2,4-DINITROTOLUENE, 2,6-DINITROTOLUENE AND 3,5-DINITROTOLUENE

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

The CAS Reg. No. for dinitrotoluene, not otherwise specified, is 25321-14-6.

2,4-Dinitrotoluene

Chem. Abstr. Serv. Reg. No.: 121-14-2

Chem. Abstr. Name: 1-Methyl-2,4-dinitrobenzene

IUPAC Systematic Name: 2,4-Dinitrotoluene

Synonyms: Dinitrotoluene; 2,4-dinitrotoluol; DNT; 2,4-DNT; 4-methyl-1,3-dinitrobenzene

2,6-Dinitrotoluene

Chem. Abstr. Serv. Reg. No.: 606-20-2

Chem. Abstr. Name: 2-Methyl-1,3-dinitrobenzene

IUPAC Systematic Name: 2,6-Dinitrotoluene

Synonyms: 2,6-DNT; 1-methyl-2,6-dinitrobenzene

3,5-Dinitrotoluene

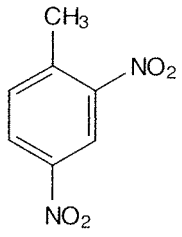
Chem. Abstr. Serv. Reg. No.: 618-85-9

Chem. Abstr. Name: 1-Methyl-3,5-dinitrobenzene

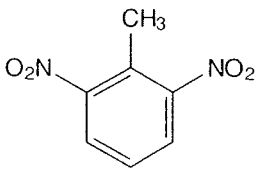
IUPAC Systematic Name: 3,5-Dinitrotoluene

Synonyms: 3,5-DNT

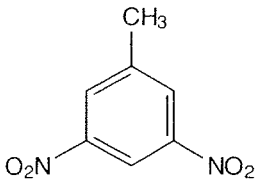
1.1.2 *Structural and molecular formulae and relative molecular mass*



2,4-Dinitrotoluene



2,6-Dinitrotoluene



3,5-Dinitrotoluene

$C_7H_6N_2O_4$ Relative molecular mass: 182.15

1.1.3 *Chemical and physical properties of the pure substance*

2,4-Dinitrotoluene

(*a*) *Description*: Yellow needles from ethanol or carbon disulfide (Booth, 1991; Lide, 1993)

(*b*) *Boiling-point*: 300 °C (decomposes) (Lide, 1993)

(*c*) *Melting-point*: 71 °C (Lide, 1993)

(*d*) *Spectroscopy data*: Infrared (prism [175, 258], grating [8017]), ultraviolet (UV) [2550], nuclear magnetic resonance (proton [3229], C-13 [4627]) and mass spectral data have been reported (Sadtler Research Laboratories, 1980)

(*e*) *Solubility*: Slightly soluble in water (270 mg/L at 22 °C); soluble in acetone, benzene, diethyl ether and ethanol (Mabey *et al.*, 1982; Lide, 1993)

(*f*) *Volatility*: Vapour pressure, 0.00011 mm Hg [0.015 Pa] at 20 °C; relative vapour density (air = 1), 6.27 (Howard, 1989; Booth, 1991)

(*g*) *Stability*: Combustible when exposed to heat or flame; can react with oxidizing materials. Decomposes when heated at ≥ 250 °C. Mixture with nitric acid is a high explosive. Mixture with sodium carbonate (or other alkalies) can decompose with significant increase in pressure at 210 °C (Sax & Lewis, 1989).

(*h*) *Octanol/water partition coefficient (P)*: log P, 1.98 (Hansch *et al.*, 1995)

(*i*) *Conversion factor*: $mg/m^3 = 7.45 \times ppm$[1]

2,6-Dinitrotoluene

(*a*) *Description*: Rhombic needles from ethanol (Lide, 1993)

(*b*) *Boiling-point*: 285 °C (Howard, 1989)

(*c*) *Melting-point*: 66 °C (Lide, 1993)

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

(*d*) *Spectroscopy data*: Infrared (prism [17378], grating [676]), UV [5514], nuclear magnetic resonance (proton [895]) and mass spectral data have been reported (Sadtler Research Laboratories, 1980)

(*e*) *Solubility*: Slightly soluble in water (180 mg/L at 20 °C); soluble in ethanol (Mabey *et al.*, 1982; Lide, 1993)

(*f*) *Volatility*: Vapour pressure, 0.018 mm Hg [2.4 Pa] at 20 °C (Mabey *et al.*, 1982)

(*g*) *Octanol/water partition coefficient (P)*: log P, 2.10 (Hansch *et al.*, 1995)

(*h*) *Conversion factor*: $mg/m^3 = 7.45 \times ppm$[1]

3,5-Dinitrotoluene

(*a*) *Description*: Yellow rhombic needles from acetic acid (Lide, 1993)

(*b*) *Boiling-point*: Sublimes (Lide, 1993)

(*c*) *Melting-point*: 93 °C (Lide, 1993)

(*d*) *Density*: 1.2772 at 11 °C/4 °C (Lide, 1993)

(*e*) *Solubility*: Soluble in benzene, chloroform, diethyl ether and ethanol (Lide, 1993)

(*f*) *Stability*: Combustible when exposed to heat or flame; can react with oxidizing materials. A moderate explosion hazard when exposed to heat (Sax & Lewis, 1989)

(*g*) *Octanol/water partition coefficient (P)*: log P, 2.28 (United States National Library of Medicine, 1995)

(*h*) *Conversion factor*: $mg/m^3 = 7.45 \times ppm$[1]

1.1.4 *Technical products and impurities*

2,4-Dinitrotoluene is available commercially at purities ranging from 95% (with content of 2,6-isomer < 1.5%) to > 99% in high-melt and super high-melt grades. 2,6-Dinitrotoluene is available commercially at purities ranging from 97% to > 99%, sometimes with 10% water added. Mixtures of 95 : 5, 80 : 20 and 65 : 35 2,4-dinitrotoluene and 2,6-dinitrotoluene, respectively, are also available commercially (Girundus Corp., 1994; Miles, 1994; TCI America, 1994; Acros Organics, 1995; Air Products and Chemicals, 1995; Lancaster Synthesis, 1995).

1.1.5 *Analysis*

Spectrophotometric determination of 2,4-dinitrotoluene in acidified urine has been used routinely in the range of 5–50 mg/L. After diazotization and coupling to 1-amino-8-naphthol-2,4-disulfonic acid (Chicago acid), the primary aromatic amine was determined as a red azo dye. An alternative method uses formamidine sulfinic acid (thiourea dioxide) to reduce the aromatic nitro compounds under alkaline conditions (Konieck & Linch, 1958).

---

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

Various instrumental techniques have been used for the determination of nitroaromatic compounds, nitramines and explosives, with gas chromatography being perhaps the most widely used. Because some of these compounds may undergo thermal degradation during analysis by gas chromatography, high-performance liquid chromatographic (HPLC) methods have been developed, which use reverse-phase chromatography and ultraviolet detection in most instances. Spectrophotometry and polarography have also been used for the detection of explosives. Thin-layer chromatography has been used to identify nitroaromatic compounds and explosives (Steuckart *et al.*, 1994).

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

Table 1. Methods for the analysis of dinitrotoluenes

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Air	Draw air through modified Tenax-GC tube; desorb with acetone	GC/TEA	20 µg/m^{3a}	US Occupational Safety and Health Administration (1990) [Method 44]
	Draw air through a filter/bubbler; extract with ethylene glycol	HPLC/UV	NR^{a}	Taylor (1978) [Method S215]
Water	Extract sample with dichloromethane or adsorb on Amberlite XAD resin and elute with dichloromethane	GC/ECD	NR^{a,b}	Feltes *et al.* (1990)
	Liquid–liquid extraction with dichloromethane; dry with anhydrous sodium sulfate; evaporate to dryness; redissolve in methanol	SFC/FID	30 ppm (mg/L)^{a}	Ong *et al.* (1992)
	Extract with toluene	GC/EC	3 ng/L^{b} 40 ng/L^{a}	Hable *et al.* (1991)
	Liquid–liquid extraction with dichloromethane	HPTLC	20 ng^{a,b}	Steuckart *et al.* (1994)
	Solid-phase microextraction with a polydimethylsiloxane-coated fibre; thermally desorb directly into GC	GC/FID	15 µg/L^{a,b}	Horng & Huang (1994)
Wastewater	Dilute sample with methanol and acetonitrile	HPLC/UV	10 µg/L^{a}	Jenkins *et al.* (1986)
	Extract with diethyl ether; dry over anhydrous magnesium sulfate; filter	GC/FID	NR^{a,b,c}	Spanggord *et al.* (1982a)

Table 1 (contd)

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Municipal and industrial discharges	Extract with dichloromethane; dry; exchange to hexane	GC/ECD	0.01 µg/L[b] 0.02 µg/L[a]	US Environmental Protection Agency (1986a, 1994) [Methods 8090 & 609]
	Extract with dichloromethane at pH > 11 and at pH < 2; dry (packed column method)	GC/MS	1.9 µg/L[b] 5.7 µg/L[a]	US Environmental Protection Agency (1986b, 1994) [Methods 8250 & 625]
	Add isotopically labelled analogue to sample; extract with methylene chloride at pH 12–13 and at pH < 2; dry (capillary column method)	GC/MS	10 µg/L[a,b]	US Environmental Protection Agency (1994) [Method 1625B]
Industrial effluents and seawater	Extract with benzene; inject into glass capillary GC column	GC/ECD	0.059 µg/L[b] 0.13 µg/L[a] 0.17 µg/L[c]	Hashimoto *et al.* (1980)
Water, soil, sediment, waste	Extract with dichloromethane (capillary column method)	GC/MS	PQL[a,b,d]	US Environmental Protection Agency (1986c) [Method 8270]
Soil	Extract with acetone in ultrasonic bath; extract with toluene, remove acetone; dry; add calcium chloride solution; extract with dichloromethane	HPTLC	20 ng[a,b]	Steuckart *et al.* (1994)
	Extract with acetone; react supernatant with potassium hydroxide/sodium sulfite; read absorbance at 570 nm	Colorimetry	2 µg/g[a]	Jenkins & Walsh (1992)
Freshwater and marine sediments	Extract with acetonitrile; clean-up with solid-phase extraction	GC/MS	NR[a,b]	Davis *et al.* (1993)
Urine	Hydrolyse metabolites; extract; derivatize	GC/MS	NR[a,b]	Turner *et al.* (1985)
	Extract with ethyl acetate	GC/ECD	NR[a,b]	Woollen *et al.* (1985)
Blood	Extract with toluene	GC/ECD	NR[a,b]	Woollen *et al.* (1985)
	Extract from separated plasma and concentrate simultaneously using 2,2,4-trimethylpentane	GC/ECD	2.0 µg/L[b]	Lewalter & Ellrich (1991)

Table 1 (contd)

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Materials exposed to explosives	Draw air through ethanol-soaked handswab; adsorb on alumina/octadecylsilylsilica; desorb with methanol/water (100 : 35 v/v)	HPLC/ED	12 pg/ sample[a]	Lloyd (1983a,b)

GC, gas chromatography; TEA, thermal energy analyser; HPLC, high-performance liquid chromatography; UV, ultraviolet detection; NR, not reported; ECD, electrolytic conductivity detection; SFC, capillary supercritical fluid chromatography; FID, flame ionization detection; EC, electron capture detection; HPTLC, high-performance thin-layer chromatography

[a] 2,4-Dinitrotoluene

[b] 2,6-Dinitrotoluene

[c] 3,5-Dinitrotoluene

[d] PQL, practical quantitation limit: groundwater, 10 µg/L; low soil/sediment, 660 µg/kg; medium-level soil and sludges by sonicator, 4.95 mg/kg; non-water-miscible waste, 49.5 kg

1.2 Production and use

1.2.1 *Production*

A 96% yield of 2,4-dinitrotoluene can be produced by continuous nitration of 4-nitrotoluene with 'mixed acid' (which contains equimolar quantities of nitric and sulfuric acids) under controlled conditions. Alternative processes yield mixed isomer products — when toluene is nitrated directly under similar conditions with 2.1 equivalents of nitric acid, the product is approximately an 80 : 20 mixture of 2,4-dinitrotoluene and 2,6-dinitrotoluene, and nitration of 2-nitrotoluene, which is sometimes present in excess, gives an approximate 67 : 33 mixture of 2,4- and 2,6-dinitrotoluenes (Levine *et al.*, 1985; Booth, 1991).

