2009).

1.2 Production and use

1.2.1 Production

Nitrotoluenes are produced by the nitration of toluene with an aqueous acidic mixture of sulfuric acid and nitric acid at an initial temperature of 25 °C that is slowly raised to 35–40 °C. The resulting product contains 55–60% 2-nitrotoluene, 3–4% *meta*-nitrotoluene and 35–40% *para*-nitrotoluene. The isomers can be separated by a combination of fractional distillation and crystallization (Dugal, 2005).

In 1993, the production of mononitrotoluenes in the United States of America was 26 000 tonnes, of which approximately 16 120 tonnes were the *ortho* isomer, 780 tonnes were the *meta* isomer and 9100 tonnes were the *para* isomer (Dugal, 2005). In 1984, the yearly production capacity for mononitrotoluenes (all isomers) in the western world was approximately 200 000 tonnes (Booth, 1991). 2-Nitrotoluene is listed as a high production volume chemical, and, according to data submitted by companies under the Inventory Update Rule, production of 2-nitrotoluene in the USA was between 10 million and 50 million pounds [4.5 million and 22.7 million tonnes] for every 4-year reporting period between 1986 and 2002 (US EPA, 2004).

2-Nitrotoluene is produced by 10 companies in the USA, five companies in the People's Republic of China, three companies in the United Kingdom, two companies each in Canada and China, Hong Kong Special Administrative Region, and one company each in the Czech Republic, Germany, India, Italy, Japan and Switzerland (Chemical Sources International, 2010). Other sources indicated that 2-nitrotoluene was produced by one company in the USA (HSDB, 2004), four companies in Germany and one company each in Belgium, Italy, Spain and the United Kingdom (IUCLID, 2000).

1.2.2 Use

2-Nitrotoluene is primarily used in the production of derivatives, including *ortho*-toluidine, 2-amino-4-chlorotoluene, 2-amino-6-chlorotoluene and *ortho*-toluidine-4-sulfonic acid, which are intermediates in the production of various azo dyes (IARC, 1996). It is also used in the manufacture of (or manufacture of intermediates for) other dyes, such as magenta, sulfur dyes, rubber chemicals, agricultural chemicals and explosives (HSDB, 2004).

Table 1.1 Estimated occupational exposure to 2-nitrotoluene

Route	Work activity	Exposure	Comment
Inhalation	All	2.85–5.61 mg/m^3	1 h
		0.35–0.7 mg/m^3	8-h TWA
Dermal	Sampling	0–21 mg/d	Exposed area of 210 cm^2
	Filling and emptying tanks	420 mg/d	Exposed area of 420 cm^2
	Maintenance	0–840 mg/d	840 cm^2
	All	420 mg/d	Reasonable worst case

d, day or days; h, hour or hours; TWA, time-weighted average
From European Commission (2008)

1.3 Occurrence

1.3.1 Natural occurrence

ortho-Nitrotoluene is not known to occur as a natural product.

1.3.2 Occupational exposure

Occupational exposure to 2-nitrotoluene may occur during its production and use. The major routes of exposure are inhalation and dermal contact (European Commission, 2008). It is estimated that approximately 150 workers are potentially exposed to 2-nitrotoluene in the European Union (EU; European Commission, 2008).

According to information on manufacturing firms in the EU, 2-nitrotoluene is typically produced and used in a closed system and is usually processed within the manufacturing plants (European Commission, 2008). Exposure to 2-nitrotoluene may occur during sampling, loading and unloading tanks and drums and maintenance. Occupational monitoring data for exposure to 2-nitrotoluene are available from the major producers for the period 1996–2000. 2-Nitrotoluene was measured in personal air samplers for a full shift. For manufacturing workers, mean concentrations were 0.057 mg/m^3 for distillation activities in 1991–2000 and 0.075 mg/m^3 for nitration activities in 1996–99. Workers involved in the processing of 2-nitrotoluene in 1995–2000 were exposed to mean levels of 0.050–0.062 mg/m^3, and those involved in filling drums or tanks in 1997–99 were exposed to mean levels of 0.030–0.052 mg/m^3. Mean short-term occupational exposure measurements ranged from 0.109 to 0.250 mg/m^3 for processing activities. Levels of occupational exposure to 2-nitrotoluene ranged from 0.01 to 0.0545 mg/m^3 (19 samples) at a company where it was used to produce dinitrotoluenes. The EU noted that detailed information on work activities, sampling and analytical methods were not reported, but estimated that the highest value for a shift (0.280 mg/m^3) could be regarded as a reasonable worst-case exposure level. Based on these data and information on sampling and maintenance activities (Table 1.1), inhalation exposure (8-hour time-weighted average) was estimated to range from 0.35 to 0.7 mg/m^3 for all activities. Dermal exposure was estimated at 420 mg per day; the highest exposures were deemed to occur during maintenance activities (Table 1.1).

2-Nitrotoluene was detected in the ambient air of a chemical manufacturing plant in New Jersey (USA) at a concentration of 47 ng/m^3, and at concentrations up to 2 mg/m^3 in the nitrotoluene production area of a chemical plant that produced pharmaceuticals and explosives (IARC, 1996). Annual average predicted levels in the air obtained from data on emissions reported by industry at three sites in Italy were below 0.01 mg/m^3 (European Commission, 2008). The

Table 1.2 Estimates of releases of 2-nitrotoluene from production and processing

Location	Air (kg per day)	Wastewater (kg per day)
Local		
Site A	0.07[a]	0.57[a]
Site B	0.125[b]	0.015
Site C	1.64[b]	1.08
Regional[c]	1.35	0.978
Continental[d]	1.05	0.758

[a] Calculated from data in kilograms per year, assuming 365 days of production and processing
[b] No data received; estimate based on general default emission factors
[c] Model used emission and production data from Site C, the largest (worst case) source
[d] Total amount used and produced in the European Union minus the regional data
From European Commission (2008)

mean 8-hour time-weighted average at a Chinese plant that manufactured di- and trinitrotoluenes was 0.75 mg/m^3 (range, undetected–4.29 mg/m^3) (Jones *et al.*, 2005a). [The Working Group noted the lack of data on worldwide occupational exposure.]

1.3.3 Environmental occurrence

(a) Releases

2-Nitrotoluene can be released into the environment (primarily into air and water) during its manufacture and use. It may also be formed from the degradation of di- or trinitrotoluenes and can be released into the environment from di- or trinitrotoluenes-manufacturing plants or industries that use these chemicals, such as munitions-production facilities.

Data on releases into the air and water from 2-nitrotoluene production and processing were obtained from three industrial sites by the European Union, and were used to estimate regional and continental releases (Table 1.2). Releases into the air at the three sites ranged from 0.07 to 1.64 kg per day, and those into water ranged from 0.015 to 1.08 kg per day. The data submitted by industry showed that no sewage sludge from industrial sewage treatment plants was spread at two sites, and that the sludge was sent off-site for composting before being spread at the third site. Modelling using worst-case values predicted emissions to the soil of 1.35 kg per day at the regional scale and 1.05 kg per day at the continental scale.

(b) Ambient air

HSDB (2004) and the European Commission (2008) reviewed information and calculated parameters related to the environmental fate of nitrotoluene in ambient air, water and soil. 2-Nitrotoluene released into the air is expected to exist entirely in the vapour phase and is degraded in the atmosphere by a reaction with photochemically produced hydroxyl radicals, with an estimated atmospheric half-life of 23 days (European Commission, 2008) or 42 days (HSDB, 2004). It may also be degraded by photolysis; the major products formed are 2-methyl-6-nitrophenol and 2-methyl-4-nitrophenol (European Commission, 2008).

Limited environmental monitoring data are available. 2-Nitrotoluene was detected in the ambient air in Japan at a concentration of 44 μg/m^3 (European Commission, 2008). In the USA, it was detected at 0.03 and 0.9 ng/m^3 in two ambient air samples collected in Boise, ID, in the winter of 1986–87 and at 0.047 μg/m^3 in ambient air at a manufacturing plant in New Jersey (Pellizzari, 1978; NTP, 2008). [The Working

Table 1.3 Environmental occurrence of 2-nitrotoluene in effluents, wastewater or surface water near industrial sites

Location	Source	Sample	Concentration (µg/L)	Reference
Effluents and wastewater				
India	Nitrotoluene-manufacturing plant	Wastewater – acid Wastewater – alkaline	87 000–102 000 53 000–80 000	Swaminathan *et al.* (1987)
Radford, VA, USA	Trinitrotoluene-manufacturing plant	Effluent	320–16 000	Nay (1972), Howard *et al.* (1976)
USA	Production and purification of 2,4,6-trinitrotoluene	Wastewater	20–140	Spanggord *et al.* (1982a)
USA	Dinitrotoluenes manufacturing plant	Raw effluent	7800	Webb *et al.* (1973), Howard *et al.* (1976)
USA	Trinitrotoluene plant	Raw effluent	150	Webb *et al.* (1973), Howard *et al.* (1976)
NR	Paper mill	Waste-treatment lagoon	Detected	Webb *et al.* (1973), Howard *et al.* (1976)
Teheran, Islamic Republic of Iran	Research facility	Wastewater	110	Ebrahimzadeh *et al.* (2007, 2009)
Ground- or surface water near or at munitions or military sites				
Germany	Near former munitions-manufacturing plant	*Hischagen/Waldhof – surface water* 2 brooks River Losse (adjacent) *Clausthal-Zellerfeld – surface water* 2 ponds River Oder (adjacent)	 0.4; 7.4 1.2 0.4; 22 < 0.01	Feltes *et al.* (1990)

Table 1.3 (continued)