3,5-Dinitrotoluene can be prepared by the nitration of nitrotoluene with mixed acid (Lewis, 1993).

Mixtures of 2,4- and 2,6-dinitrotoluenes were produced in the United States of America at a rate of over 227 thousand tonnes per year in 1982 (Howard, 1989).

2,4-Dinitrotoluene is produced by three companies each in Japan and the United States and one company each in China, the Czech Republic, Egypt, Germany, Italy, Portugal, Turkey and the United Kingdom. 2,6-Dinitrotoluene is produced by one company each in Egypt, Germany and Italy. The mixture of 2,4- and 2,6-dinitrotoluenes is produced by two companies in the United States and by one company each in China, the Czech Republic, Germany, Poland, the Republic of Korea, Romania and Turkey (Chemical Information Services, 1994).

1.2.2 *Use*

Most of the 2,4-dinitrotoluene produced is hydrogenated (nickel catalyst) to 2,4-diaminotoluene (see IARC, 1978, 1987a) for conversion to toluene diisocyanate (see IARC, 1986, 1987b), which is a monomer in the production of polyurethane (see IARC, 1979). A much smaller amount is used in explosives and for further nitration to trinitrotoluene (see this volume). Crude mixtures of 2,4- and 2,6-dinitrotoluenes are also used to produce mixed toluene diamines (80 : 20 or 67 : 33, depending on the nitration process used), which in turn are converted to mixed toluene diisocyanates for polyurethane production. The use of these mixed isomers in polyurethane production has considerable cost benefits (Booth, 1991).

Dinitrotoluenes, including the 2,4-, 2,6- and 3,5-isomers, are used in organic synthesis in the production of toluidines and dyes. In the production of explosives, dinitrotoluenes are used to manufacture trinitrotoluene and gelatin explosives, to plasticize cellulose nitrate, to moderate the burning rate of propellants and to waterproof some smokeless powders (Howard, 1989; Lewis, 1993).

1.3 Occurrence

1.3.1 *Natural occurrence*

Dinitrotoluenes are not known to occur as natural products.

1.3.2 *Occupational exposure*

Exposure to dinitrotoluenes may occur from their use in the manufacture of toluene diisocyanate, in the production of explosives, in the manufacture of azo dye intermediates and in organic synthesis in the preparation of toluidines and dyes (Howard, 1989).

However, few reports of occupational exposures to dinitrotoluene exist. Levine *et al.* (1985) monitored in 1983 7-h time-weighted average (TWA) personal exposure to dinitrotoluene and urinary metabolites of dinitrotoluene in a dinitrotoluene manufacturing plant constructed in 1973. Exposures of production unit operators to both 2,4- and 2,6- dinitrotoluenes averaged 0.26 mg/m^3 (range, 0.05–0.59 mg/m^3). Exposures of loaders, who load storage tanks, collect samples and perform cleaning tasks, averaged 0.32 mg/m^3 (range, 0.14–0.49 mg/m^3). Exposures of maintenance mechanics averaged 0.12 mg/m^3 (range, 0.08–0.15 mg/m^3) and the exposure of acid stripper operators was 0.06 mg/m^3. The highest personal air concentrations and levels of urinary metabolites were found to be for loaders, followed by process operators. The levels of urinary metabolites of dinitrotoluene in loaders and operators exceeded those that would have resulted from the inhaled concentrations (as indicated by personal air monitoring), although the workers wore gloves for operations in which dermal exposures were possible.

A study of explosives manufacture in the United Kingdom also compared personal airborne exposures to dinitrotoluene with levels of urinary metabolites of dinitrotoluene (Woollen *et al.*, 1985). Personal exposures ranged from 'not detected' to 0.1 mg/m^3 dinitrotoluene. Area samples positioned near dusty parts of the process ranged from 0.02

to 2.68 mg/m^3. However, atmospheric concentrations could not account for the observed excretion levels of the metabolite 2,4-dinitrobenzoic acid, indicating probable dermal uptake.

Quantitative occupational exposure data were not available for a mortality study of munitions workers producing rocket propellants in the United States (Stayner *et al.*, 1993). Workers were classified into three exposure categories based on their opportunity for exposure to dinitrotoluenes.

1.3.3 *Environmental occurrence*

(*a*) *Water*

2,4-Dinitrotoluene has been detected in seawater, river water and in wastewater from 2,4,6-trinitrotoluene production. 2,4-Dinitrotoluene was found in Dokai Bay, Japan, at concentrations of up to 206 μg/L (Hashimoto *et al.*, 1982), in water from the River Rhine in the Netherlands at 0.3 μg/L (Zoeteman *et al.*, 1980), in water samples obtained from the River Potomac near Quantico, VA, United States, at a concentration of < 10 μg/L (Hall *et al.*, 1987) and in Waconda Bay, Lake Chickamauga, TN, United States (range of means, < 0.10–22.1 μg/L) (Putnam *et al.*, 1981). It has also been detected in groundwater at levels ranging from 2 to 90 500 μg/L (0.002 to 90.5 ppm) near a nitroaromatic manufacturing facility in Pasadena, TX, United States (Matson, 1986), and was detected but not quantified, in groundwater (one sample) at the Hawthorne Naval Ammunition Depot, NV, United States (Pereira *et al.*, 1979).

2,4-Dinitrotoluene has also been found in condensate wastewater from 2,4,6-trinitrotoluene manufacture at an unspecified concentration (Liu *et al.*, 1984) and in wastewater from 2,4,6-trinitrotoluene production at an average concentration of 9700 μg/L in 29/54 samples (Spanggord & Suta, 1982). It has been detected in effluents from coal mining (18 μg/L in 1/49 samples), iron and steel manufacture (530 μg/L in 1/5 samples), aluminium forming (77 μg/L in 1/2 samples), foundries (mean, 26 μg/L; range, 7–50 μg/L in 4/4 samples) and organic chemical manufacture (mean, 14 000 μg/L in 4 samples) (United States Environmental Protection Agency, 1980; Howard, 1989).

2,4-Dinitrotoluene concentrations of 3.1 and 13.0 μg/L were detected in surface-water samples collected from two brooks near Hischagen/Waldhof, Germany, which were close to sites used for Second World War munitions manufacture; the river into which the brooks fed (River Losse) was found to have a concentration of 0.5 μg/L. Two ponds in the Clausthal-Zellerfeld region of Germany, again near sites of previous munitions manufacture, had levels of 1.2 and 0.8 μg/L; the ponds feed into the River Oder, which was found to have a level of 0.02 μg/L. Concentrations at three locations (Brunsbüttel, Brokdorf, Lauenburg) of the River Elbe ranged from 0.1 to 1.3 μg/L (Feltes *et al.*, 1990).

Concentrations of 2,4-dinitrotoluene ranged from 700 to 1180 μg/L in groundwater samples collected near a former explosives factory in Elsnig, Germany (Steuckart *et al.*, 1994).

In wastewaters generated in the manufacture of 2,4,6-trinitrotoluene, 2,4-dinitrotoluene was found in all 54 samples at concentrations ranging from 40 to 48 600 μg/L over a 12-month sampling period (Spanggord *et al.*, 1982a).

In 1993, estimated quantities of 2,4-dinitrotoluene released into the environment by industrial facilities in the United States were 850 kg into air and 150 kg into water (United States National Library of Medicine, 1995).

2,6-Dinitrotoluene has also been detected in seawater, in raw wastewater from a textile plant and in wastewater from 2,4,6-trinitrotoluene production. It was found in Dokai Bay, Japan, at concentrations ranging from 'not detected' to 14.8 µg/L (Hashimoto *et al.*, 1982). It was found at concentrations ranging from 1.3 to 38.7 µg/L (mean, 19.4 µg/L) at Waconda Bay, Lake Chichamauga, TN, United States (Putnam *et al.*, 1981) and was detected at concentrations ranging from not detected to 76 800 µg/L (mean, 16 763 µg/L) in groundwater near a nitroaromatic plant in Pasadena, TX, United States (Matson *et al.*, 1986). The concentration of 2,6-dinitrotoluene in raw wastewater from a textile plant was 50 µg/L in one sample (Rawlings & Samfield, 1979) and averaged 4300 µg/L in 12/54 samples in wastewater from 2,4,6-trinitrotoluene production (Spanggord & Suta, 1982). It was also detected (5 µg/L) in wastewater from a nitrobenzene plant (Shafer, 1982). It was detected in effluents from coal mining (30 µg/L in 1/49 samples), iron and steel manufacture (range, 47–140 µg/L in 2/8 samples), nonferrous metals manufacture (max., 16 µg/L), foundries (mean, 20 µg/L in 6/6 samples; range 4–50 g/L), organic chemical manufacture (mean, 3800 µg/L in 4 samples), paint and ink formulations (max., 10 µg/L) and textile mills (54 µg/L in 1 sample) (United States Environmental Protection Agency, 1980; Howard, 1989).

2,6-Dinitrotoluene concentrations of 4.1 and 7.6 µg/L were detected in surface-water samples collected from two brooks near Hischagen/Waldhof, Germany, in the vicinity of munitions manufacture during the Second World War; the river into which the brooks fed (River Losse) had a concentration of 0.1 µg/L. Two ponds in the Clausthal-Zellerfeld region of Germany, again near previous munitions manufacture, had levels of 0.07 and 0.3 µg/L; the ponds feed into the River Oder which had a level of 0.02 µg/L. Concentrations at three locations (Brunsbüttel, Brokforf, Lauenburg) of the River Elbe ranged from 0.04 to 0.5 µg/L.

2,6-Dinitrotoluene was found in all 54 samples taken from wastewaters generated in the manufacture of 2,4,6-trinitrotoluene at a concentration ranging from 60 to 14 900 µg/L over a 12-month sampling period (Spanggord et al., 1982a).

The concentration of 2,6-dinitrotoluene ranged from not detected to 510 µg/L in groundwater samples collected near a former explosives factory in Elsnig, Germany (Steuckart et al., 1994).

In 1993, estimated quantities of 2,6-dinitrotoluene released into the environment by industrial facilities in the United States were 210 kg into air and 100 kg into water (United States National Library of Medecine, 1995).

3,5-Dinitrotoluene has been detected at concentrations ranging from 162 to 339 µg/L in condensate effluent resulting from the production of 2,4,6-trinitrotoluene (Spanggord & Suta, 1982).

In wastewaters generated in the manufacture of 2,4,6-trinitrotoluene, 3,5-dinitrotoluene was found in 51/54 samples with a range of 140–6480 µg/L over a 12-month sampling period (Spanggord *et al.*, 1982a).

(b) Soil and sediments

In the United States, both 2,4- and 2,6-dinitrotoluenes were detected in one of two soil samples taken near the Buffalo River at the former site of a dye manufacturing plant (Nelson & Hites, 1980). 2,4- and 2,6-Dinitrotoluenes were detected in sediment from Waconda Bay, Lake Chickamauga, TN, at concentrations ranging from < 2.5 to ≤ 7.9 µg/kg and < 1.3–17 µg/kg, respectively (Putnam *et al.*, 1981).

Concentrations of 2,4- and 2,6-dinitrotoluenes ranged from 3.4 to 226 mg/kg and 0.2 to 12.1 mg/g, respectively, in soil samples collected from two explosive ordnance sites in Mississippi and Alaska, United States (Jenkins & Walsh, 1992).

1.4 Regulations and guidelines

Occupational exposure limits and guidelines in several countries are presented in Table 2.