Location	Source	Sample	Concentration (µg/L)	Reference
USA	Munitions-manufacturing plants	*Groundwater*		ATSDR (2007)
		Texas	4600	
		Illinois	21 000	
		Tennessee	140 000	
		Tennessee (same plant)		
		Well-water	42 600	Best *et al.* (2001)
		Groundwater	2900	NTP (2008)
		Wisconsin (former plant)		ATSDR (2007)
		Off-site well	0.095	
		Ground-water (4/17 samples)	0.16–17[b]	
		Missouri – surface water	0.12 (max)	
USA	Former munitions plant/current nuclear weapons assembly/disassembly plant 1999–2005	Texas – groundwater[a]		Pantex (2005)
		Ogallala aquifer	ND–2.9	
		Perched aquifer	ND–5	
Massachussetts, USA	Military training facility	Groundwater	25	ATSDR (2007)

[a] Not detected every year, and not detected in 2005, last year of monitoring
[b] *ortho*-Nitrotoluene and *para*-nitrotoluene combined
ND, not detected; NR, not reported

Table 1.4 Environmental occurrence of 2-nitrotoluene in water or sediments

Country	Year	Location	Concentration (μg/L)	Reference
Surface water				
Netherlands	1972	*Waal River*		European Commission (2008)
		Brakel	3.1–16	
		Rhine River		
	1978	Maassluis	1–10	
	1978	Lobith	3–10	
	1979		1–3	
	1981–84		max., 0.8–3.0	
	1981	Gorinchem	3 (max)	
Germany		*Rhine River*		European Commission (2008)
	1987	Wiesbaden	< 0.02–0.35	
	1987	Köln	0.05–0.37	
	1987	Düsseldorf	0.08–0.46	
	1989	Lippe	≤ 2.45	
		River Elbe		
	1997	Schmika	0.15–0.20	
	NR	*River Elbe*		Feltes *et al.* (1990)
		Brunsbüttel	0.05	
		Brokdorf	0.08	
		Lauenburg	0.4	
Europe	1992–93	River Elbe	40 (mean)	Götz *et al.* (1998)
Europe	1999	River Danube	< 0.02 (90%)	European Commission (2008)
	1999	River Rhine	0.5 (90%)	
	1999	River Elbe	0.06 (max)	
China	NR	Yellow River	72–483	He *et al.* (2006)
	NR	Soughua River	150–8600	Lang *et al.* (1993)
	2006	Daliao River watershed[a] (surface water and sediment)	ND–3708	Men *et al.* (2010)

Table 1.4 (continued)

Country	Year	Location	Concentration (µg/L)	Reference
Groundwater				
France	1987	Degrémont	90–165	Duguet *et al.* (1988)
Netherlands	1974 1974	River Waal River Maas	4.5 (average)[b] 18.1 (max)[b] 0.3 (max)[b]	Meijers & van der Leer (1976)
Germany	NR	River Rhine	10	
Sediment				
Japan	1976		3.4–140	European Commission (2008)
Drinking-water				
Germany	NR	NR	Detected	Zoeteman (1980)

[a] Sampling from surface water (aqueous and particles) and sediment from 28 sites (main river and branch streams) in the watershed including the Hun River, the Taizi River and the Daliao River; some of the branches were contaminated by wastewater from various industries, including oil refining iron ore and cement and chemical engineering.
[b] Includes 2- and 4-nitrotoluene
ND, not detected; NR, not reported

Table 1.5 Estimated human daily intake of 2-nitrotoluene

Source	Regional intake (mg/kg bw per day)
Air	7390×10^{-12}
Drinking-water	$96\,700 \times 10^{-12}$
Fish	$100\,000 \times 10^{-12}$
Leaf crops	3720×10^{-12}
Root crops	8820×10^{-12}
Meat	4.44×10^{-12}
Milk	13.1×10^{-12}
Total	$217\,000 \times 10^{-12}$

From European Commission (2008)

Group noted the large differences in concentration data, which reflect the nature and location of the sampling.]

(c) Water

2-Nitrotoluene released into water is not expected to adsorb (or only slightly) to suspended solids and sediment. Because 2-nitrotoluene absorbs UV light strongly, it is susceptible to photochemical degradation. Volatilization is expected to play a minor role in its environmental fate in water and sediments. Chemical hydrolysis is not expected to be important in the removal of 2-nitrotoluene from aquatic environments, and its bioaccumulation is low. It may be removed from aquatic environments by biodegradation, but not under conditions in which acclimation of the same population of bacteria or other micro-organisms is unlikely to occur, such as surface or running waters (HSDB, 2004).

Environmental monitoring data on 2-nitrotoluene in effluents, wastewater or surface water near industrial sites are provided in Table 1.3 and those in water or sediments are given in Table 1.4. 2-Nitrotoluene was found at highest concentrations (up to 102 000 µg/L) in the wastewater of a manufacturing plant in India (Swaminathan *et al.*, 1987).

2-Nitrotoluene is a break-down product of di- and trinitrotoluenes. It was detected in the wastewater or effluent (concentrations up to 16 000 µg/L) from di- and trinitrotoluenes-manufacturing plants, in surface water near former munitions-manufacturing plants in Germany (concentrations up to 22 µg/L), in ground- (concentrations up to 140 000 µg/L) and surface water (concentrations up to 0.12 µg/L) at several munitions-manufacturing plants in the USA, in groundwater at a military training facility (maximum concentration, 25 µg/L), and in wastewater from a paper mill (40 µg/L) and from a research facility (181 µg/L) (Table 1.3).

Numerous studies have detected 2-nitrotoluene in rivers, namely in the River Waal, the River Rhine, the River Maas and the River Elbe (Table 1.4). Most, but not all, of the reported values were below 1 µg/L; one study reported a mean concentration of 40 µg/L in samples from the River Elbe taken in Germany (Götz *et al.*, 1998). Much higher levels (ranging up to 8600 µg/L) have been reported in rivers in China (Men *et al.*, 2010). 2-Nitrotoluene was also detected in groundwater samples taken in France (Duguet *et al.*, 1988) and in drinking-water in Germany (Kool *et al.*, 1982).

(d) Soil

In soil, 2-nitrotoluene is predicted to have moderate mobility based on an estimated soil absorption coefficient of 420. Volatilization of 2-nitrotoluene is expected from moist soil (based on a Henry' Law constant of 1.25×10^{-5} atm.m^3/mol) but not from dry soil (based on its vapour pressure of 0.188 mm Hg) (HSDB, 2004). However, it is unlikely to be removed from the soil by oxidation, chemical hydrolysis or biodegradation. The European Commission (2008) estimated that the half-lives for biodegradation were 300 days in soil and 3014 days in sediment.

2-Nitrotoluene is suspected to be a pollutant at munitions sites, but no data are available. It was found at a concentration of 1.4 ppm in soil contaminated with trinitrotoluene at a historical military testing site (Radtke *et al.*, 2002).

Table 1.6 Occupational exposure limits and guidelines for 2-nitrotoluene

Country	Year	Concentration (mg/m^3)	Interpretation
Argentina[a]	2007	11 (sk)	TWA
Australia	2008	11 (sk)	TWA
Austria	2006	0.5	TRK
Belgium	2002	11 (sk)	TWA
Bulgaria[a]	2007	11 (sk)	TWA
Colombia[a]	2007	11 (sk)	TWA
Denmark	2002	12 (sk)	TWA
Germany	NR	5 (sk)	MAK
		10	STEL
Jordan[a]	2007	11 (sk)	TWA
Mexico	2004	30	TWA
		60	STEL
New Zealand	2002	11 (sk)	TWA
Norway	1999	5.5	TWA
Republic of Korea	2006	11 (sk)	TWA
Singapore[a]	2007	11 (sk)	TWA
Sweden	2005	6	TWA
		11 (sk)	STEL
United Kingdom	2000	30 (sk)	TWA
		60	STEL
USA			
ACGIH (TLV)	2007	11 (sk)	TWA
		60 (sk)	STEL
OSHA (PEL)	1994	30 (sk)	TWA
NIOSH (REL)	1992	11 (sk)	TWA
Viet Nam[a]	2007	11 (sk)	TWA

[a] These countries follow the recommendations of the ACGIH threshold limit values.
ACGIH, American Conference of Governmental Industrial Hygienists; MAK, Maximale Arbeitsplatz-Konzentration; NIOSH, National Institute for Occupational Safety and Health; OSHA, Occupational Safety and Health Administration; PEL, permissible exposure limit; REL, recommended exposure limit; sk, absorption through the skin may be a significant source of exposure; STEL, short-term exposure limit; TLV, threshold limit value; TRK, Technische Richtkonzentrationen; TWA, time-weighted average
From IUCLID (2000); RTECS (2009); ACGIH (2010)

1.3.4 *Estimated human intake of* ortho-*nitrotoluene*

The European Commission (2008) developed a model to predict indirect exposure to 2-nitrotoluene from the environment using data collected from three industrial sites in Italy (see Section 1.3.2). The estimated daily dose for the region was 2.17×10^{-7} mg/kg body weight (bw); the estimated intakes from various other sources are provided in Table 1.5.

1.4 Regulations and guidelines

Occupational exposure limits and guidelines for 2-nitrotoluene in several countries are presented in Table 1.6.

Table 2.1 Cohort study of dyestuff workers

Study location and period	Study population	Follow-up period	Exposure assessment	Organ site	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Comments
Northern Italy 1922–70	906 male dyestuff workers	1946–76	Work histories accessed from personal and factory records; workers assigned to 10 categories related to the type of dyes that they produced	All cancers	Total cohort	96	2.65 [2.15–3.24]	National rates; workers in the total cohort exposed to benzidine and α- and β-naphthylamine; 2-nitrotoluene was used to manufacture fuchsin and safranine T.
				Bladder cancer	Total cohort	36	29.27 [20.50–40.52]	
					Fushsin and safranine T	5	62.5 [20.29–145.85]	

CI, confidence interval
From Rubino *et al.* (1982)

2. Cancer in Humans

No studies of human cancer were identified that evaluated specific exposure to 2-nitrotoluene. Occupational exposure to 2-nitrotoluene may occur during the manufacture of magenta. Although the Working Group was aware of studies that reported exposure to dinitrotoluene and trinitrotoluene, they did not specifically mention exposure to 2-nitrotoluene and were therefore not reviewed.

2.1 Cohort studies

2.1.1 Background

Magenta production is classified by IARC as *carcinogenic to humans (Group 1)*, based on *sufficient evidence* that the production of magenta causes bladder cancer in humans. IARC also concluded that there was *inadequate evidence* in humans for the carcinogenicity of magenta itself (IARC, 2010). The IARC evaluation was based on a review of one case report (Rehn, 1895), two cohort studies (Case & Pearson, 1954; Rubino *et al.*, 1982) of magenta-manufacturing workers (not exposed to 1- or 2-naphthylamine or benzidine) and one case–control study of occupational exposure to magenta (Vineis & Magnani, 1985). One of the cohort studies discussed the process used to manufacture New Magenta (New Fuchsin, a component of magenta) at a dyestuff factory in northern Italy (Rubino *et al.* 1982; IARC, 2010). In this factory, fuchsin was manufactured by two different processes, both of which involved exposure to 2-nitrotoluene. In the first process, 2-nitrotoluene was produced as an intermediate (during the conversion of toluene to *ortho*-toluidine); in the second process, it was used as a raw ingredient (Rubino *et al.*, 1982). The other studies of magenta production did not describe the manufacturing process, and are not reviewed here because it is not known whether the subjects were exposed to 2-nitrotoluene (for a review of these studies, see IARC, 2010).

2.1.2 Dyestuff manufacturing workers

See Table 2.1

Rubino *et al.* (1982) conducted a retrospective cohort study of dyestuff workers in a plant that manufactured aromatic amines and used them in azo dyes. The factory began its operations in 1922, and the manufacture of β-naphthylamine was discontinued in 1960. The cohort comprised 906 men who had worked at the plant for at a least 1 month between 1922 and 1970. Vital status (95.8% complete) was accessed using personal records and from municipal registries, and cause of death was obtained from death certificates. Deaths were observed from 1946 to 1976, and expected numbers of deaths were calculated using national rates for 1951–76. Information on exposure was obtained from personal and factory records, and workers were classified into 10 different dye-manufacturing categories, including the manufacture of α- and β-naphthylamine and benzidine, which are known bladder carcinogens. Workers involved in the manufacture of these dyes were excluded from the other dye categories and workers involved in the other manufacturing categories did not change jobs. Of the 868 men with available personal records, 53 were involved in the manufacture of fuchsin and safranine T, and were potentially exposed to 2-nitrotoluene, and other known or suspected carcinogens such as *ortho*-toluidine and 4,4’-methylenebis(2-methylaniline).

Among the total cohort, a statistically significant excess of mortality from all cancers was found (ratio of observed versus expected [standardized mortality ratio (SMR)], 2.65 [95% confidence interval (CI): 2.15–3.24]; 96 observed deaths) due primarily to cancers of the bladder (SMR, 29.27 [95%CI: 20.50–40.52]; 36 exposed deaths), but also cancers of the lung (SMR, 1.78 [95%CI: 0.97–2.98]; five exposed deaths), larynx (SMR, 3.55 [95%CI: 1.15–8.28]; five exposed deaths) and oesophagus (SMR, 4.72 [95%CI: 1.53–11.01]; 10 exposed deaths).

Among fuchsin- and safranine T-manufacturing workers potentially exposed to 2-nitrotoluene, a large excess of mortality from bladder cancer was found (SMR, 62.5 [95%CI: 20.29–145.85]; five exposed cases). Findings for other cancer sites (such as the lung, larynx and oesophagus) were not reported. [It is not possible to evaluate whether 2-nitrotoluene played a causal role in bladder cancer in this study because the workers were also exposed to *ortho*-toluidine, which causes bladder cancer in humans and is classified by IARC as *carcinogenic to humans (Group 1*, IARC, 2010).]

3. Cancer in Experimental Animals

Carcinogenicity studies of oral administration of 2-nitrotoluene in the diet to mice and rats have been conducted (NTP, 2002), the results of which are summarized in Table 3.1 (see also Dunnick *et al.*, 2003).

3.1 Oral administration

3.1.1 Mouse

Groups of 60 male and 60 female B6C3F$_1$ mice were fed diets containing 0 (controls), 1250, 2500 or 5000 ppm 2-nitrotoluene (equivalent to average daily doses of approximately 0, 165, 360 or 700 and 150, 320 or 710 mg/kg bw in males and females, respectively) for 105 weeks. All males in the 2500- and 5000-ppm groups died before the end of the study. Treated males and females had an increased incidence of haemangiosarcoma located in the skeletal muscle, subcutaneous tissue or mesentery and carcinoma of the large intestine (caecum). In treated female mice, the incidence of hepatocellular adenoma and carcinoma was increased (NTP, 2002; Dunnick *et al.*, 2003).

Table 3.1 Carcinogenicity studies of 2-nitrotoluene by oral administration in the diet to mice (2-year study) and rats (2-year study and 3-month stop-exposure study)

Species, strain (sex) Duration	Dosing regimen, Animals/group at start	Incidence of tumours	Significance (poly-3-test)
Mouse, B6C3F$_1$ (M) 2 yr	0, 1250, 2500 or 5000 ppm 60 animals/group	Circulatory system (haemangiosarcoma): 4/60, 17/60, 55/60, 60/60	$P < 0.001$ (1250 ppm) $P < 0.001$ (2500 ppm) $P < 0.001$ (5000 ppm) $P < 0.001$ (trend)
		Large intestine (caecum, carcinoma: 0/60, 12/60, 9/60, 0/60	$P < 0.001$ (1250 ppm) $P < 0.001$ (2500 ppm) $P < 0.001$ (trend)
Mouse, B6C3F$_1$ (F) 2 yr	0, 1250, 2500 or 5000 ppm 60 animals/group	Circulatory system (haemangiosarcoma): 0/60, 2/60, 3/60, 50/60	$P < 0.001$ (5000 ppm) $P < 0.001$ (trend)
		Large intestine (caecum, carcinoma): 0/60, 1/60, 4/60, 3/60	$P = 0.024$ (trend)
		Liver (hepatocellular adenoma): 7/60, 5/59, 19/59, 29/60	$P = 0.006$ (2500 ppm) $P < 0.001$ (5000 ppm) $P < 0.001$ (trend)
		Liver (hepatocellular carcinoma): 2/60, 4/59, 6/59, 16/60	$P < 0.001$ (2500 ppm) $P < 0.001$ (5000 ppm) $P = 0.024$ (trend)
		Liver (hepatocellular adenoma or carcinoma): 9/60, 9/59, 24/59, 39/60	$P < 0.001$ (2500 ppm) $P < 0.001$ (5000 ppm) $P < 0.001$ (trend)

Table 3.1 (continued)

Species, strain (sex) Duration	Dosing regimen, Animals/group at start	Incidence of tumours	Significance (poly-3-test)
Rat, F344 (M) 2 yr	0, 625, 1250 or 2000 ppm; 2000 or 5000 ppm 3 mo stop-exposure 60 animals/group	Mesothelium (malignant mesothelioma): 2/60, 20/60, 29/60, 44/60, 44/60, 54/60	$P < 0.001$ (625 ppm) $P < 0.001$ (1250 ppm) $P < 0.001$ (2000 ppm) $P < 0.001$ (trend) $P < 0.001$ (2000 ppm, stop-exposure) $P < 0.001$ (5000 ppm, stop-exposure) $P < 0.001$ (trend, stop-exposure)
		Skin (subcutaneous lipoma): 0/60, 4/60, 13/60, 13/60, 10/60, 12/60	$P = 0.041$ (625 ppm) $P < 0.001$ (1250 ppm) $P < 0.001$ (2000 ppm) $P < 0.001$ (trend) $P < 0.01$ (2000 ppm, stop-exposure) $P < 0.01$ (5000 ppm, stop-exposure) $P < 0.01$ (trend, stop-exposure)
		Skin (fibroma): 5/60, 46/60, 52/60, 59/60, 45/60, 52/60	$P < 0.001$ (625 ppm) $P < 0.001$ (1250 ppm) $P < 0.001$ (2000 ppm) $P < 0.001$ (trend) $P < 0.001$ (2000 ppm, stop-exposure) $P < 0.001$ (5000 ppm, stop-exposure) $P < 0.001$ (trend, stop-exposure)
		Skin (fibrosarcoma): 0/60, 7/60, 17/60, 20/60, 8/60, 12/60	$P < 0.001$ (625 ppm) $P < 0.001$ (1250 ppm) $P < 0.001$ (2000 ppm) $P < 0.001$ (trend) $P < 0.001$ (2000 ppm, stop-exposure) $P < 0.001$ (5000 ppm, stop-exposure) $P < 0.001$ (trend, stop-exposure)
		Skin (fibroma or fibrosarcoma): 5/60, 47/60, 55/60, 59/60, 47/60, 53/60	$P < 0.001$ (625 ppm) $P < 0.001$ (1250 ppm) $P < 0.001$ (2000 ppm) $P < 0.001$ (trend) $P < 0.001$ (2000 ppm, stop-exposure) $P < 0.001$ (5000 ppm, stop-exposure) $P < 0.001$ (trend, stop-exposure)

Table 3.1 (continued)