Table 2. Occupational exposure limits and guidelines for dinitrotoluenes (all isomers unless otherwise noted)

Country	Year	Concentration (mg/m^3)	Interpretation
Australia	1993	1.5 (Sk)	TWA
Belgium	1993	1.5 (Sk)	TWA
Bulgaria[a]	1995	0.15 (Sk)	TWA
Colombia[a]	1995	0.15 (Sk)	TWA
Czech Republic	1993	1	TWA
		2	STEL
Denmark	1993	1.5 (Sk)	TWA
Egypt	1993	1.5 (Sk)	TWA
Finland	1993	1.5 (Sk)	TWA
		3 (Sk)	STEL
Germany	1995	None (III A2) (Sk)	
India	1993	1.5 (Sk)	TWA
		5 (Sk)	STEL
Jordan[a]	1995	0.15 (Sk)	TWA
Netherlands	1994	1.5 (Sk)[b]	TWA
New Zealand[a]	1995	0.15 (Sk)	TWA
Philippines	1993	1.5 (Sk)	TWA
Poland	1991	1 (Sk)	TWA
Republic of Korea[a]	1995	0.15 (Sk)	TWA
Singapore[a]	1995	0.15 (Sk)	TWA
Switzerland	1993	1.5 (Sk,C)	TWA
Turkey	1993	1.5 (Sk)	TWA
United Kingdom	1995	None	
USA			
ACGIH (TLV)	1995	0.15 (Sk, A2)[c,d]	TWA
OSHA (PEL)	1994	1.5 (Sk)	TWA
NIOSH (REL)	1994	1.5 (Sk,Ca)	TWA

Table 2 (contd)

Country	Year	Concentration (mg/m^3)	Interpretation
Viet Nam[a]	1995	0.15 (Sk)	TWA

From International Labour Office (1991); Arbeidsinspectie (1994); United States National Institute for Occupational Safety and Health (NIOSH) (1994a,b); United States Occupational Safety and Health Administration (OSHA) (1994); American Conference of Governmental Industrial Hygienists (ACGIH) (1995); Deutsche Forschungsgemeinschaft (1995); Health and Safety Executive (1995); United Nations Environmental Programme (1995)

Sk, absorption through the skin may be a significant source of exposure; TWA, time-weighted average; STEL, short-term exposure limit; III A2, substances shown to be clearly carcinogenic only in animal studies but under conditions indicative of carcinogenic potential at the workplace; C, suspected of being a carcinogen; TLV, threshold limit value; A2, suspected human carcinogen; PEL, permissible exposure level; REL, recommanded exposure level; Ca, potential occupational carcinogen

[a] Follows ACGIH values

[b] 2,4-Dinitrotoluene only

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

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

2. Studies of Cancer in Humans

A retrospective cohort mortality study conducted by Levine *et al.* (1986) included workers exposed to dinitrotoluene at one of two munitions facilities in Joliet, IL, and Radford, VA, United States. The aim of this study was to investigate a possible interaction between exposure to dinitrotoluenes and incidence of liver cancer, as had been demonstrated in studies on animals (see Section 3). The study included 156 men from the Joliet facility and 301 men from the Radford facility with at least one month of exposure to dinitrotoluene during the 1940s and 1950s. The vital status of this cohort was ascertained to the end of 1980. Expected deaths and standardized mortality ratios (SMRs) were estimated using United States mortality rates for white men. The study failed to detect an increased risk for any cancer site. No death from liver or gall-bladder cancer was observed (0.5 expected). [The Working Group noted the low statistical power of this study.]

A study by Stayner *et al.* (1993) was conducted in one of the above two facilities (Radford, VA), although it differed in its identification of dinitrotoluene-exposed jobs and in the time period studied. This investigation included 4989 white male workers who

were exposed to dinitrotoluene for at least one day and had worked for at least five months at the Radford facility between 1949 and 1980. Workers in this study were exposed to a mixture of approximately 98% 2,4-dinitrotoluene and 2% 2,6-dinitrotoluene. The vital status of this cohort was ascertained until the end of 1982. SMRs were estimated using United States mortality rates and standardized rate ratios (SRRs) were estimated using mortality rates from an internal unexposed cohort of 7436 workers identified at the Radford facility. An excess of cancer of the 'biliary passages, liver and gall-bladder' (six cases) was observed in this study based upon comparisons with both the United States population (SMR, 2.7; 95% confidence interval (CI), 1.0–5.8) and the internal unexposed cohort (SRR, 3.9; 95% CI, 1.0–14.4). No other cancer site was at increased risk. Hospital or pathology records were available for five of the six cases and all were validated. It was not possible to conduct informative duration-response analysis because very few workers had more than five years of exposure to dinitrotoluene.

3. Studies of Cancer in Experimental Animals

2,4-Dinitrotoluene

3.1 Oral administration

3.1.1 *Mouse*

Groups of 50 male and 50 female B6C3F1 mice, approximately six weeks of age, were fed diets containing 0 (control), 0.008 or 0.04% [0, 80 or 400 mg/kg diet (ppm)] 'practical-grade' 2,4-dinitrotoluene [purity stated to be > 95% by the supplier] for 78 weeks. This was followed by 13 weeks of control diet for all groups, after which time the mice were killed and subjected to complete histopathological examination. Studies with the low and high doses of 2,4-dinitrotoluene were run at different times, and, therefore, each had its own control group. Mean body weights of treated mice were reduced compared to controls during the study; final body-weight reductions were 9% and 18% in low- and high-dose males and 11% and 24% in low- and high-dose females, respectively. Survival was 78% in high-dose males compared to 74% in concurrent controls and 90% in low-dose males compared to 82% in concurrent controls. The respective figures in females were 72% at the high dose (concurrent controls, 70%) and 84% at the low dose (concurrent controls, 78%). No increase in tumour incidence was reported for any site in treated mice (United States National Cancer Institute, 1978).

In a screening assay based on increased multiplicity and incidence of lung tumours in a strain of mice highly susceptible to the development of this neoplasm, groups of 26 male and 26 female strain A mice, six to eight weeks old, were given 2,4-dinitrotoluene (at a purity of 92–95%, with the major impurity being 2,6-dinitrotoluene) in tricaprylin by gavage twice a week for 12 weeks (total doses, 0, 1200, 3000 and 6000 (maximal tolerated dose, MTD) mg/kg bw). Surviving mice were killed 18 weeks after the last treatment and examined for the gross appearance of lung tumours. Survival was 45/50 in controls, 47/52 at the low dose, 48/52 at the mid dose and 44/52 at the high dose. There

was no increase in lung tumour incidence or in the number of lung tumours per mouse when compared to controls. The incidence of lung tumours in survivors was 27% in controls, 28% at the low dose, 31% at the mid dose and 23% at the high dose (Stoner *et al.*, 1984).

After two weeks of acclimatization, groups of 38 male and 38 female weanling Charles River CD-1 mice received diets containing 0, 0.01%, 0.07% and 0.5% [100, 700 and 5000 mg/kg diet (ppm)] 2,4-dinitrotoluene (98.5–99% 2,4-dinitrotoluene, 1–1.5% 2,6-dinitrotoluene; Lee *et al.*, 1985) for 24 months. After 12 months, eight males and eight females from each group were killed and necropsied. The remaining mice were killed at 24 months. Estimates of 2,4-dinitrotoluene intake for controls and low-, mid- and high-dose mice were 0, 14, 95 and 898 mg/kg bw per day, respectively. Body weights of low- and mid-dose females did not differ from those of controls. After three months, weight gains of mid-dose males were lower than those of controls [approximately 10%]; high-dose males and females were also lighter than controls [by approximately 22–35%]. Survival of high-dose mice began to decrease after month 8; median survival was reached in 21, 21, 19 and 9 months for control, low-, mid- and high-dose males and 20.5, 19, 20.5 and 10 months for control, low-, mid- and high-dose females, respectively. Tumours, described as cystic adenoma, cystic papillary adenoma, cystic papillary carcinoma and solid carcinoma, were observed in the renal tubular epithelium. The incidence of these tumours in males was 0/24 in controls, 6/22 at the low dose, 16/19 at the mid dose and 10/29 at the high dose; 2/8 high-dose males examined at one year also had a renal tumour. One of 23 high-dose females developed a renal tumour at 23 months (Hong *et al.*, 1985).

3.1.2 *Rat*

Groups of 50 male and 50 female Fischer 344 rats, approximately six weeks of age, were fed diets containing 0, 0.008 (0.0075% for the first 19 weeks) or 0.02% [0, 80 (75) or 200 mg/kg diet (ppm)] 'practical-grade' 2,4-dinitrotoluene [purity stated to be > 95% by the supplier] for 78 weeks followed by control diet for 26 weeks. After this time they were killed and subjected to complete histopathological examination. The studies with low- and high-dose rats were run at different times and, therefore, each had its own control group. Mean body weights of treated rats were reduced compared to controls during the study; compared to their concurrent controls, body-weight reductions were very slight in low-dose males, 20–25% in high-dose males, variable in low-dose females and up to 18% in high-dose females. Survival was 58% in high-dose males compared to 52% in concurrent controls and 58% in low-dose males compared to 64% in concurrent controls. The respective figures in females were 52% at the high dose (concurrent controls, 48%) and 62% at the low dose (concurrent controls, 62%). No integumentary tumours were seen in controls, but the incidences of these tumours were increased in low- and high-dose males — fibromas of the skin/subcutaneous tissue occurred in 7/49 low-dose and 13/49 high-dose males; lipomas occurred in 3/49 high-dose males; fibrosarcomas occurred in 1/49 low-dose and 2/49 high-dose males, and squamous-cell papillomas and basal-cell carcinomas each occurred in single low-dose males. In treated

females, the incidence of fibroadenomas of the mammary gland was increased (23/50 at the high dose versus 4/23 in controls; $p < 0.016$, Fisher's exact test) (United States National Cancer Institute, 1978).

After two weeks acclimatization, groups of 38 male and 38 female weanling Charles River CD rats received diets containing 0%, 0.0015%, 0.01% and 0.07% [15, 100 and 700 mg/kg diet (ppm)] 2,4-dinitrotoluene (98.5–99% 2,4-dinitrotoluene, 1-1.5% 2,6-dinitrotoluene) for up to 24 months. After 12 months, eight males and eight females from each group were killed and necropsied. The remaining rats were killed at 24 months. Estimates of 2,4-dinitrotoluene intake for control, low-, mid- and high-dose males were 0, 0.57 ± 0.02, 3.92 ± 0.15 and 34.5 ± 0.8 mg/kg bw per day, respectively, and those for females were 0, 0.71 ± 0.02, 5.14 ± 0.18 and 45.3 ± 1.4 mg/kg bw per day, respectively. Body weights of low-dose rats did not differ from those of controls. Weight gains of mid-dose rats were lower after month 9 [by as much as approximately 12% in males and females]. [High-dose rats were up to approximately 33–38% lighter than controls.] Survival of low- and mid-dose rats did not differ from that of controls, whereas all high-dose males and all but one high-dose females died before the end of the study, with 50% mortality being reached between 19 and 20 months for each sex. [Survival was approximately 40–45% for the controls.] At 12 months, neoplastic nodules of the liver were diagnosed in 1/8 low-dose males, 6/7 high-dose males and 7/8 high-dose females with hepatocellular carcinoma in 1/8 high-dose females. None was found in the other groups. In rats surviving longer than 12 months, the incidences of hepatocellular neoplastic nodules and hepatocellular carcinomas were as follows: males — hepatocellular neoplastic nodules: 1/25 in controls, 2/27 at the low dose, 1/19 at the mid dose, 2/27 at the high dose; hepatocellular carcinomas: 1/25, 0/27, 1/19, 6/27, respectively [$p = 0.06$; Fisher's exact test]; females — hepatocellular neoplastic nodules: 0/23 in controls, 2/28 at the low dose, 2/26 at the mid dose, 5/25 at the high dose; hepatocellular carcinomas: 0/23, 0/28, 1/26, 10/25, respectively [$p = 0.03$; Fisher's exact test]. The incidences of mammary gland tumours (predominantly fibroadenomas) in females were 11/23 in controls, 1/28 at the low dose, 16/26 at the mid dose and 21/25 at the high dose [$p < 0.05$; Dunnett's test]; the incidences of skin tumours (mostly fibromas) in males were 2/25 in controls, 4/27 at the low dose, 3/19 at the mid dose and 15/27 at the high dose [$p < 0.05$; Dunnett's test] (Lee *et al.*, 1985).

Groups of 28 male CDF (Fischer 344)/CrlBR rats, weighing 130–150 g and having been in quarantine for four weeks, received a control diet or a diet containing a sufficient quantity of 2,4-dinitrotoluene ['purified', but purity unspecified] to provide a dose of 27 mg/kg bw per day for 52 weeks. The rats were then killed and the livers and lungs evaluated histopathologically. Body-weight gain of the treated group was about 25% less than that of the controls at the end of the study. No early death was indicated. Hepatocyte degeneration and vacuolization were apparent in the majority of treated animals; acidophilic and basophilic foci were reported in 70% and 10% of the livers of treated rats, respectively. One treated rat had a hepatic neoplastic nodule and no liver tumour was noted in the control group (Leonard *et al.*, 1987). [The Working Group noted the small numbers of animals used, the short duration of the study and the incomplete histopathology.]