Species, strain (sex) Duration	Dosing regimen, Animals/group at start	Incidence of tumours	Significance (poly-3-test)
Rat, F344 (M) (contd)		Mammary gland (fibroadenoma): 0/60, 7/60, 10/60, 2/60, 13/60, 20/60	*P* = 0.004 (625 ppm) *P* < 0.001 (1250 ppm) *P* < 0.001 (trend) *P* < 0.001 (2000 ppm, stop-exposure) *P* < 0.001 (5000 ppm, stop-exposure) *P* < 0.001 (trend, stop-exposure)
		Liver (hepatocellular adenoma): 2/60, 3/60, 3/60, 7/60, 3/60, 4/60	*P* = 0.006 (2000 ppm) *P* = 0.007 (trend)
		Liver (cholangiocarcinoma): 0/60, 0/60, 0/60, 0/60, 0/60, 3/60	*P* = 0.034 (5000 ppm, stop-exposure) *P* = 0.025 (trend, stop-exposure)
		Liver (hepatocellular adenoma or carcinoma): 3/60, 3/60, 3/60, 8/60, 3/60, 6/60	*P* = 0.007 (2000 ppm) *P* = 0.009 (trend) *P* = 0.029 (5000 ppm, stop-exposure) *P* = 0.03 (trend, stop-exposure)
		Lung (alveolar/bronchiolar adenoma): 1/60, 5/60, 1/60, 2/60, 3/60, 8/60	*P* < 0.001 (5000 ppm, stop-exposure) *P* < 0.001 (trend, stop-exposure)
		Lung (alveolar/bronchiolar adenoma or carcinoma) 2/60, 5/60, 1/60, 2/60, 3/60, 11/60	*P* < 0.001 (5000 ppm, stop-exposure) *P* < 0.001 (trend, stop-exposure)
		Circulatory system (haemangioma or haemangiosarcoma): 1/60, 3/60, 1/60, 2/60, 0/60, 4/60	*P* = 0.037 (5000 ppm, stop-exposure) *P* = 0.041 (trend, stop-exposure)
Rat, F344 (F) 2 yr	0, 625, 1250 or 2000 ppm 60 animals/group	Skin (fibroma): 3/60, 3/60, 18/60, 20/60	*P* < 0.001 (1250 ppm) *P* < 0.001 (2000 ppm) *P* < 0.001 (trend)
		Skin (fibroma or fibrosarcoma): 3/60, 3/60, 21/60, 22/60	*P* < 0.001 (1250 ppm) *P* < 0.001 (2000 ppm) *P* < 0.001 (trend)
		Mammary gland (fibroadenoma): 23/60, 47/60, 52/60, 56/60	*P* < 0.001 (625 ppm) *P* < 0.001 (1250 ppm) *P* < 0.001 (2000 ppm) *P* < 0.001 (trend)
		Liver (hepatocellular adenoma): 1/60, 0/59, 1/60, 6/60	*P* = 0.048 (2000 ppm) *P* = 0.005 (trend)

F, female; M, male; mo, month or months; yr, year or years
From NTP (2002), Dunnick *et al.* (2003)

3.1.2 Rat

2-Nitrotoluene caused early tumour formation in a 13-week study in male F344/N rats fed 0, 625, 1250, 2500, 5000 or 10 000 ppm in the diet; mesothelioma of the tunica vaginalis (3/10) was observed in the 5000-ppm group (Dunnick, 1993; Dunnick *et al.*, 1994).

In a subsequent 26-week study in male F344/N rats fed 0 or 5000 ppm 2-nitrotoluene in the diet, mesothelioma of the tunica vaginalis (5/20 at 5000 ppm) and cholangiocarcinoma (2/20 at 5000 ppm) occurred after 13 weeks of treatment followed by a 13-week recovery period. In the 26-week exposure arm of this study, mesothelioma of the tunica vaginalis (7/20 at 5000 ppm) and cholangiocarcinoma (1/20 at 5000 ppm) also developed (NTP, 2000).

A 105-week feeding study was conducted in male and female F344/N rats. A stop-exposure study (exposure for 13 weeks then no further treatment until the end of the 105 weeks) was also conducted in male F344/N rats. In the main study, groups of 60 males and 60 females were fed diets containing 0 (controls), 625, 1250 or 2000 ppm 2-nitrotoluene (equivalent to average daily doses of approximately 0, 25, 50 or 90 and 0, 30, 60 or 100 mg/kg bw in males and females, respectively) for 105 weeks. In the 13-week stop-exposure study, groups of 60 male rats were fed diets containing 2000 or 5000 ppm 2-nitrotoluene (equivalent to average daily doses of approximately 125 or 315 mg/kg bw) for 13 weeks and were maintained on control diet for the remainder of the 105-week study period. The control group of 60 male rats that received untreated feed in the main study served as the control group for the stop-exposure study. All males in the 2000-ppm group in the main study, all males in the 5000-ppm stop-exposure group and all but three males in the 1250-ppm group in the main study died before the end of the 105 weeks. In the main study, 2-nitrotoluene increased the incidence of mesothelioma, skin lipoma, hepatocellular adenoma or carcinoma (combined), cholangiocarcinoma and alveolar/bronchiolar adenoma or carcinoma (combined) in males, and that of mammary gland fibroadenoma, skin fibroma and/or fibrosarcoma and hepatocellular adenoma in males and females. At the end of 2 years, the incidence of mesothelioma, skin lipoma, fibroma and fibrosarcoma, hepatocellular adenoma or carcinoma (combined), cholangiocarcinoma, alveolar/bronchiolar adenoma or carcinoma (combined), haemangioma or haemangiosarcoma (combined), and mammary gland fibroadenoma was also increased in male rats in the stop-exposure study (NTP, 2002; Dunnick *et al.*, 2003).

4. Other Relevant Data

4.1 Absorption, distribution, metabolism and excretion

4.1.1 Humans

(a) Absorption, distribution and excretion

Ahlborg *et al.* (1985) studied workers in a chemical factory that produced pharmaceuticals and explosives, including primarily trinitrotoluene but also small amounts of 2-nitrotoluene as a contaminant, and found that only those workers exposed to trinitrotoluene had urine that was mutagenic in *Salmonella typhimurium* TA98 in the absence of metabolic activation; the addition of a metabolic activation system increased the mutagenic potency of the urine, but not significantly. Only trinitrotoluene, and none of the other nitrotoluenes, induced the observed mutagenic activity. A follow-up study showed the presence of diazo-positive metabolites in the urine, but the assay method could not ascribe these to any specific compound (Ahlborg *et al.*, 1988).

(b) Metabolism

Jones *et al.* (2005a) analysed urinary metabolites in workers who manufactured dinitrotoluenes and trinitrotoluene in Liaoning, Liaoning Province, China. Nitrobenzoic acids were found in 96% and 73% of the urine samples from workers exposed to 2-nitrotoluene, and 4-nitrotoluene respectively, and air concentrations of neither dinitrotoluenes nor 2-nitrotoluene correlated with the concentrations of nitrobenzoic acids. The air concentrations of 2-nitrotoluene and 4-nitrotoluene were 759 ± 836 μg/m^3 and 685 ± 500 μg/m^3, respectively, and the concentrations (μmol/L) of the urinary metabolites were 4.25 ± 5.76 2-nitrobenzoic acid, 0.33 ± 0.65 2-nitrobenzyl alcohol, 0.12 ± 0.40 4-nitrobenzoic acid and 0.01 ± 0.03 4-nitrobenzyl alcohol (Jones *et al.*, 2005a; Sabbioni *et al.*, 2006). Thus, nitrobenzoic acids are the primary urinary metabolites in humans exposed to 2-nitrotoluene and 4-nitrotoluene.

4.1.2 Experimental systems

(a) Absorption, distribution, and excretion

Chism *et al.* (1984) collected excreta 72 hours after administration of a single oral dose (200 mg/kg bw) of radiolabeled 2-nitrotoluene to male F344 rats. The radiolabel was excreted rapidly (86% within 24 hours). The major route of excretion was urinary; 70–85% of the dose was excreted in the urine in 72 hours. Within that period, 5–13% and 0–0.1% of the dose were excreted in the faeces and in exhaled air, respectively. 2-Nitrobenzoic acid was the major metabolite excreted in the urine 72 hours after administration (29% of the dose); other metabolites identified included 2-nitrobenzyl glucuronide (14% of the dose) and *S*-(2-nitrobenzyl)-*N*-acetylcysteine (12% of the dose).

(b) Metabolism

deBethizy & Rickert (1984) incubated 2-nitrotoluene with isolated male F344 rat hepatocytes for up to 1 hour and identified the following metabolites: 2-nitrobenzyl alcohol (52%), 2-nitrobenzyl glucuronide (28%), an unidentified metabolite (20%) and 2-nitrobenzoic acid (3%). Chism & Rickert (1985) administered 200 mg/kg bw 2-nitrotoluene to male and female F344 rats; 12 hours later, males had excreted 28.6% and females had excreted 9.6% of the dose in the bile. Of the material excreted in the bile, 77% in males (22% of the dose) and 86% in females (8.3% of the dose) was 2-nitrobenzyl glucuronide. Inhibition of enterohepatic circulation by bile-duct cannulation resulted in a decrease in liver macromolecular covalent binding of 98 and 85%, respectively, in males and females, showing that enterohepatic circulation is necessary for the hepatic macromolecular covalent binding of 2-nitrotoluene in both sexes of F344 rats.

In rodents, 2-nitrotoluene undergoes oxidation and conjugation in the liver to form *ortho*-nitrobenzyl alcohol and nitrobenzyl glucuronide, which are then excreted into the intestine via the bile and converted to aminobenzyl alcohol by intestinal microflora through reduction of the nitro group. The aminobenzyl alcohol is then re-absorbed and metabolized in the liver, resulting in an electrophilic compound that can bind to hepatic DNA — possibly a nitrenium ion (Chism & Rickert, 1985) specific to 2-methylaniline. Hepatic DNA adducts and haemoglobin adducts, together with hepatic DNA damage (assessed by the unscheduled DNA synthesis assay), have been found in 2-nitrotoluene-treated rats (see Section 4.2.2a; Doolittle *et al.*, 1983; Jones & Sabbioni, 2003).

4.2 Genetic and related effects

4.2.1 Humans

(a) Haemoglobin adducts

Jones *et al.* (2005b) found a wide variety of haemoglobin adducts among workers in a factory that produced di- and trinitrotoluenes, one of which was a 2-methylaniline–haemoglobin adduct, which derives specifically and only from 2-nitrotoluene.