3.2 Intraperitoneal injection

Mouse: In a screening assay based on increased multiplicity and incidence of lung tumours in a strain of mice highly susceptible to the development of this neoplasm, groups of 52 or 53 male and female strain A mice, six to eight weeks old, were given thrice weekly intraperitoneal injections of 2,4-dinitrotoluene (at a purity of 92–95%, with the major impurity being 2,6-dinitrotoluene) in tricaprylin for eight weeks (total doses, 0, 600, 1500 and 3000 (MTD) mg/kg bw). Surviving mice were killed 22 weeks after the last injection and lung tumours appearing grossly were counted. Survival was 52/52 in controls, 52/53 at the low dose, 52/52 at the mid dose and 50/52 at the high dose. There was no increase in lung tumour incidence or in the number of lung tumours per mouse when compared to controls. The incidence of lung tumours in surviving mice was 29% in controls, 44% at the low dose, 19% at the mid dose and 26% at the high dose (Schut *et al.*, 1982; Stoner *et al.*, 1984).

2,6-Dinitrotoluene

3.1 Oral administration

3.1.1 *Mouse*

In a screening assay based on increased multiplicity and incidence of lung tumours in a strain of mice highly susceptible to the development of this neoplasm, groups of 26 male and 26 female strain A mice, six to eight weeks old, were given 2,6-dinitrotoluene (at a purity of 98%) in tricaprylin twice a week by gavage for 12 weeks (total doses, 0, 1200, 3000 and 6000 (MTD) mg/kg bw). Surviving mice were killed 18 weeks after the last dose and examined for the gross appearance of lung tumours. Survival was 45/50 in controls, 49/52 at the low dose, 50/52 at the mid dose and 38/52 at the high dose. There was no increase in lung tumour incidence or in the number of lung tumours per animal when compared to controls. The incidence of lung tumours in surviving mice was 27% in controls, 18% at the low dose, 22% at the mid dose and 34% at the high dose (Stoner *et al.*, 1984).

3.1.2 *Rat*

In a study designed to evaluate the influence of pectin-induced changes in gut microflora on the hepatocarcinogenicity of 2,6-dinitrotoluene, groups of 30 male CDF (Fischer 344)/CrlBR rats, weighing 130–150 g and having been in quarantine for two weeks, were placed on one of three diets containing sufficient quantities of 2,6-dinitrotoluene (at a purity of 99.9%) to produce daily doses of 0, 0.6–0.7 or 3–3.5 mg/kg bw. Ten animals from each treatment group were killed at three, six and 12 months and livers were evaluated histopathologically. The diets used were open formula, cereal-based NIH-07, purified AIN-76A and AIN-76A with 5% added pectin. Groups receiving the high dose of 2,6-dinitrotoluene gained about 10% less weight than the respective control groups. No early death was reported. The number and size of γ-glutamyl transpeptidase (γ-GT)-staining foci increased in a time- and dose-dependent manner in animals given

2,6-dinitrotoluene in the NIH-07 diet. The group on the NIH-07 diet receiving the high dose of 2,6-dinitrotoluene exhibited a 100% incidence of liver tumours (6/10 with hepatocellular carcinomas and/or 6/10 with neoplastic nodules) at 12 months. Three of 10 rats receiving the low dose in NIH-07 diet had neoplastic nodules at 12 months. No tumour was observed in rats receiving the control diets or 2,6-dinitrotoluene in the AIN-76A diet with or without added pectin (Goldsworthy *et al.*, 1986). [The Working Group noted that pectin did not influence the tumour outcome in this experiment].

Groups of 28 male CDF (Fischer 344)/CrlBR rats, weighing 130–150 g and having been in quarantine for four weeks, received a control diet or a diet containing a sufficient quantity of 2,6-dinitrotoluene ['purified', but purity unspecified] to provide doses of 7 or 14 mg/kg bw per day for 52 weeks. After this time, the rats were killed and the livers and lungs evaluated histopathologically. Body-weight gains of the low-dose and high-dose groups were about 18% and 32% less than those of the controls at the end of the study. No early death was indicated. Hepatocyte degeneration and vacuolization were apparent in the majority of treated animals; acidophilic and basophilic foci were reported in over 90% of treated rats. No liver tumour was noted in the control group. Neoplastic nodules were found in 18/20 rats at the low dose and 15/19 at the high dose. Hepatocellular carcinomas, described as trabecular, occurred in 17/20 at the low dose and 19/19 at the high dose, and one tumour described as an adenocarcinoma was found in a low-dose rat. Cholangiocarcinomas occurred in 2/20 low-dose rats. Liver tumours metastasized to the lung in 3/20 rats at the low dose and 11/19 at the high dose (Leonard *et al.*, 1987).

3.2 Intraperitoneal injection

Mouse: In a screening assay based on increased multiplicity and incidence of lung tumours in a strain of mice highly susceptible to the development of this neoplasm, groups of 26 male and 26 female strain A mice, six to eight weeks old, were given thrice weekly intraperitoneal injections of 2,6-dinitrotoluene (at a purity of 98%) in tricaprylin for eight weeks (total doses, 0, 600, 1500 and 3000 (MTD) mg/kg bw). Surviving mice were killed 22 weeks after the last injection and were examined for the gross appearance of lung tumours. Survival was 52/52 in controls, 50/52 at the low dose, 51/52 at the mid dose and 47/52 at the high dose. There was no increase in lung tumour incidence or in the number of lung tumours per mouse when compared to controls. The incidences of lung tumours in surviving mice were 29% in controls, 34% at the low dose, 45% at the mid dose and 30% at the high dose (Stoner *et al.*, 1984).

3,5-Dinitrotoluene

No adequate data were available to the Working Group.

Technical grades of dinitrotoluene

3.1 Oral administration

3.1.1 *Mouse*

In a screening assay based on increased multiplicity and incidence of lung tumours in a strain of mice highly susceptible to the development of this neoplasm, groups of 26 male and 26 female strain A mice, six to eight weeks old, were given 2 : 1 mixtures of 2,4-dinitrotoluene (at a purity of 92–95%, with the major impurity being 2,6-dinitrotoluene) and 2,6-dinitrotoluene (at a purity of 98%) in tricaprylin twice a week by gavage for 12 weeks (total doses, 0, 1200, 3000 and 6000 (MTD) mg/kg bw). Surviving mice were killed 18 weeks after the last dose and were examined for the gross appeareance of lung tumours. Survival was 45/50 in controls, 48/52 at the low dose, 48/52 at the mid dose and 48/52 at the high dose. There was no increase in lung tumour incidence or in the number of lung tumours per mouse when compared to controls. The incidence of lung tumours in survivors was 27% in controls, 35% at the low dose, 35% at the mid dose and 33% at the high dose (Stoner *et al.*, 1984).

3.1.2 *Rat*

Popp and Leonard (1982), Rickert *et al.* (1984) and Leonard *et al.* (1987) reported various aspects of a study on the carcinogenicity of technical-grade dinitrotoluene (76.5% 2,4-dinitrotoluene, 18.8% 2,6-dinitrotoluene, 2.43% 3,4-dinitrotoluene, 1.54% 2,3- dinitrotoluene, 0.69% 2,5-dinitrotoluene and 0.04% 3,5-dinitrotoluene) in Fischer 344 rats. In Rickert *et al.* (1984), data that outlined the incidence of hepatic neoplastic lesions in Fischer 344 rats at all sample intervals were presented in tabular form. The compound was given in the diet at doses of 0, 3.5, 14 and 35 mg/kg bw per day. Due to the mortality in animals at the high-dose level, the final kill of these animals was carried out at 55 weeks. At the high-dose level, the incidences of hepatocellular carcinomas in males were 2/10 at 26 weeks, 10/10 at 52 weeks and 20/20 at 55 weeks, and in females were 0/10, 4/10 and 11/20, respectively; the incidences of neoplastic nodules in males were 0/10 at 26 weeks, 3/10 at 52 weeks and 5/20 at 55 weeks, and in females were 0/10, 8/10 and 12/20, respectively. In the mid-dose group sampled at 26, 52, 78 and 104 weeks, the incidences of hepatocellular carcinomas in males were 0/10, 3/10, 19/20 and 22/23, and in females were 0/10, 0/10, 10/20 and 41/63, respectively; similarly, the incidences of neoplastic nodules were 0/10, 4/10, 11/20 and 16/23 in males, and 0/10, 0/10, 0/20 and 53/69 in females. In the low-dose group, no liver tumour was observed in any animal at 26 or 52 weeks. At 104 weeks, the incidences of hepatocellular carcinomas were 9/70 and 12/61 in males and females, respectively (neoplastic nodules, 11/70 and 12/61, respectively). In control animals, a single hepatocellular carcinoma was observed in males (1/61) at 104 weeks, at which time neoplastic nodules were also seen in 9/61 males and 5/57 females.

Groups of 28 male CDF (Fischer 344)/CrlBR rats, weighing 130–150 g and having been in quarantine for four weeks, received a control diet or a diet containing a sufficient quantity of technical-grade dinitrotoluene (76.5% 2,4-dinitrotoluene, 18.8% 2,6-dinitro-

toluene, 2.43% 3,4-dinitrotoluene, 1.54% 2,3-dinitrotoluene, 0.69% 2,5- dinitrotoluene and 0.04% 3,5-dinitrotoluene) to provide a dose of 35 mg/kg bw per day for 52 weeks. After this time the rats were killed and the livers and lungs were evaluated histopathologically. Body-weight gain of the treated rats was about 26% less than that of the controls at the end of the study. Hepatocyte degeneration and vacuolization were apparent in the majority of treated animals; acidophilic and basophilic foci were reported in over 90% of treated animals. No liver tumour was noted in the control group. Neoplastic nodules were found in 10/19 treated rats, hepatocellular carcinomas (trabecular) in 9/19 and cholangiocarcinomas in 2/19 (Leonard *et al.*, 1987).

3.2 Intraperitoneal injection

Mouse: In a screening assay based on increased multiplicity and incidence of lung tumours in a strain of mice highly susceptible to the development of this neoplasm, groups of 26 male and 26 female strain A mice, six to eight weeks old, were given thrice weekly intraperitoneal injections of a 2 : 1 mixture of 2,4-dinitrotoluene (92–95% pure with the major impurity being 2,6-dinitrotoluene) and 2,6 dinitrotoluene (with a purity of 98%) in tricaprylin for eight weeks (total doses, 0, 960, 2400 and 4800 (MTD) mg/kg bw). Surviving mice were killed 22 weeks after the last injection and were examined for the gross appearance of lung tumours. Survival was 52/52 in controls, 48/52 at the low dose, 50/52 at the mid dose and 40/52 at the high dose. There was no increase in lung tumour incidence or in the number of lung tumours per mouse when compared to controls. The incidence of lung tumours in surviving mice was 29% in controls, 33% at the low dose, 28% at the mid dose and 23% at the high dose (Stoner *et al.*, 1984).

Initiation–promotion experiments with various dinitrotoluenes

Oral administration

Rat: Popp and Leonard (1982) and Leonard *et al.* (1983) summarized results with several standard initiation–promotion assays in CDF (Fischer 344)/CrlBR rats, using technical-grade dinitrotoluene (76% 2,4-dinitrotoluene, 18% 2,6-dinitrotoluene and less than 3% of each 2,3-dinitrotoluene, 2,5-dinitrotoluene, 3,4-dinitrotoluene and 3,5-dinitrotoluene) and purified 2,6-, 2,3-, 2,4-, 2,5-, 3,4- and 3,5-dinitrotoluene isomers [of unspecified purity]. They reported weak hepatocyte-initiating activity (scored as γ-GT-positive foci) of technical-grade dinitrotoluene and 2,6-dinitrotoluene. No initiating activity was demonstrated with the other isomers.

The liver foci-promoting activity of technical-grade dinitrotoluene (76.5% 2,4-dinitrotoluene, 18.8% 2,6-dinitrotoluene, 1.5% 2,3-dinitrotoluene, 0.7% 2,5-dinitrotoluene, 2.4% 3,4-dinitrotoluene and 0.1% 3,5-dinitrotoluene) was compared to that of purified 2,4-dinitrotoluene and 2,6-dinitrotoluene (purity > 99.4% for both isomers) in a male CDF (Fischer 344)/CrlBR rat hepatic initiation–promotion protocol. Rats weighing 130–150 g were given a single dose of 150 mg/kg bw *N*-nitrosodiethylamine by intraperitoneal injection. Two weeks later, the animals were placed on diets containing 0.47% 2,4-dinitrotoluene, 0.06, 0.12 or 0.24% 2,6-dinitrotoluene or 0.55 or 0.2% technical-

grade dinitrotoluene. Rats receiving technical-grade dinitrotoluene were killed after three or six weeks of feeding and those receiving the purified isomers after six or 12 weeks of feeding. Liver foci scored as γ-GT-positive were increased in all treatments suggesting that 2,4- and 2,6-dinitrotoluenes and technical-grade dinitrotoluene all have promoting potential (Leonard *et al.*, 1986).

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

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

The metabolism of dinitrotoluenes has been reviewed (Rickert *et al.*, 1984; Rickert, 1987).

Metabolites of 2,4- and 2,6-dinitrotoluenes have been identified in the urine of workers exposed to technical-grade dinitrotoluene (about 80% 2,4-dinitrotoluene and 20% 2,6-dinitrotoluene). Metabolites of 2,4-dinitrotoluene were 2,4-dinitrobenzoic acid, 2-amino-4-nitrobenzoic acid, 2,4-dinitrobenzyl glucuronide and 2-(*N*-acetyl)amino-4-nitrobenzoic acid; and metabolites of 2,6-dinitrotoluene were 2,6-dinitrobenzoic acid and 2,6-dinitrobenzyl glucuronide (2-amino-6-nitrobenzoic acid was not detected). In addition, the urine contained unchanged dinitrotoluenes. 2,4-Dinitrobenzoic acid and 2-amino-4-nitrobenzoic acid accounted collectively for 74–86% of the dinitrotoluene metabolites detected. The elimination half-life of total dinitrotoluene-related material detected in urine ranged from 1.0 to 2.7 h (Turner *et al.*, 1985).

In 17 workers exposed to technical-grade dinitrotoluene in a dinitrotoluene production plant, personal air sampling revealed levels ranging from 0.6 to 5.9 mg/10 m^3 (0.06–0.59 mg/m^3) 2,4- and 2,6-dinitrotoluenes. The wiping of skin suspected of being contaminated (mainly hands and forehead) showed levels of 'not detected' (< 2 μg) to 179.5†μg 2,4-dinitrotoluene [area unspecified]. 2,4- and 2,6-Dinitrobenzoic acids, 2,4- and 2,6-dinitrobenzyl glucuronides, 2-amino-4-nitrobenzoic acid and 2-(*N*-acetyl)amino-4-nitrobenzoic acid (but not 2-amino-6-nitrobenzoic acid) were found in the urine. Possibly, there was a sex difference as regards the pattern of metabolites; three women appeared to excrete relatively more dinitrobenzyl glucuronides than 14 men (33.3 versus 9.5% of all metabolites). The urine contained more metabolites than would have resulted from the dinitrotoluene present in the inhaled air, which strongly indicated dermal absorption. A rough estimate of the maximal absorbed daily dose encountered indicated an exposure of 0.24–1.00 mg/kg bw in one worker (Levine *et al.*, 1985).

Urinary metabolites have been used for biomonitoring of workers exposed to technical grade dinitrotoluene. 2,4-Dinitrobenzoic acid was the major metabolite found in urine, and appeared within hours of the onset of exposure. In the 28 workers studied (Woolen *et al.*, 1985), the highest concentrations were found in post-shift samples, which contained 17 mg/L 2,4-dinitrobenzoic acid on average. The levels varied considerably

between workers and in individual workers from day to day. The atmospheric levels of dinitrotoluene were so low (range, 0.03–0.1 mg/m^3 personal sampling) that the authors concluded that the skin may be a major route of absorption. Pre-shift urinary samples contained very low levels of metabolites (< 1 mg/L), which shows that a large fraction has a rapid turn over — the elimination half-life of 2,4-dinitrobenzoic acid was 2–5 h. However, traces could still be detected after several days, which indicates the presence of a slow compartment. In addition to the metabolites described by Turner *et al.* (1985) and Levine *et al.* (1985), the urine also contained 2-amino-6-nitrobenzoic acid. 2,4- and 2,6-Dinitrotoluenes were found in blood samples.

Guest *et al.* (1982) showed that the faecal material and ileal contents from a patient with ileostomy were capable of metabolizing 2,4-dinitrotoluene, producing nitrosonitrotoluenes and aminonitrotoluenes. Mori *et al.* (1984a) found that all six dinitrotoluene isomers were metabolized to aminonitrotoluenes by *Escherichia coli* isolated from human intestinal contents.

No data on 2,3-dinitrotoluene were available to the Working Group.

4.1.2 *Experimental systems*

(*a*) *Whole animals*

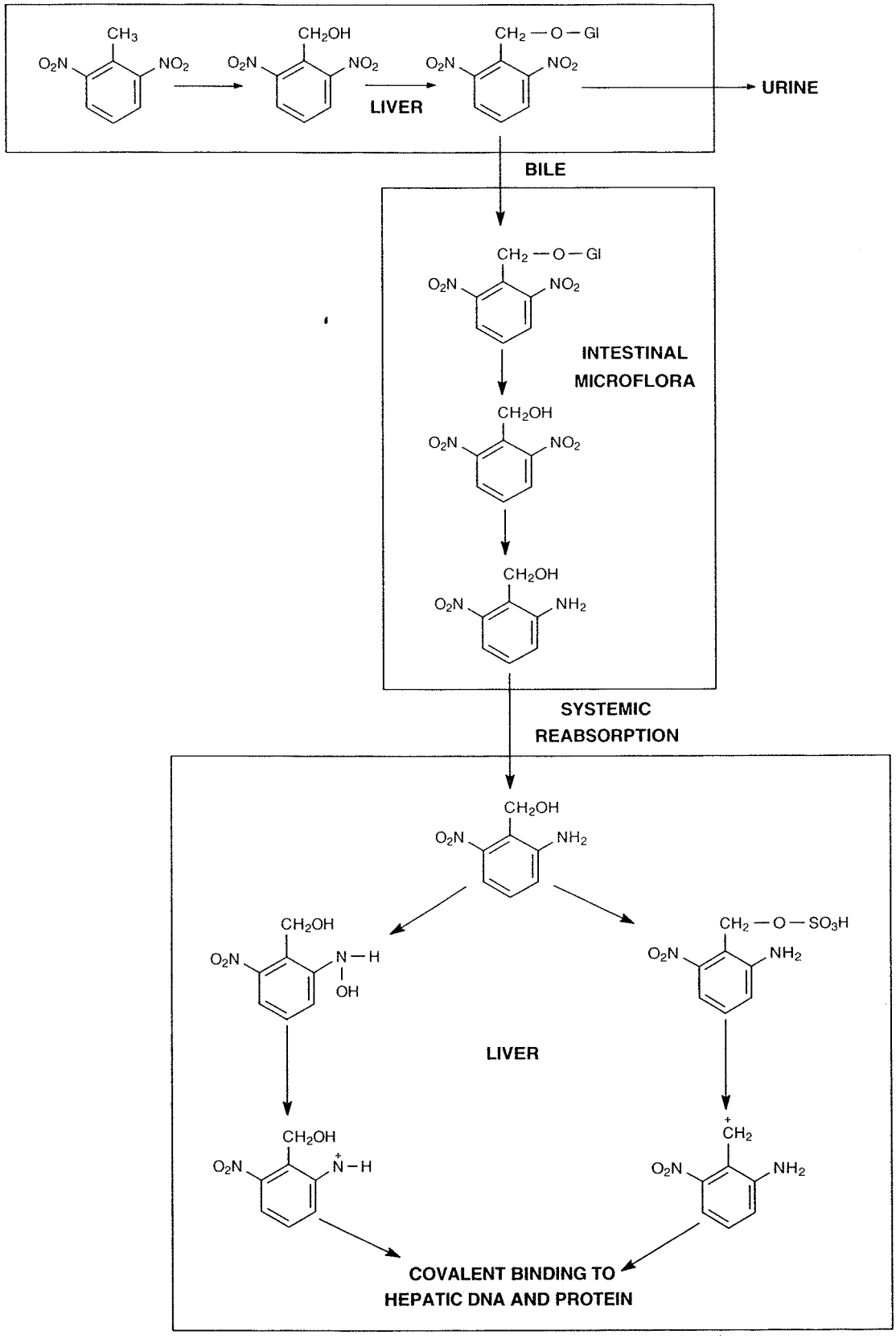
A scheme for the metabolism of 2,6-dinitrotoluene is presented in Figure 1.

Male Wistar rats (200 g) were given an oral dose of 22.2 mg/kg bw 2,4-dinitro-[^{3}H]-toluene in salad oil [not identified] (Mori *et al.*, 1978). Radioactivity in blood and liver reached a peak at 6 h after administration with an elimination half-life from blood of 22 h [liver elimination half-life unspecified]. Approximately 10% of the radioactivity administered was excreted in the bile within 24 h. After seven days, about 46% of the radioactivity was excreted in urine or faeces (Mori *et al.*, 1977).

The distribution of uniformly [^{14}C]-ring-labelled 2,4-dinitrotoluene after oral gavage in corn oil was investigated in male Fischer 344 rats (80–90 days old) at three doses (10, 35 and 100 mg/kg bw) and in females at a dose of 100 mg/kg bw (Rickert & Long, 1980). In male rats, terminal elimination half-lives of total radioactivity ranged from 61 (for the two lowest doses) to 27 h (for the high dose) in plasma and from 51 to 36 h in livers, respectively. Terminal elimination half-lives of radioactivity were similar in male and female rats; however, in female livers, concentrations of radioactivity were only one-half of those found in males. The tissues of both sexes contained unmetabolized 2,4-dinitrotoluene, 2,4-dinitrobenzoic acid and 2-amino-4-nitrobenzoic acid. No evidence of the presence of 2,4-diaminotoluene was found in any tissue examined.

Rickert and Long (1981) also investigated the metabolism and excretion of 2,4-dinitrotoluene in a separate study of male and female Fischer 344 rats given 10, 35 or 100 mg/kg bw uniformly [^{14}C]-ring-labelled 2,4-dinitrotoluene by oral gavage in corn oil. In both males and females, urine was the major route of excretion of 2,4-dinitrotoluene metabolites at all doses: 4-(*N*-acetyl)amino-2-nitrobenzoic acid, 2,4-dinitrobenzoic acid, 2-amino-4-nitrobenzoic acid and 2,4-dinitrobenzyl alcohol glucuronide were the major metabolites, comprising > 85% of the radioactivity excreted in rat urine. Both male and

Figure 1. Metabolism of 2,6-dinitrotoluene



From Chism & Rickert (1985)

female rats showed dose-dependent changes in urinary excretion of 2,4-dinitrotoluene metabolites, with a smaller percentage of the dose being excreted in urine at the higher concentrations. The most significant sex-dependent difference was the greater percentage of 2,4-dinitrotoluene excreted as 2,4-dinitrobenzyl alcohol glucuronide by female rats after the 10- or 35-mg/kg dose. The finding that urine was the major route for elimination of ^{14}C is in agreement with a report by Lee *et al.* (1975); however, Mori *et al.* (1978) found that the faeces were the major route of 2,4-dinitrotoluene excretion. This difference among studies may be due to strain differences or to the use of 2,4-dinitro-^{3}H-toluene by Mori *et al.* (1978).

Rickert *et al.* (1981) explored the role of gut metabolism in the activation of 2,4-dinitrotoluene in Fischer 344 rats (80–90 days old) weighing 120–150 g (females) or 200–250 g (males). Both conventional and axenic rats received a single oral dose of 35 mg/kg bw uniformly [^{14}C]-ring-labelled 2,4-dinitrotoluene by gavage. Throughout the study, axenic rats, which are lacking in intestinal microflora, were housed in sterile isolation units. Conventional rats were housed in typical temperature- and humidity-controlled exposure rooms. Axenic males and females excreted a smaller fraction of the 2,4-dinitrotoluene dose in the urine than did conventional animals. Most notably, amounts of 4-(*N*-acetyl)amino-2-nitrobenzoic acid and 2-amino-4-nitrobenzoic acid excreted by axenic animals were one-tenth to one-fifth of those excreted by conventional animals. Additionally, hepatic covalent binding was decreased by one-half in axenic animals. These data suggested to the authors that intestinal microflora play a major role in the appearance of reduced urinary metabolites and of covalently bound material after 2,4-dinitrotoluene administration.