(b) Mutations

There were no reports of 2-nitrotoluene-induced mutations in humans. However, workers exposed to nitrotoluenes (including 2-nitrotoluene) in a factory in China had highly mutagenic urine, with up to 141.6 revertants/mL-equivalent for enzymatically hydrolysed urine (with β-glucuronidase and arylsulfatase) for the 75th percentiles in *S. typhimurium* strain YG1041 in the absence of metabolic activation (Sabbioni *et al.*, 2006). [The Working Group noted that this value is higher than that generally found in the urine of unexposed subjects (< 10 revertants/mL-equivalent)].

(c) Chromosomal effects

Sabbioni *et al.* (2006) found an increased frequency of chromosomal aberrations (including gaps) in the circulating blood lymphocytes of factory workers exposed to a variety of nitrotoluenes; the concentration of 2-nitrotoluene in air was 759 ± 836 μg/m^3. Exposure to 2-nitrotoluene resulted in the excretion of 2-nitrotoluene metabolites in the urine (see Section 4.1.1*b*).

4.2.2 Experimental systems

(a) DNA and haemoglobin adducts

A single oral dose of radiolabelled 2-nitrotoluene given to male F344 rats resulted in the covalent binding of radioactivity to liver DNA, which was maximal 12 hours after administration. Prior administration of inhibitors of sulfotransferase (SULT) markedly reduced the level of bound material, indicating that 2-nitrotoluene requires SULT for its conversion to a form that binds to liver DNA *in vivo* (Rickert *et al.*, 1984). After incubation of radiolabelled 2-aminobenzyl alcohol with calf-thymus DNA, male F344 rat hepatic cytosol and 3′-phosphoadenosine 5′-phosphosulfate, the radiolabel bound covalently to DNA; this binding was prevented by the addition of a SULT inhibitor, 2,6-dichloro-4-nitrophenol. The authors suggested that 2-aminobenzyl sulfate was probably the metabolite that bound to DNA (Chism & Rickert, 1989).

Although a single oral administration of 2-nitrotoluene by gavage to female Wistar rats did not produce any detectable DNA adducts in the liver, it did produce hydrolysable haemoglobin adducts (Jones & Sabbioni, 2003). When male WELS-Fohm rats were given 2-nitrotoluene on 5 days a week for 12 weeks, haemoglobin adducts were formed, which were identified as 2-methylaniline after mild base treatment. Hepatic DNA adducts of methylaniline at guanosine and adenosine were also formed, and there was a strong linear relationship between the frequencies of both haemoglobin and DNA adducts and dose, suggesting that haemoglobin adducts could be a useful surrogate marker for hepatic DNA adducts (Jones *et al.*, 2003).

(b) DNA damage

2-Nitrotoluene administered by gavage to male F344 rats induced unscheduled DNA synthesis, which is a measure of DNA damage. However, unscheduled DNA synthesis was not observed in female rats or germ-free male rats (Doolittle *et al.*, 1983). This study highlighted the obligatory role of intestinal bacteria in the metabolic activation of 2-nitrotoluene, most probably via nitroreductase activity provided by the gut flora, as well as sex differences in the ability of 2-nitrotoluene to cause DNA damage.

In standard cell-culture methods that include serum, 2-nitrotoluene did not induce unscheduled DNA synthesis in the spermatocytes of rats exposed *in vivo* (Working & Butterworth, 1984). Similarly, it did not induce unscheduled DNA synthesis *in vitro* in rat hepatocytes or hepatocytes from six human subjects (Butterworth *et al.*, 1989). However, in defined serum-free media, 2-nitrotoluene did induce unscheduled DNA synthesis in primary rat hepatocytes (Parton *et al.*, 1995). The positive results in this study probably reflected the ability of serum-free medium to retain the integrity of the cell membrane and cell-surface receptors more efficiently than serum-containing medium.

2-Nitrotoluene did not induce DNA damage in the *rec* assay (deficiency in recombination) in *Bacillus subtilis* (Shimizu & Yano, 1986).

(c) Mutations

Numerous studies have confirmed that 2-nitrotoluene is not mutagenic in a variety of *Salmonella* strains in the presence or absence of metabolic activation (Chiu *et al.*, 1978; Tokiwa *et al.*, 1981; Spanggord *et al.*, 1982b; Haworth *et al.*, 1983; Shimizu & Yano, 1986).

(d) Chromosomal effects

In Chinese hamster ovary cells, 2-nitrotoluene induced sister chromatid exchange in the presence of metabolic activation, but gave an equivocal response in its absence, and did not induce chromosomal aberrations in the presence or absence of metabolic activation (Galloway *et al.*, 1987). It did not induce chromosomal aberrations but did induce polyploidy in Chinese hamster lung cells (Ishidate *et al.*, 1988). 2-Nitrotoluene did not induce micronuclei in the bone marrow of male mice or rats injected intraperitoneally, but gave equivocal results for the induction of micronuclei in the peripheral blood of male mice. No micronuclei were seen in the peripheral blood erythrocytes of female mice administered 2-nitrotoluene in the diet for 13 weeks (NTP, 2002).

(e) Alterations in oncogenes and suppressor genes in tumours

Several studies have investigated mutations and altered gene expression in various types of tumour induced by 2-nitrotoluene in the National Toxicology Program (NTP, 2002) rodent bioassay. The predominant tumours in mice were haemangiosarcomas. Hong *et al.* (2003) found that 13/15 (87%) of the haemangiosarcomas of the skeletal muscle, subcutaneous tissue or mesentery had mutations in at least one of the following genes: *ras*, *Tp53* or β-catenin (*Catnb*). In particular, 11/15 (73%) had misssense mutations in *Tp53* exons 5–8 and 7/15 (47%) had deletions at exon 2 splice-sites or smaller deletions in the *Catnb* gene. In contrast, only 1/15 (7%) of the tumours had mutations in the K-*ras* gene. Spontaneous haemangiosarcomas lacked both p53 and β-catenin protein (encoded by *Catnb*) expression and *ras* mutations. The mutations in *Tp53* and *Catnb* may have resulted from the genotoxic effects of 2-nitrotoluene and played a role in the pathogenesis of the haemangiosarcomas in 2-nitrotoluene-treated mice.

Tumours of the large intestine (caecum) were the other type of tumour induced by 2-nitrotoluene in mice; this was especially unusual, because it had not been observed among either controls or treated mice in more than 500 carcinogenicity studies conducted by the NTP (Sills *et al.*, 2004). These gland-forming tumours were positive for cytokeratin 20 and negative for cytokeratin 7. Accumulation of β-catenin protein was found in 8/10 (80%) of the caecal carcinomas, and increased cyclin D1 and p53 protein expression was detected in 8/11 (73%); there was no difference in the expression of adenomatous polyposis coli protein between caecal tumours and normal colon tissue. All tumours had mutations in exon 2 in the *Catnb* gene (which corresponds to exon 3 in the human gene), 9/11 (82%) had mutations in

exon 7 of *Tp53* and 9/11 (82%) had specific G→T transversions at codons 10 or 12 in K-*ras*. These mutations and alterations in proteins activate signal-transduction pathways (K-*ras* and *Catnb*), disrupt the cell cycle and by-pass G_1-arrest (*Tp53*, cyclin D1). These changes are common features of human colon cancer and probably contribute to the pathogenesis of the large intestinal cancers in mice treated with 2-nitrotoluene.

(f) Changes in gene expression

Starting at 6 weeks of age, mice were fed 2-nitrotoluene for 2 weeks; livers were then collected and microarray analysis was performed among 20 842 genes. Several cancer-related genes, including the fragile histidine triad gene, the WW domain-containing oxidoreductase gene and the epidermal growth factor receptor gene, were downregulated, whereas P21, Cyclin G1, the nicotinamide adenine dinucleotide phosphate:quinone oxidoreductase gene and the regulator of G-protein signalling 16 gene were upregulated. The early changes in gene expression observed in this study involved apoptosis- and cell cycle-related genes (Iida *et al.*, 2005).

Kim *et al.* (2005) evaluated the ability of 2-nitrotoluene, and various other agents, to alter gene expression in mouse lymphoma L5178Y cells, which do not have a functional *Tp53* gene and are more sensitive to DNA damage. Differences were found between genotoxic and non-genotoxic (such as 2-nitrotoluene) agents for genes involved in cell-cycle control, stress response and immune response; however, no gene cluster with altered expression was found that was specific for the four carcinogenic agents studied.

Microarray analysis of peritoneal mesotheliomas induced by 2-nitrotoluene in male rats found alterations in the expression of the insulin-like growth factor 1 gene, the *p38* mitogen-activated protein-kinase gene, *Wnt/Catnb* and the integrin signalling pathways gene; these changes are also observed in human mesotheliomas (Kim *et al.*, 2006).

4.3 Other mechanistic considerations

4.3.1 Effects on cell physiology

Sabbioni *et al.* (2006) evaluated a wide variety of biomarkers among nitrotoluene workers in a factory in China, and found that haemoglobin adduct levels were higher in workers who were positive for glucose and/or protein in their urine, and for urobilinogen. The levels of bilirubin and urea in serum were significantly lower in workers with high levels of haemoglobin adducts, and their white blood cell count was lower. [The positive results for protein and glucose in the urine could be caused by tubular or glomerular damage induced by nitrotoluenes.] The nephrotoxicity of nitrotoluenes has been documented in miners exposed to dinitrotoluene (Brüning *et al.*, 1999, 2002) and in rodents exposed to 2-nitrotoluene (NTP, 1992).

Sabbioni *et al.* (2006) also found that increased exposure to nitrotoluenes among these workers resulted in increased levels of alkaline phosphatase and decreased levels of alanine aminotransferase. Albumin and total protein decreased, but the concentration of haemoglobin and red blood cell count increased with increased exposure to nitrotoluenes. [The high levels of alkaline phosphatase and the low level of serum proteins could indicate hepatotoxicity, which, together with liver cancer, were noted in the NTP (2002) rodent study of 2-nitrotoluene.]