The biliary excretion and enterohepatic circulation of 2,4-dinitrotoluene was investigated in male Fischer 344 rats (200 g) given oral doses of 35, 63 or 100 mg/kg bw uniformly [^{14}C]-ring-labelled 2,4-dinitrotoluene in corn oil or female rats (160 g) given 35 mg/kg bw uniformly [^{14}C]-ring-labelled 2,4-dinitrotoluene (Medinsky & Dent, 1983). The excretion of ^{14}C in bile of male rats was related linearly to dose, with biliary elimination of ^{14}C accounting for approximately 25% of the dose. After a dose of 35 mg/kg bw 2,4-dinitrotoluene, females excreted less ^{14}C in the bile than males (18% of the dose). Over 90% of the radioactivity in the bile was identified as the glucuronide conjugate of 2,4-dinitrobenzyl alcohol. Biliary elimination half-lives for ^{14}C ranged from 3.3 to 5.3 h.

Sayama *et al.* (1989a) also investigated the biliary excretion of 2,4-dinitrotoluene, 2,4-dinitrobenzyl alcohol and 2,4-dinitrobenzaldehyde in male Wistar rats (180–220 g). 2-Acetylamino-4-nitrotoluene, 2,4-dinitrobenzyl alcohol, 2,4-dinitrobenzaldehyde and unchanged 2,4-dinitrotoluene were detected in the nonhydrolysed neutral basic fraction of bile from rats dosed orally with 2,4-dinitrotoluene (40 mg/kg bw in salad oil [mixed vegetable oil]). The major biliary metabolite of 2,4-dinitrotoluene in male Wistar rats was 2,4-dinitrobenzyl alcohol glucuronide (11.8% of the dose). A variety of minor metabolites, most notably 2,4-dinitrobenzaldehyde (0.27%) and 4-amino-2-nitro(2-amino-4-nitro)benzyl alcohol sulfate (1.5%), were also formed. Similar metabolites were eliminated in the bile after oral administration of 2,4-dinitrobenzyl alcohol and 2,4-dinitrobenzaldehyde.

The metabolism and excretion of the isomer 2,6-dinitrotoluene was investigated by Long and Rickert (1982) in male and female Fischer 344 rats (80–90 days old, 200 and 150 g, respectively). Uniformly [^{14}C]-ring-labelled 2,6-dinitrotoluene (> 99% radiochemically pure) was dissolved in corn oil and administered by oral gavage (10 mg/kg bw). The major route of excretion of ^{14}C after a single dose was via the urine (males, 53.6 ± 2.6%; females, 54.0 ± 4.8%). Faecal excretion accounted for 17.9% (males) and 19.8% (females) of the dose. Analysis of the urine by HPLC revealed three major metabolites that accounted for 95% of the urinary ^{14}C. These metabolites were identified as 2,6-dinitrobenzoic acid, 2,6-dinitrobenzyl alcohol glucuronide and 2-amino-6-nitrobenzoic acid. No sex-dependent difference in the total amounts of the individual metabolites was noted. These results were analogous to those found after administration of 10 mg/kg bw 2,4-dinitrotoluene to male and female Fischer 344 rats. The only major difference in the disposition of the two isomers is that no *N*-acetylaminonitrobenzoic acid was found after administration of 2,6-dinitrotoluene *in vitro*. This may reflect steric hindrance to *N*-acetylation of an amino group adjacent to a methyl group.

Mori *et al.* (1989a) also reported on the metabolism of 2,6-dinitrotoluene in male Wistar rats (180–200 g). Rats were dosed orally with a solution of 2,6-dinitrotoluene (75 mg/kg bw) in 1 ml of salad oil [type unspecified]. The bile ducts of some rats were cannulated prior to dosing. The major urinary metabolite identified by chromatography with standards was 2,6-dinitrobenzyl alcohol, which represented 1.5% of the dose excreted in 24 h. The glucuronide conjugate of 2,6-dinitrobenzyl alcohol was eliminated in the bile and accounted for 30% of the dose excreted in 24 h. Small percentages (< 0.1%) of other metabolites were also detected in bile. In these studies, no 2,6-dinitrobenzoic acid was detected in the urine. [This discrepancy between the findings of Mori *et al.* (1989a) and those of Long and Rickert (1982) may be explained by methodological differences, or may indicate strain differences in the metabolism of 2,6-dinitrotoluene.]

Chadwick *et al.* (1990) examined the gastrointestinal enzyme activity and activation of 2,6-dinitrotoluene in male CD-1 mice (38.2 g) and male Fischer 344 rats (204 g) dosed orally with 75 mg/kg bw in dimethyl sulfoxide (DMSO). Mice metabolized significantly more 2,6-dinitrotoluene to mutagenic urinary metabolites than did Fischer 344 rats. Mutagenicity was evaluated by adding β-glucuronidase-hydrolysed urine samples to cultures of *Salmonella typhimurium* strain TA98 and quantitating revertants detected in urine. The investigators noted that native intestinal nitroreductase activity was markedly higher in the CD-1 mouse than in the Fischer 344 rat and that this correlated with the increase in revertants. In contrast to these observations, a comparison of the metabolism and binding of 2,6-dinitrotoluene in Fischer 344 rats and A/J mice indicated a higher hepatic DNA binding of 2,6-dinitrotoluene in the rats (Dixit *et al.*, 1986). In addition, the metabolism of 2,6-dinitrotoluene was very slow in the small intestines of A/J mice (Schut *et al.*, 1983).

The hepatic macromolecular covalent binding and intestinal disposition of 2,6-dinitrotoluene was compared with that of 2,4-dinitrotoluene (Rickert *et al.*, 1983). Male Fischer 344 rats, 80–90 days old, received 10 or 35 mg/kg bw of each uniformly [^{14}C]-ring-labelled isomer orally by gavage in corn oil. Covalent binding of radioactivity

after administration of 2,6-dinitrotoluene was always two- to five-fold higher than binding after administration of 2,4-dinitrotoluene.

Further studies comparing the hepatic macromolecular covalent binding of 2,6- and 2,4-dinitrotoluenes were conducted by Kedderis *et al.* (1984) in male Fischer 344 rats (180–260 g). Animals were administered intraperitoneal injections of the sulfotransferase inhibitors 2,6-dichloro-4-nitrophenol or pentachlorophenol (40 μmol/kg bw) in propane-1,2-diol or vehicle alone. Subsequently, animals were dosed orally with 2,6-dinitro[3-^{3}H]toluene or uniformly [^{14}C]-ring-labelled 2,4-dinitrotoluene dissolved in corn oil. At 12 h after dinitrotoluene administration, the livers were removed and processed for isolation of DNA, and urine was analysed by HPLC. Prior administration of the sulfotransferase inhibitors resulted in a significant decrease in the hepatic macromolecular covalent binding of both isomers with the decrease being more pronounced for 2,6-dinitrotoluene. Covalent binding to hepatic DNA was reduced by > 95% (2,6-dinitrotoluene) or > 84% (2,4-dinitrotoluene). The authors suggested that metabolites formed via a sulfotransferase-dependent pathway were responsible for the majority of the covalent binding of 2,6-dinitrotoluene to hepatic DNA.

(b) In-vitro studies

(i) *Subcellular hepatic fractions*

Postmitochondrial supernatants from the livers of CD rats, CD-1 mice, New Zealand albino rabbits, beagle dogs and rhesus monkeys were compared in their relative capacities to metabolize 1 mM 2,4-dinitro[^{14}C]toluene (Short *et al.*, 1979). All species metabolized 2,4-dinitrotoluene. The production of dinitrobenzyl alcohol was generally greater in rabbits, dogs and monkeys than in rats and mice.

Decad *et al.* (1982) examined the hepatic microsomal metabolism and covalent binding of 2,4-dinitro[^{14}C]toluene in hepatic microsomes from adult male Fischer 344 rats (180–200 g). Incubations contained a microsomal protein concentration of 2 mg/mL and a substrate concentration of 200 nmol. The pattern of 2,4-dinitrotoluene metabolism was dependent on oxygen tension. Under aerobic conditions, 2,4-dinitrobenzyl alcohol was the major metabolite formed. In contrast, under anaerobic conditions no dinitrobenzyl alcohol was detected and 4-amino-2-nitrotoluene and 2-amino-4-nitrotoluene were the major metabolites. The authors suggested that oxidative metabolism of 2,4-dinitrotoluene to dinitrobenzyl alcohol was mediated by cytochrome P450-dependent mixed-function oxidases, and that the capacity to oxidize the alcohol further probably resided in the cytosol and might be catalysed by alcohol and aldehyde dehydrogenase.

Chapman *et al.* (1992) investigated the metabolism of 100 μM 2,6-dinitro[^{3}H]toluene by liver microsomal and cytosolic fractions obtained from male Fischer 344 rats (200–300 g) under aerobic (100% oxygen) and anaerobic (100% nitrogen) incubation conditions for 20 min. With liver microsomes, the major metabolites were 2,6-dinitrobenzyl alcohol under aerobic conditions and 2-amino-6-nitrotoluene under anaerobic conditions. According to the authors, the results using metabolic inhibitors indicated that xanthine oxidase contributes to the hypoxanthine-supported anaerobic metabolism of 2,6-dinitrotoluene in cytosol but that, in microsomes, reductive metabolism is mediated by

cytochromes P450. There was a relative absence of reduced 2,6-dinitrotoluene metabolites in microsomal and cytosolic incubations under aerobic conditions, suggesting to the authors that, under fully oxygenated conditions, hepatic reduction of 2,6-dinitrotoluene probably does not contribute significantly to the activation of 2,6-dinitrotoluene *in vivo*.

Mori *et al.* (1989b) investigated the metabolism of 2,4- and 2,6-dinitrotoluenes and their dinitrobenzyl alcohols and benzaldehydes using liver microsomal and cytosolic fractions prepared from male Wistar (180–200 g) and Sprague-Dawley (180–200 g) rats. Incubation mixtures contained 1 mM of substrates and 1 or 2 mg of microsomal or cytosolic protein, respectively. The authors concluded that the dinitrobenzaldehydes were intermediates in the oxidation of dinitrobenzyl alcohols to dinitrobenzoic acids and that the oxidation of dinitrobenzyl alcohols to dinitrobenzaldehydes and the reduction of dinitrobenzaldehydes to dinitrobenzyl alcohols were reversible reactions. The investigators also determined that the oxidation of 2,6-dinitrotoluene to 2,6-dinitrobenzyl alcohol was higher than that of 2,4-dinitrotoluene to 2,4-dinitrobenzyl alcohol in both strains and that the rate of oxidation in Wistar rat microsomes was higher than that in Sprague-Dawley rat microsomes. The reduction of dinitrobenzaldehydes to dinitrobenzyl alcohols was the highest reaction for both strains, and the reduction of 2,4-dinitrobenzaldehyde to 2,4-dinitrobenzyl alcohol in Wistar rats was particularly high.

Mori *et al.* (1984b) investigated the reduction of 2,4-dinitrotoluene in male Wistar rat (190–220 g) liver microsomal and cytosol fractions. 2,4-Dinitrotoluene was reduced by nicotinamide-adenine-dinucleotide-phosphate hydrolase (NADPH)-dependent microsomal activity to 2-amino-4-nitrotoluene and 4-amino-2-nitrotoluene under anaerobic conditions. However, further reduction to 2,4-diaminotoluene could not be demonstrated, whereas 2,4-diaminotoluene could be produced in incubations using hepatic cytosol fractions. Reduction of 2,4-diaminotoluene by cytosolic enzymes was blocked by the xanthine oxidase inhibitor allopurinol. The authors suggested that diaminotoluene production by cytosol enzymes is due to both cytosolic xanthine oxidase and NADPH-dependent cytosolic enzymes.

Using hepatic microsomes obtained from 200–250-g male or 175–225-g female Fischer 344 rats, Kedderis and Rickert (1985) characterized the oxidation of 2-amino-4-nitrobenzyl alcohol and 2-amino-6-nitrobenzyl alcohol, metabolites of 2,4- and 2,6-dinitrotoluenes, respectively. The investigators found that further metabolism of 2-amino-6-nitrobenzyl alcohol resulted in the formation of two metabolites, both of which were reducing agents. One was identified as 2-hydroxylamino-6-nitrobenzyl alcohol and the other metabolite was tentatively identified as 2-amino-5-hydroxy-6-nitrobenzyl alcohol.