4.3.2 Structure-activity relationships

Many studies have investigated the metabolism, DNA binding, DNA damage, and carcinogenicity associated with various nitrotoluenes. For example, 2- but not 3- or 4-nitrotoluene induces unscheduled DNA synthesis (Doolittle *et al.*, 1983), and 2-nitrotoluene binds covalently

to liver DNA *in vivo* (Jones *et al.*, 2003). In addition, 2-nitrotoluene, similarly to 2,6-dinitrotoluene, requires SULT for its conversion to a form that binds covalently to liver DNA (Rickert *et al.*, 1984).

4.4 Susceptibility

4.4.1 Genetic polymorphisms and enzyme induction

Sabbioni *et al.* (2006) evaluated various genotypes among a group of workers exposed to nitrotoluenes (including 2-nitrotoluene) and found that the glutathione *S*-transferase M1 (*GSTM1*)-positive genotype was significantly more common in controls than in the exposed group [an example of the 'healthy-worker effect']. This probably reflected an elevated susceptibility of the *GSTM1*-null genotype to the adverse effects of exposure to dinitrotoluene, such as nausea (odds ratio, 8.8; 95%CI: 2.4–32.2). The levels of methylaniline–haemoglobin adducts, which are derived only from 2-nitrotoluene, were elevated among workers with the *GSTM1*-positive genotype, as well as among those with the *N*-acetyltransferase 1 (*NAT1)* rapid genotype. When the concentration of 2-nitrotoluene in the air was also considered, the correlation between the *NAT2* slow genotype and methylaniline–haemoglobin adducts was quite high ($r = 0.97$; $P < 0.01$). There was a lesser association ($P < 0.1$) between the levels of methylaniline–haemoglobin adducts and the *SULT1A1 Arg/Arg* genotype and the *SULT1A2 Asn/Asn* genotype. The results suggest that *N*-hydroxyarylamine sulfonation may be more important than *N*-sulfonation for the formation of haemoglobin adducts following exposure to nitrotoluene. The high-activity genotype, which induces high levels of the sulfuric acid ester, may cause most of the metabolite to solubilize back to the *N*-hydroxy derivative, thus giving an additional opportunity for the formation of the haemoglobin adducts.

4.5 Mechanisms of carcinogenesis

A study in rats found a high correlation between levels of 2-nitrotoluene-induced hepatic DNA adducts and haemoglobin adducts, indicating that haemoglobin adducts are a reasonable surrogate for hepatic DNA adducts in rats (Jones *et al.*, 2003) and, by extension, possibly in humans. Thus, the high levels of haemoglobin adducts measured in Chinese factory workers (Sabbioni *et al.*, 2006; see Section 4.4.1), including methylaniline–haemoglobin adducts, which derive directly from 2-nitrotoluene, may indicate the presence of nitrotoluene-derived hepatic DNA adducts, although these were not measured directly. The association with SULT enzymes, based on the genotyping data among the factory workers and nitrotoluene–haemoglobin adducts, also supports the possible presence of nitrotoluene–DNA adducts — the formation of which in rats requires SULT activity.

Nearly all of the Chinese factory workers exposed to a mixture of nitrotoluenes had nitrobenzoic acids in their urine, and 2-nitrobenzoic acid, which is derived directly from 2-nitrotoluene, is mutagenic in the absence of metabolic activation in *Salmonella* (Sundvall *et al.*, 1984). A wide variety of other nitrobenzoic acids, nitrobenzyl alcohols and nitrosulfonic acids are also mutagenic (Grummt *et al.*, 2006). The factory workers also had highly mutagenic urine, indicative of systemic exposure to genotoxins, which was probably due to benzyl alcohol metabolites of nitrotoluenes, especially those of the dinitrotoluenes, because of the high correlation between dinitrotoluene-derived haemoglobin adducts and urinary mutagenicity (Sabbioni *et al.*, 2006). Thus, there is strong evidence that a genotoxic mechanism underlies the induction of tumours by 2-nitrotoluene in rodents and possibly humans.

5. Summary of Data Reported

5.1 Exposure data

2-Nitrotoluene is isolated from mixtures of nitrotoluene stereoisomers and is produced by nitration of toluene with an aqueous sulfuric/nitric acid mixture. Occupational exposure to 2-nitrotoluene can occur during its production or use in the production of azo dyes, such as *ortho*-toluidine, magenta and sulfur dyes, as well as rubber, agricultural chemicals and explosives.

2-Nitrotoluene can be released into the environment (primarily in air and water) during its manufacture and use. It has been detected in wastewater near 2-nitrotoluene-manufacturing plants and in ambient air and surface water. It may also be formed from the degradation of di- or trinitrotoluenes and has been detected in wastewater, effluents, groundwater, surface water and soil near facilities that produced or used these chemicals, namely munitions-manufacturing plants.

5.2 Human carcinogenicity data

No studies of human cancer were identified that evaluated specific exposure to 2-nitrotoluene; however, one cohort study of dyestuff workers stated that exposure to 2-nitrotoluene occurred (from its use as an raw ingredient or formation as an intermediate) during the manufacturing process of New Fuchsin (a component of magenta). Magenta production, but not magenta, is classified by IARC as *carcinogenic to humans (Group 1)*. In this study, a very large excess of mortality from bladder cancer was found among fuchsin- and safranine T-manufacturing workers, who were potentially exposed to 2-nitrotoluene. These workers were also exposed to *ortho*-toluidine, which causes bladder cancer and is classified by IARC as *carcinogenic to humans (Group 1)*; thus, it is not possible to evaluate whether 2-nitrotoluene played a causal role in the incidence of bladder cancer.

5.3 Animal carcinogenicity data

In a 2-year study, male and female mice fed 2-nitrotoluene in the diet had an increased incidence of haemangiosarcoma and carcinoma of the large intestine (caecum). The incidence of hepatocellular adenoma and carcinoma was also increased in females.

2-Nitrotoluene caused mesothelioma of the tunica vaginalis and cholangiocarcinomas after 13 or 26 weeks of oral administration in the diet to male rats. In a 2-year study in male and female rats, administration of 2-nitrotoluene in the diet increased the incidence of lipoma, fibroma and fibrosarcoma of the skin, mesothelioma, mammary gland fibroadenoma, hepatocellular adenoma or carcinoma (combined), cholangiocarcinoma and alveolar bronchiolar adenoma or carcinoma (combined) in male rats. Treatment-related tumours in female rats included skin fibroma, and skin fibroma or fibrosarcoma (combined), mammary gland fibroadenoma and hepatocellular adenoma. In a 2-year stop-exposure study in which male rats were exposed to 2-nitrotoluene for 13 weeks and then maintained on untreated diet until the end of the experiment, an increase in the incidence of mesothelioma, skin lipoma, fibroma and fibrosarcoma, cholangiocarcinoma, hepatocellular adenoma or carcinoma (combined), alveolar/bronchiolar adenoma or carcinoma (combined), and haemangioma or haemangiosarcoma (combined) was also observed. Thus, even these short exposures to 2-nitrotoluene induced cancer.

There was consistency in the tumour response across the 13-week, 26-week, 2-year and stop-exposure studies.

5.4 Other relevant data

In rodents, 2-nitrotoluene undergoes oxidation and conjugation in the liver to form 2-nitrobenzyl alcohol glucuronide, which is then excreted into the intestine via the bile and converted to aminobenzyl alcohol by intestinal microflora through reduction of the nitro group. The aminobenzyl alcohol is then re-absorbed and metabolized in the liver, resulting in an electrophilic compound that can bind to liver DNA and form DNA adducts. A similar pathway probably operates in humans, because 2-nitrotoluene is primarily metabolized to nitrobenzoic acids, which have been found as urinary metabolites in both humans and rodents.

Urine from workers exposed to a mixture of nitrotoluenes containing 2-nitrotoluene was mutagenic in bacteria. 2-Nitrobenzoic acid, which is derived directly from 2-nitrotoluene, is mutagenic in bacteria in the absence of metabolic activation. Many other nitrobenzoic acids, nitrobenzylalcohols and nitrosulfonic acids are also mutagenic.

Exposure of rats to 2-nitrotoluene induced a specific protein adduct, 2-methylaniline–haemoglobin, and liver DNA adducts of 2-methylaniline at guanosine and adenosine, which were found to be well correlated. This observation suggests that the haemoglobin adducts may serve as a reasonable biomarker of a systemic genotoxic effect.

In workers exposed to a mixture of nitrotoluenes containing 2-nitrotoluene, elevated levels of chromosomal aberrations were found in circulating blood lymphocytes, as were 2-methylaniline–haemoglobin adducts. Because this protein biomarker is specific to exposure to 2-nitrotoluene and was found to be correlated with the formation of DNA adducts in rat liver, and because the urine of exposed workers is mutagenic, there is strong evidence to suggest that genotoxic metabolites of 2-nitrotoluene are formed in exposed humans. Furthermore, the levels of haemoglobin adducts are also influenced by genotype, including those of glutathione *S*-transferase, *N*-acetyltransferase (rapid and slow) and sulfotransferase 1A1 genes.

Although exposure of humans to a mixture of nitrotoluenes containing 2-nitrotoluene results in genotoxic effects (i.e. mutagenic urine), 2-nitrotoluene is generally not mutagenic in standard in-vitro mutagenicity assays in the presence or absence of metabolic activation, presumably because the complex metabolism required for its activation is not present in such assays.

Analysis of mutations and changes in gene expression in 2-nitrotoluene-induced haemangiosarcomas in mice found that mutations in *Tp53* and β-catenin probably resulted from the genotoxic effects of 2-nitrotoluene and played a role in the formation of the tumours. Similarly, mutations in β-catenin, *Tp53* and K-*ras* were found in 2-nitrotoluene-induced tumours of the large intestine in mice, and these are common features of human colon cancer. In 2-nitrotoluene-induced mesotheliomas in rats, alterations in expression were found in various genes such as insulin-like growth factor, p38 mitogen-activated protein-kinase, *Wnt/β-catenin* and integrin-signalling pathways. Alterations in the expression of these genes are also found in human mesotheliomas.