The in-vitro activation of 2-amino-6-nitrobenzyl alcohol was investigated using incubations of cytosol and microsomes prepared from male Fischer 344 rats (250 g, 100 days old) (Chism & Rickert, 1989). Subcellular fractions were incubated in the presence of NADPH, 3′-phosphoadenosine-5′-phosphosulfate and acetyl coenzyme A, 2 mg calf thymus DNA and 84 μM substrate. 2-Amino-6-nitrobenzyl alcohol was converted enzymatically to metabolites capable of binding to calf thymus DNA when incubated with

cytosol and 3′-phosphoadenosine-5′-phosphosulfate or with microsomes and NADPH. However, when cytosol and microsomes were incubated together, activation of 2-amino-6-nitrobenzyl alcohol appeared to require only 3′-phosphoadenosine-5′-phosphosulfate, which suggested a minor role for NADPH-dependent enzymes in the activation of 2-amino-6-nitrobenzyl alcohol.

(ii) *Isolated hepatocytes and whole livers*

Bond and Rickert (1981) investigated the metabolism of 2,4-dinitrotoluene by freshly isolated primary hepatocytes from male Fischer 344 rats (200–250 g) at incubation concentrations of 100 μM. The primary metabolite formed was 2,4-dinitrobenzyl alcohol, which accounted for 75–80% of the total. Much smaller amounts of 2,4-dinitrobenzyl alcohol glucuronide or the amino or acetylamino metabolites occurred. The small amounts of these reduced dinitrotoluene metabolites formed suggested to these investigators that, under physiological conditions, the hepatic reductive metabolism of 2,4-dinitrotoluene probably plays a minor role in the overall metabolism of 2,4-dinitrotoluene.

The sex-dependent metabolism and biliary excretion of 2,4-dinitrotoluene was evaluated in isolated perfused livers from male and female Fischer 344 rats (80–90 days old, weighing approximately 200 and 160 g, respectively). Isolated livers were exposed for 90 min to 2,4-dinitro[^{14}C]toluene added to a recirculating perfusate at initial concentrations of 20 or 70 μM. Isolated perfused livers from both male and female rats displayed a capacity for oxidation, reduction, acetylation and conjugation of 2,4-dinitrotoluene or its metabolites. Oxidation of 2,4-dinitrotoluene to 2,4-dinitrobenzyl alcohol followed by glucuronidation to 2,4-dinitrobenzyl alcohol glucuronide was the major route of 2,4-dinitrotoluene metabolism (Bond *et al.*, 1981).

The metabolism of 2,6-dinitrotoluene was investigated in isolated perfused livers of Fischer 344 rats (Long & Rickert, 1982). Male and female rats (80–90 days old) weighing approximately 200 and 150 g, respectively, were anaesthetized and their livers were removed, placed in a recirculating perfusion apparatus for up to 90 min and exposed to 20 or 70 μM 2,6-dinitro[^{14}C]toluene. 2,6-Dinitrobenzyl alcohol glucuronide was the major metabolite found in both the perfusate and bile. Biliary excretion of 2,6-dinitrobenzyl alcohol glucuronide by livers from male rats was 3.3- and 8.6-fold greater than that for female rats at 20 and 70 μM, respectively. The results of studies using isolated perfused livers and the 2,6-dinitrotoluene isomer paralleled those described above for 2,4-dinitrotoluene (Bond *et al.*, 1981).

(iii) *Intestinal microflora*

The metabolism of 2,4-dinitrotoluene by caecal microflora from male Fischer 344 rats was investigated by Dent *et al.* (1981) using a substrate concentration of 2,4-dinitro-[^{14}C]-toluene of 100 μM. The authors reported that rat caecal microflora metabolized 2,4-dinitrotoluene via an ordered sequence of reductive steps. The reductive metabolic capacity of the caecal contents on a per gram weight basis exceeded that of the liver by a factor of 1000, suggesting that the caecum represents a major site for reductive metabolism of dinitrotoluene. Similarly, Sayama *et al.* (1993) investigated the intestinal

biotransformation of 2,4- or 2,6-dinitrotoluene in male Wistar rats (180–200 g). The microflora were prepared from the entire intestinal (duodenum to rectum) contents of the rats. The incubation solutions contained 15 μmol 2,6- or 2,4-dinitrotoluene/4 mL incubation medium. Anaerobic incubation of 2,6-dinitrotoluene with intestinal microflora indicated that 2,6-dinitrotoluene was transformed to 2-nitroso-6-nitrotoluene, 2-hydroxylamino-6-nitrotoluene, 2-amino-6-nitrotoluene and 2,6-diaminotoluene. The presumed intermediates, the aminonitrosotoluenes and aminohydroxylaminotoluene, were not detected (Guest *et al.*, 1982).

Mori *et al.* (1985) also examined the intestinal metabolism of 2,4-dinitro[^{3}H]toluene in male Wistar rats (200–220 g). These studies were carried out under anaerobic conditions with 2,4-dinitrotoluene concentrations of 25, 50 or 100 μM, using preparations of microflora from the contents of the caecum (20 mg caecal contents/mL incubation mixture). The formation of the reduced metabolites was found to proceed in an ordered fashion, with the formation of 4-hydroxylamino-2-nitrotoluene and 2-hydroxylamino-4-nitrotoluene followed by 4-amino-2-nitrotoluene and 2-amino-4-nitrotoluene. As the concentrations of these two intermediates began to disappear in the incubation mixture, the formation of the final product, 2,4-diaminotoluene, occurred.

Products detected in the anaerobic and aerobic incubation of 2,4-dinitrotoluene with *S. typhimurium* strains TA98 and TA98/1,8-DNP$_6$ were nitrosonitrotoluenes, hydroxylaminonitrotoluenes, aminonitrotoluenes and dimethyl dinitroazoxybenzene (Sayama *et al.*, 1991).

Data on absorption, distribution, metabolism and excretion of dinitrotoluenes in whole animals suggest that, upon absorption, these compounds are metabolized extensively in the liver primarily by oxidation to dinitrobenzyl alcohol followed by conjugation with glucuronic acid. The glucuronide conjugate can be eliminated in the bile, which represents a significant route of elimination for these metabolites, or it can be eliminated in the urine. Reduction of the nitro groups occurs primarily in the intestine. The intestinal microflora are responsible for both cleavage of the glucuronic acid and reduction of one or both of the nitro groups. The resultant metabolite can be absorbed from the gut and returned to the liver where subsequent oxidation, or conjugation with sulfate, can result in the production of unstable species that can bind covalently to hepatic DNA and protein (Fig. 1).

Overall, studies with subcellular fractions and hepatocytes prepared from various species have shown that the dinitrotoluene isomers can be oxidized to dinitrobenzyl alcohol. Very little reduction of the nitro groups takes place in the hepatocytes or subcellular hepatic fractions, unless these samples are incubated under anaerobic conditions. In contrast, reductive metabolism of dinitrotoluenes occurs in incubations with intestinal microflora. The reductive capacity of intestinal microflora is approximately 1000-fold greater than that of the liver. Studies with isolated perfused livers support the hypothesis that oxidation and conjugation are the primary routes for hepatic metabolism of these isomers, and that biliary excretion of the glucuronide conjugate is an important pathway in dinitrotoluene disposition.

4.2 Toxic effects

4.2.1 *Humans*

Using medical examinations, a total of 154 workers in a military plant manufacturing powder containing technical dinitrotoluene were followed for 12 months. During that period, 112 reported complaints, and 84 had objective evidence of sickness. The most common complaints were unpleasant taste (62%), weakness (51%), headache (49%), loss of appetite (47%), dizziness (44%), nausea (37%), insomnia (37%), pains in the extremities (26%), vomiting (23%) and numbness and tingling (19%). Among the findings were pallor (36%), cyanosis (34%), anaemia (23%), leucocytosis (12%), leucopoenia (3.2%) and acute toxic hepatitis with jaundice (1.4%) (McGee *et al.*, 1942).

In the study of Levine *et al.* (1986), described in Section 2, of workers who had been employed in the 1940s and 1950s for at least one month in two munitions plants where they had had the possibility of substantial exposure to dinitrotoluene [but only marginally to nitroglycerine or ethylene glycol dinitrate], there was an increase in total mortality as compared to the general population (SMR, 1.29; $p \leq 0.001$; 164 deaths). This was mainly due to ischaemic heart disease (SMR, 1.41; $p \leq 0.01$; 64 deaths). The increase occurred more than 15 years after onset of employment (SMR, 1.54; $p \leq 0.001$). Further, such deaths occurred mainly among those with high intensity of exposure to dinitrotoluene and employment lasting longer than five months (SMRs, 2.24 and 2.05 in the two factories; both $p \leq 0.05$). There was no information on smoking habits or other established risk factors for cardiovascular disease. However, the authors stress that smoking was prohibited in the plants and that there was no increase in mortality from lung cancer or respiratory diseases. Further, at a military examination near the time of cohort entry, the workers in one of the factories did not differ from other young men in the state. Cerebrovascular mortality was not increased. There was no death from non-malignant diseases of the blood and blood-forming organs [expected number not stated], nor were there deaths attributed to diseases of the liver, besides cirrhosis (SMR, 1.03; 4 deaths). The mortality from accidents, poisonings and violence was high (SMR, 1.91; $p = 0.0007$; 28 deaths; Levine *et al.*, 1986). [The workers were subject to pre-employment medical examination and an extensive medical surveillance programme; those subjects with abnormal electrocardiogram or hypertension could be restricted from work in operations with potential exposure to dinitrotoluene (Stayner *et al.*, 1992). This may have detracted from the effect on cardiovascular disease.]

In an extended study of one of the munitions factories studied by Levine *et al.* (1986) (described in Section 2), 4989 workers were employed for more than five months in jobs with probable exposure to dinitrotoluene (> 1 day of exposure) and total deaths were as expected (SMR, 1.00; 95% CI, 0.9–1.1; 747 deaths). There was an increase in mortality from mental and personality disorders (SMR, 2.2; 95% CI, 1.2–3.9; 12 deaths), which was primarily due to alcoholism. Ischaemic heart disease was not increased (SMR, 0.98; 253 deaths). The authors stress that the results may have been affected by an extensive medical screening programme and that the apparent discrepancy versus the results obtained by Levine *at al.* (1986) in the same factory may be due to differences in the

definition of exposure intensity (lower here) and the length of the follow-up (Stayner *et al.*, 1992).

Single cases of patch test (Kanerva *et al.*, 1991) and photopatch test (Emtestam & Forsbeck, 1985) positivity have been reported in patients with eczema of the hand and occupational exposure to dynamite.

4.2.2 *Experimental systems*

(a) Single-dose studies

Data on acute toxicity following a single oral dose of five dinitrotoluene isomers in rats and mice were reported by Vernot *et al.* (1977). LD_{50} values and 95% CIs were estimated using the moving-average technique. Values obtained for 2,4- and 2,6-dinitrotoluenes are presented below (Table 3). The animals used in these studies were male Sprague-Dawley rats (200–300 g) and CF-1 mice (22–28 g). The vehicle in which the materials was administered was not specified.

Table 3. Acute toxicity in rats and mice following single oral dose of dinitrotoluenes

Material studied	LD_{50} mg/kg (95% CI)	
	Male rats	Mice
2,4-Dinitrotoluene	270 (180–400)	1630 (1180–2240)
2,6-Dinitrotoluene	180 (130–240)	1000 (590–1700)

From Vernot *et al.* (1977)

Male Fischer 344 rats (180–200 g) were injected intraperitoneally with 2,6-dinitrotoluene and its metabolites 2,6-diaminotoluene and 2-amino-6-nitrotoluene dissolved in DMSO at doses of 1.2 mmol/kg bw (219 mg/kg) or 0.3 mmol/kg (55 mg/kg) (La & Froines, 1993). Intraperitoneal administration of 0.3 mmol/kg 2,6-dinitrotoluene to rats resulted in 50% lethality within two days. 2,6-Diaminotoluene and 2-amino-6-nitrotoluene did not produce the death of any animal even at 1.2 mmol/kg. Histopathological examination of livers showed that 2,6-dinitrotoluene induced extensive centrilobular haemorrhagic necrosis, whereas no evidence of necrosis was observed in rat livers at either dose level of 2,6-diaminotoluene or 2-amino-6-nitrotoluene.