Overall, there is strong evidence that a genotoxic mechanism underlies the induction of tumours in 2-nitrotoluene-exposed rodents. The evidence for the relevance of this mechanism to humans is strong.

6. Evaluation

6.1 Cancer in humans

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

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

2-Nitrotoluene is *probably carcinogenic to humans (Group 2A).*

6.4 Rationale

In making the overall evaluation, the Working Group took into consideration that there is strong evidence that a mutational mechanism underlies the induction of tumours in both rodents and humans exposed to 2-nitrotoluene.

References

ACGIH (2010) *2010 TLVs and BEIs* [CD-ROM]. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

Ahlborg G Jr, Bergström B, Hogstedt C *et al.* (1985). Urinary screening for potentially genotoxic exposures in a chemical industry. *Br J Ind Med*, 42: 691–699. PMID:3899158

Ahlborg G Jr, Ulander A, Bergström B, Oliv Å (1988). Diazo-positive metabolites in urine from workers exposed to aromatic nitro-amino compounds. *Int Arch Occup Environ Health*, 60: 51–54. doi:10.1007/BF00409379 PMID:3350604

ATSDR (2007). Internet HazDat - Contaminant Site List Agency for Toxic Substances and Disease Registry. Available at: http://www.atsdr.cdc.gov/toxprofiles/index.asp

Best EP, Miller JL, Larson SL (2001). Tolerance towards explosives, and explosives removal from groundwater in treatment wetland mesocosms. *Water Sci Technol*, 44: 515–521. PMID:11804143

Booth G (1991). *Nitro compounds, aromatic*. In: *Ullmann's Encyclopedia of Industrial Chemistry*, Elvers B, Hawkins S & Schulz G, editors. New York: VCH Publishers.

Brüning T, Chronz C, Thier R *et al.* (1999). Occurrence of urinary tract tumors in miners highly exposed to dinitrotoluene. *J Occup Environ Med*, 41: 144–149. doi:10.1097/00043764-199903000-00003 PMID:10091137

Brüning T, Thier R, Bolt HM (2002). Nephrotoxicity and nephrocarcinogenicity of dinitrotoluene: new aspects to be considered. *Rev Environ Health*, 17: 163–172. doi:10.1515/REVEH.2002.17.3.163 PMID:12462481

Butterworth BE, Smith-Oliver T, Earle L *et al.* (1989). Use of primary cultures of human hepatocytes in toxicology studies. *Cancer Res*, 49: 1075–1084. PMID:2917345

Case RA & Pearson JT (1954). Tumours of the urinary bladder in workmen engaged in the manufacture and use of certain dyestuff intermediates in the British chemical industry. II. Further consideration of the role of aniline and of the manufacture of auramine and magenta (fuchsine) as possible causative agents. *Br J Ind Med*, 11: 213–216. PMID:13182161

Chemical Sources International (2010). *Chem Sources-Online*, Clemson, SC. Available at: http://www.chem-sources.com/index.html

Chism JP & Rickert DE (1985). Isomer- and sex-specific bioactivation of mononitrotoluenes. Role of entero-hepatic circulation. *Drug Metab Dispos*, 13: 651–657. PMID:2867866

Chism JP & Rickert DE (1989). In vitro activation of 2-aminobenzyl alcohol and 2-amino-6-nitrobenzyl alcohol, metabolites of 2-nitrotoluene and 2,6-dinitrotoluene. *Chem Res Toxicol*, 2: 150–156. doi:10.1021/tx00009a005 PMID:2519719

Chism JP, Turner MJ Jr, Rickert DE (1984). The metabolism and excretion of mononitrotoluenes by Fischer 344 rats. *Drug Metab Dispos*, 12: 596–602. PMID:6149910

Chiu CW, Lee LH, Wang CY, Bryan GT (1978). Mutagenicity of some commercially available nitro compounds for Salmonella typhimurium. *Mutat Res*, 58: 11–22. doi:10.1016/0165-1218(78)90090-3 PMID:362191

deBethizy JD & Rickert DE (1984). Metabolism of nitrotoluenes by freshly isolated Fischer 344 rat hepatocytes. *Drug Metab Dispos*, 12: 45–50. PMID:6141911

Doolittle DJ, Sherrill JM, Butterworth BE (1983). Influence of intestinal bacteria, sex of the animal, and position of the nitro group on the hepatic genotoxicity of nitrotoluene isomers in vivo. *Cancer Res*, 43: 2836–2842. PMID:6850595

Dugal (2005). *Nitrobenzene and nitrotoluenes*. In: *Kirk-Othmer Encyclopedia of Chemical Technology*. M. Grayson & D. Eckroth, editors, Vol. 17. New York: John Wiley and Sons.

Duguet JP, Anselme C, Mazounie P, *et al.* (1988). *Application of the ozone hydrogen peroxide combination for the removal of toxic compounds from a groundwater*. In: *Organic Micropollutants in the Aquatic Environment, Proceedings of the Fifth European Symposium*. Angeletti G & Bjorseth A, editors. Dordrecht, the Netherlands: Kluwer Academic Publishers, pp.299–309.

Dunnick J (1993). NTP technical report on the toxicity studies of ortho-, meta-, and para- Nitrotoluenes (CAS Nos. 88–72–2, 99–08–1, 99–99–0) Administered in

Dosed Feed to F344/N Rats And B6C3F$_1$ Mice. *Toxic Rep Ser*, 23: 1–E4. PMID:12209183

Dunnick JK, Burka LT, Mahler J, Sills R (2003). Carcinogenic potential of o-nitrotoluene and p-nitrotoluene. *Toxicology*, 183: 221–234. doi:10.1016/S0300-483X(02)00543-7 PMID:12504353

Dunnick JK, Elwell MR, Bucher JR (1994). Comparative toxicities of o-, m-, and p-nitrotoluene in 13-week feed studies in F344 rats and B6C3F$_1$ mice. *Fundam Appl Toxicol*, 22: 411–421. doi:10.1006/faat.1994.1047 PMID:7519572

Ebrahimzadeh H, Yamini Y, Kamarei F (2009). Optimization of dispersive liquid-liquid microextraction combined with gas chromatography for the analysis of nitroaromatic compounds in water. *Talanta*, 79: 1472–1477. doi:10.1016/j.talanta.2009.06.022 PMID:19635386

Ebrahimzadeh H, Yamini Y, Kamarei F, Shariati S (2007). Homogeneous liquid-liquid extraction of trace amounts of mononitrotoluenes from waste water samples. *Anal Chim Acta*, 594: 93–100. doi:10.1016/j.aca.2007.05.013 PMID:17560390

European Commission (2008). *2-Nitrotoluene. Summary risk assessment report, EU 23517EN/2*. Available at: http://esis.jrc.ec.europa.eu/doc/existing-chemicals/risk_assessment/REPORT/2nitrotoluenereport403.pdf

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

Galloway SM, Armstrong MJ, Reuben C *et al.* (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals. *Environ Mol Mutagen*, 10: Suppl 101–35. doi:10.1002/em.2850100502 PMID:3319609

Götz R, Bauer OH, Friesel P, Roch K (1998). Organic trace compounds in the water of the River Elbe near Hamburg. Part II. *Chemosphere*, 36: 2103–2118. doi:10.1016/S0045-6535(98)00009-5 PMID:9532733

Grummt T, Wunderlich HG, Chakraborty A *et al.* (2006). Genotoxicity of nitrosulfonic acids, nitrobenzoic acids, and nitrobenzylalcohols, pollutants commonly found in ground water near ammunition facilities. *Environ Mol Mutagen*, 47: 95–106. doi:10.1002/em.20172 PMID:16180207

Haworth S, Lawlor T, Mortelmans K *et al.* (1983). Salmonella mutagenicity test results for 250 chemicals. *Environ Mutagen*, 5: Suppl 13–142. doi:10.1002/em.2860050703 PMID:6365529

He MC, Sun Y, Li XR, Yang ZF (2006). Distribution patterns of nitrobenzenes and polychlorinated biphenyls in water, suspended particulate matter and sediment from mid- and down-stream of the Yellow River (China). *Chemosphere*, 65: 365–374. doi:10.1016/j.chemosphere.2006.02.033 PMID:16580044

Hong HL, Ton TV, Devereux TR *et al.* (2003). Chemical-specific alterations in ras, p53, and β-catenin genes in hemangiosarcomas from B6C3F$_1$ mice exposed to o-nitrotoluene or riddelliine for 2 years. *Toxicol Appl Pharmacol*, 191: 227–234. doi:10.1016/S0041-008X(03)00165-0 PMID:13678655

Howard PH, Santodonato J, Saxena J *et al.* (1976). *Investigation of selected environmental contaminents: nitroaromatics*. Washington D.C.: Office of Toxic Substances, US Environmental Protection Agency. Available at: http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=9101327P.txt

HSDB (2004). *2-Nitrotoluene*. National Library of Medicine's TOXNET system. Research Triangle Park, NC: National Toxicology Program. Available at: http://toxnet.nlm.nih.gov/

IARC (1996). Printing processes and printing inks, carbon black and some nitro compounds. *IARC Monogr Eval Carcinog Risks Hum*, 65: 1–578.

IARC (2010). Some Aromatic Amines, Organic Dyes, and Related Exposures. *IARC Monogr Eval Carcinog Risks Hum*, 99: 1–678.

Iida M, Anna CH, Holliday WM *et al.* (2005). Unique patterns of gene expression changes in liver after treatment of mice for 2 weeks with different known carcinogens and non-carcinogens. *Carcinogenesis*, 26: 689–699. doi:10.1093/carcin/bgi005 PMID:15618236

Ishidate M Jr, Harnois MC, Sofuni T (1988). A comparative analysis of data on the clastogenicity of 951 chemical substances tested in mammalian cell cultures. *Mutat Res*, 195: 151–213. PMID:3277036

IUCLID (2000). *2-nitrotoluene. IUCLID database*. Bruxelles: European Commission, European Chemicals Bureau. Available at: http://ecb.jrc.ec.europa.eu/IUCLID-DataSheets/88722.pdf

Jones CR, Beyerbach A, Seffner W, Sabbioni G (2003). Hemoglobin and DNA adducts in rats exposed to 2-nitrotoluene. *Carcinogenesis*, 24: 779–787. doi:10.1093/carcin/24.4.779 PMID:12727807

Jones CR, Liu Y-Y, Sepai O *et al.* (2005b). Hemoglobin adducts in workers exposed to nitrotoluenes. *Carcinogenesis*, 26: 133–143. doi:10.1093/carcin/bgh286 PMID:15471893

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: 1251–1263. doi:10.1021/tx020064i PMID:14565767

Jones CR, Sepai O, Liu Y-Y *et al.* (2005a). Urinary metabolites of workers exposed to nitrotoluenes. *Biomarkers*, 10: 10–28. doi:10.1080/13547500500079670 PMID:16097390

Kim J-Y, Kwon J, Kim JE *et al.* (2005). Identification of potential biomarkers of genotoxicity and carcinogenicity in

L5178Y mouse lymphoma cells by cDNA microarray analysis. *Environ Mol Mutagen*, 45: 80–89. doi:10.1002/em.20077 PMID:15612046

Kim Y, Ton T-V, DeAngelo AB *et al.* (2006). Major carcinogenic pathways identified by gene expression analysis of peritoneal mesotheliomas following chemical treatment in F344 rats. *Toxicol Appl Pharmacol*, 214: 144–151. doi:10.1016/j.taap.2005.12.009 PMID:16460773

Kool HJ, van Kreijl CF, Zoeteman BCJ (1982). Toxicology assessment of organic compounds in drinking water. Criteria Rev. environ. *Control*, 12: 307–357.

Lang PZ, Long FS, Yuan X *et al.* (1993). [The research on the toxic organic pollutants in water of middle stream of Songhua River (Shaokou-Songhua River Section)] *Advances in Environmental Science*, 1: 47–55.

Meijers AP & van der Leer RC (1976). The occurrence of organic micropollutants in the river Rhine and the river Maas in 1974. *Water Res*, 10: 597–604. doi:10.1016/0043-1354(76)90140-8

Men B, Wang H, He M *et al.* (2010). 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*.

Nay MW Jr (1972). *A biodegradabillity and treatability study of TNT manufacturing wastes with activated sludge system*. Soctoral Thesis. Blacksburg, Virginia: Polytechnic Institute and State University, pp. 367.

NIOSH (1998). *Nitroaromatic Compounds*. In: *Manual of Analytical Methods (NMAM),* issue 3. Schlect PC & PF O'Connor editors. Pub. No. 2003–154. Cincinnati, OH: National Institute for Occupational Safety and Health. Available at: http://www.ede.gov/niosh/docs/2003-154/pdfs/2005.pdf

NTP (1992). *Toxicity Studies of o-, m-, and p- Nitrotoluenes (CAS Nos. 88-72-2, 99-08-1, 99-99-0) Administered in Dosed Feed to F344/N Rats And B6C3F$_1$ Mice.*, No. *Toxic Rep Ser*, 23:

NTP (2000). NTP Comparative Toxicity and Carcinogenicity Studies of o-Nitrotoluene and o-Toluidine Hydrochloride (CAS Nos. 88–72–2 and 636–21–5) Administered in Feed to Male F344/N Rats. *Toxic Rep Ser*, 44: 1–C8. PMID:12118263

NTP (2002). Toxicology and carcinogenesis studies of o-nitrotoluene sulfone (CAS no. 88–72–2) in F344/N rats and B6C3F(1) mice (feed studies). *Natl Toxicol Program Tech Rep Ser*, 504: 1–357. PMID:12087420

NTP (2008). Final Report on Carcinogens background document for o-nitrotoluene. *Rep Carcinog Backgr Doc*: i-102.

O'Neil MJ, Heckelman PE, Koch CB, Roman KJ, editors (2006). *The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals*, 14th ed., Version 14:3 [CD-ROM]. Whitehouse Station, NJ: Merck & Co.

Pantex (2005). *2004 Environmental report for Pantex Plant: chapter 6*. Amarillo, TX: L.L.C. pp. 6–16

Parton JW, Yount DJ, Garriott ML (1995). Improved sensitivity of the unscheduled DNA synthesis assay in primary rat hepatocytes following culture in serum-free defined media. *Environ Mol Mutagen*, 26: 147–154. doi:10.1002/em.2850260208 PMID:7556111

Pellizzari ED (1978) *Quanitifcation of Chlorinated Hydrocarbons in Previously Collected Air Samples (US EPA Report No. EPA-450/3-78-112).* Washington D.C.: US Environmental Protection Agency

Radtke CW, Gianotto D, Roberto FF (2002). Effects of particulate explosives on estimating contamination at a historical explosives testing area. *Chemosphere*, 46: 3–9. doi:10.1016/S0045-6535(01)00107-2 PMID:11806529

Rehn L (1895). [Bladder Tumour in fuchsin workers]*Arch Klin Chir*, 50: 588–600.

Rickert DE, Long RM, Dyroff MC, Kedderis GL (1984). Hepatic macromolecular covalent binding of mononitrotoluenes in Fischer-344 rats. *Chem Biol Interact*, 52: 131–139. doi:10.1016/0009-2797(84)90067-X PMID:6595071

RTECS (2009). *Toluene, -o-nitro-*. In: *Registry of Toxic Effects of Chemical Substances Database* RTECS# XT3150000. San Diego, CA: Accelrys, Inc.

Rubino GF, Scansetti G, Piolatto G, Pira E (1982). The carcinogenic effect of aromatic amines: an epidemiological study on the role of o-toluidine and 4,4′-methylene bis (2-methylaniline) in inducing bladder cancer in man. *Environ Res*, 27: 241–254. doi:10.1016/0013-9351(82)90079-2 PMID:7084156

Sabbioni G, Jones CR, Sepai O *et al.* (2006). Biomarkers of exposure, effect, and susceptibility in workers exposed to nitrotoluenes. *Cancer Epidemiol Biomarkers Prev*, 15: 559–566. doi:10.1158/1055-9965.EPI-05-0677 PMID:16537716

Sadtler Research Laboratories (1980). *Sadtler Standard Spectra. 1980 Cumulative Index*, Philadelphia, PA.

Sax NI, Lewis RJ Jr (1989). *Dangerous Properties of Industrial Materials*, 7th ed, New York: Van Nostrand Reinhold Co., pp. 2344–2345.

Shimizu M & Yano E (1986). Mutagenicity of mononitrobenzene derivatives in the Ames test and rec assay. *Mutat Res*, 170: 11–22. doi:10.1016/0165-1218(86)90077-7 PMID:3083245

Sills RC, Hong HL, Flake G *et al.* (2004). o-Nitrotoluene-induced large intestinal tumors in B6C3F$_1$ mice model human colon cancer in their molecular pathogenesis. [PMID:14688030.]*Carcinogenesis*, 25: 605–612. doi:10.1093/carcin/bgh044 PMID:14688030

Spanggord R, Gibson BW, Keck RG *et al.* (1982a). Effluent analysis of wastewater generated in the manufacture of 2,4,6-trinitrotoluene. 1. Characterization study. *Environ Sci Technol*, 16: 229–232. doi:10.1021/es00098a012

Spanggord RJ, Mortelmans KE, Griffin AF, Simmon VF (1982b). Mutagenicity in Salmonella typhimurium and structure-activity relationships of wastewater

components emanating from the manufacture of trinitrotoluene. *Environ Mutagen*, 4: 163–179. doi:10.1002/em.2860040207 PMID:7042329

Sundvall A, Marklund H, Rannug U (1984). The mutagenicity on Salmonella typhimurium of nitrobenzoic acids and other wastewater components generated in the production of nitrobenzoic acids and nitrotoluenes. *Mutat Res*, 137: 71–78. doi:10.1016/0165-1218(84)90094-6 PMID:6381997

Swaminathan K, Kondawar VK, Chakrabarti T, Subrahmanyam PVR (1987). Identification and quantification of organics in nitro aromatic manufacturing wastewaters. 1ndian *J Environ Health*, 29: 32–38.

Tokiwa H, Nakagawa R, Ohnishi Y (1981). Mutagenic assay of aromatic nitro compounds with Salmonella typhimurium. *Mutat Res*, 91: 321–325. doi:10.1016/0165-7992(81)90008-7 PMID:7022203

US EPA (2004). *Non-confidential IUR production volume information*. US Environmental Protection Agency. Available at: http://www.epa.gov/oppt/iur/tools/data/2002-vol.html and search on CAS number

US EPA (2006). *Nitroaromatics, Nitramines and Nitrate Esters by High Performance Liquid Chromatography* - Method 8330B. Washington D.C.:US Environmental Protection Agency.

Vineis P & Magnani C (1985). Occupation and bladder cancer in males: a case-control study. *Int J Cancer*, 35: 599–606. doi:10.1002/ijc.2910350506 PMID:3997281

Webb RG, Garrison AW, Keith LH, McGuire JM (1973). *Current practice in GS-MS analysis of organics in water*. EPA-R2-73-277

Working PK & Butterworth BE (1984). An assay to detect chemically induced DNA repair in rat spermatocytes. *Environ Mutagen*, 6: 273–286. doi:10.1002/em.2860060304 PMID:6734543

Zoeteman BCJ (1980). *Sensory assessment of water quality*. Oxford: Pergamon Press.