Studies by La and Froines (1992a) investigated the toxicity of 2,4- and 2,6-dinitrotoluenes. All rats administered 150 mg/kg bw 2,6-dinitrotoluene either intraperitoneally or by gavage died within 24 h. None of the rats administered 375 mg/kg bw 2,4-dinitrotoluene died. Only the 2,6-isomer exhibited hepatotoxicity producing extensive centrilobular haemorrhagic necrosis. According to the authors, their findings were consistent with previous studies that compared the toxicities of 2,4- and 2,6-dinitrotoluenes. For example, Leonard *et al.* (1987) demonstrated increased activities of γ-glutamyltransferase and alanine aminotransferase only in rats treated with 2,6-dinitro-

toluene. Increased activities of these serum enzymes are indicative of liver injury. Additionally, Mirsalis and Butterworth (1982) demonstrated differences in toxicity between 2,4- and 2,6-dinitrotoluenes *in vitro*. Hepatocytes isolated from rats pretreated with 2,6-dinitrotoluene (100 mg/kg) did not survive in culture because of its toxicity.

(b) Repeated-dose studies

Kozuka *et al.* (1979) investigated the subchronic toxicity of 2,4-dinitrotoluene incorporated into a standard commercial diet at a concentration of 0.5% and fed *ad libitum* for six months to male Wistar rats, seven weeks old at the start of the study. The average consumption of 2,4-dinitrotoluene calculated from the amount of diet consumed was approximately 66 mg per day during the first three months and 75 mg per day in the subsequent three months. [The estimated daily dose of 2,4-dinitrotoluene ranged from 330 mg/kg bw at the beginning of the study to 500 mg/kg bw at the end.] A variety of adverse clinical signs were noted in the test group, including piloerection, whitened skin colour, humpback, incoordination, decreases in spontaneous movements and general weakness. The mortality of the treated rats was about 71% after a 26-week period. There were significant reductions in body-weight gain. The relative weights of liver, spleen and kidney were increased significantly (approximately two-fold compared with controls), while testes weights were significantly decreased. The formation of puruloid matter was noted in the livers of treated rats. The percentage of methaemoglobin was significantly increased in treated rats. At the end of the study, methaemoglobin levels of 7% were noted in the treated rats compared with 1.13% in the controls. In rats fed 0.5% 2,4-dinitrotoluene for one month, triglycerides and glucose levels in serum were elevated compared with controls. Serum enzymes (GOT, lactate dehydrogenase (LDH) and alkaline and acid phosphatase) were also increased, suggesting evidence of hepatotoxicity. No histopathology was reported.

Weanling CD rats were fed 2,4-dinitrotoluene at concentrations of 0, 0.07, 0.2 or 0.7% of the diet for 13 weeks (Lee *et al.*, 1985). The low dose, estimated to be 34 mg/kg bw per day in males and 38 mg/kg bw per day in females, caused depressed weight gain. The mid dose, estimated to be 93 or 108 mg/kg bw per day day, was toxic and caused reticulocytosis, splenic haemosiderosis and decreased spermatogenesis. The high dose, 266 or 145 mg/kg bw per day, was progressively more toxic, causing death in all of the animals by the end of the 13-week period. Anaemia, reticulocytosis, excessive pigment deposits in the spleen and decreased spermatogenesis were the most marked findings.

Weanling CD-1 mice were fed 0, 0.07, 0.2 or 0.7% 2,4-dinitrotoluene in the diet for 13 weeks (Hong *et al.*, 1985). The highest dose caused mild anaemia with concurrent reticulocytosis, mild degeneration of the seminiferous tubules, mild hepatocellular dysplasia and pigmentation in the Kupffer cells of the liver in males and females. Decreased erythrocyte counts, haematocrit and haemoglobin concentration were also noted.

The chronic toxicity of 2,4-dinitrotoluene was investigated in beagle dogs (Ellis *et al.*, 1985), five to six months old at the time of study, that were given 2,4-dinitrotoluene in a hard gelatin capsule. The major adverse effect of 2,4-dinitrotoluene in dogs was a neuropathy characterized by incoordination and paralysis. Vacuolation, endothelial prolife-

ration and gliosis of the cerebella of some of the affected dogs was observed. These effects were seen within two years in one dog given 1.5 mg/kg bw per day, within six months in all dogs given 10 mg/kg bw per day and within two months in all dogs given 25 mg/kg bw per day. Some dogs progressed to complete paralysis leading to death. Methaemoglobinaemia was also noted and the presence of Heinz bodies was common. Biliary tract hyperplasia was also noted.

In studies conducted for up to two years in which 2,4-dinitrotoluene was added to the feed of CD rats (weanlings at start of study) at doses of 0.0015, 0.01 and 0.07%, the highest dose resulted in a shortened lifespan (Lee *et al.*, 1985). Toxic anaemia, atrophy of the testes and depression of spermatogenesis were observed. After feeding for 12 months, lesions occurred in the liver. The initial lesions were small foci or areas of altered hepatocytes referred to by the authors 'hyperplastic foci'. In these animals, the liver architecture was preserved. The lesions progressed to 'hyperplastic nodules'.

Weanling CD-1 mice were fed 0.01%, 0.07% or 0.5% 2,4-dinitrotoluene in the diet for up to two years (Hong *et al.*, 1985). The authors estimated that these concentrations provided doses equal to 14, 95 or 898 mg/kg bw per day. Toxic anaemia was observed in high-dose males and females after feeding of 2,4-dinitrotoluene for 12 months. Methaemoglobin formation and the presence of numerous Heinz bodies were noted. By 21 months, all high-dose mice had died. In this group, generalized abnormal pigmentation was noted in many tissues, which was dose-dependent and increased with the length of treatment. Liver and kidney were the organs most affected. Hepatocellular dysplasia was observed. The authors use this term to characterize metabolic, degenerative and hyperplastic alterations of the cells. In males, the incidence of this lesion was increased in all three dose groups. In females, the incidence was only increased in the high-dose group.

(c) In-vitro toxicity

A reduction in the incorporation of tritiated thymidine was observed in primary cultures of aortic smooth muscle cells from dinitrotoluene-exposed animals relative to vehicle controls (Ramos *et al.*, 1991a,b). Male Sprague-Dawley rats (150–180 g) were injected intraperitoneally on five days a week for eight weeks with 2,4- or 2,6-dinitrotoluene (0.5, 5 or 10 mg/kg bw) or vehicle oil (control). Histopathological evaluation of aortae from animals exposed to either isomer showed dysplasia and rearrangement of aortic smooth muscle cells at all doses tested. Exposure of smooth muscle cells from naïve animals to dinitrotoluene *in vitro* (1, 10 or 100 μM) did not alter the extent of thymidine incorporation into DNA (Ramos *et al.*, 1991b).

The relation of various structural parameters to hepatotoxic potential was investigated by exposing isolated rat hepatocytes from young adult male Sprague-Dawley rats (320–410 g) to six dinitrotoluene isomers (Spanggord *et al.*, 1990). Dinitrotoluene-induced hepatotoxicity correlated with inhibition of protein synthesis and increases in lactate dehydrogenase release but not with lipid peroxidation. *ortho*- and *para*-Substituted isomers were more hepatotoxic at the same concentration than *meta*-substituted isomers. One group, 2,3-, 3,4- and 2,5-dinitrotoluenes, in which the nitro substituents are oriented either *ortho* or *para* to each other, was toxic at appreciably lower concentrations than the other group, which included 2,6-, 2,4- and 3,5-dinitrotoluenes in which the nitro groups

are oriented *meta* to each other. It was concluded that the reducibility of the nitro groups of the parent chemicals is the principal factor for determining their hepatotoxic potential. The results indicated that dinitrotoluene isomers and/or their metabolites produced by the hepatocytes are directly cytotoxic to the cells without the need for metabolic activation by gut microflora. Comparison of the effects of 2,6-dinitrotoluene and 2,4-dinitrotoluene indicated that the inhibition of protein synthesis was similar between the two chemicals. However, at all concentrations tested, the release of LDH from the hepatocytes was higher after 2,6-dinitrotoluene administration compared to 2,4-nitrotoluene administration *in vitro*. Thus, the LDH release correlates with differences in hepatotoxicity between 2,6- and 2,4-dinitrotoluenes observed in in-vivo studies.

4.3 Reproductive and developmental effects

4.3.1 *Humans*

In the United States, an employee at a toluene diamine/dinitrotoluene facility in Kentucky voiced to his union a concern about excessive miscarriages; this led to an investigation by the United States National Institute for Occupational Safety and Health (Hamill *et al.*, 1982). The sperm counts of nine men who were exposed to the compounds (technical grade) were significantly lower than those of nine control men who had never been exposed; however, the sperm count of the control group was unaccountably high. As the authors concluded that there was a strong suggestion of a reproductive problem amongst male workers exposed to the compounds, a larger study was commissioned by the company operating the plant. This study was carried out in a different plant operated by the company in Louisiana. The operating procedures and types of exposure to the compounds were stated to be almost identical to those of the Kentucky plant and environmental monitoring showed that average levels both in area and personal samples were comparable between the two plants. Each worker was subjected to a physician's urogenital examination, an estimation of testicular volume, an assessment of serum follicle-stimulating hormone, an analysis of semen for sperm count and morphology, and an interview about reproductive history and factors related to fertility. The intensity, frequency and timing of exposure was assessed during this interview by a review of company and union work rosters and discussions with supervisors. Ninety-four men were identified by supervisors and work rosters as ever having been exposed to dinitrotoluene/toluene diamine, of whom 78 (83%) participated in the study. Of a total of 203 workers interviewed [total number eligible not specified], 200 provided blood for follicle-stimulating hormone determination and 150 of the 175 workers who had not had a vasectomy provided at least one semen specimen. Of workers classified as having the lowest level of exposure, 12% reported that they had tried to conceive for a year without success compared with 13.5% who reported 'low to high' intensity of exposure, more than six months prior to the study and 17% who reported 'low to high' exposure within six months of the study. The corresponding proportions who reported miscarriages were 19.3%, 27.0% and 13.3%. These differences were not statistically significant. There was no noteworthy difference in physical examination characteristics between the groups. Analysis of fertility rates was stated to show no decrease related to

exposure to dinitrotoluene, either in the comparison of exposed workers with unexposed workers or by comparison of fertility rates of exposed workers during time periods when they held jobs that incurred exposure to dinitrotoluene and rates during other jobs held at the plant at which there was no exposure. No statistically significant difference between the different groups was observed in terms of levels of follicle-stimulating hormone, mean sperm count or mean percentage of normal morphology, although exposed workers had higher mean sperm counts and mean percentage of normal morphologies compared with colleagues who were not or slightly exposed.

4.3.2 *Experimental systems*

Groups of 13–23 pregnant Fischer 344 rats were administered 14, 35, 37.5, 75, 100 or 150 mg/kg bw per day technical-grade dinitrotoluene (76% 2,4-dinitrotoluene, 19% 2,6-dinitrotoluene, < 1% 3,5-dinitrotoluene and other isomers) by gavage on gestational days 7–20 (Price *et al.*, 1985). The mortality rates of the treated females were 4.5, 7.7, 0.0, 0.0, 4.3 and 46.2%, respectively. A group of 37 control females received the corn oil vehicle only, and 36 females received 200 mg/kg bw per day hydroxyurea and served as a positive control group for the evaluation of embryotoxic and teratogenic effects. No maternal death occurred in these groups. As the mortality rate in the group receiving the highest dose of dinitrotoluene was unexpectedly high, mated animals from the second and third of three sequential breedings were treated with 14, 37.5 or 100 mg/kg bw per day of the compound. Thus, the distribution of females was not balanced across the three breedings for this study. At sacrifice on gestational day 20, the haematological profile for dams in the 100-mg/kg bw per day group exhibited characteristic signs of dinitrotoluene toxicity [the haematological profile was not assessed for groups receiving other doses]. Maternal body-weight gain (minus the uterus) was decreased at 100 mg/kg, while there was weight loss at 150 mg/kg. Treatment-related increases in maternal liver and spleen weight as percentages of body weight during gestation were also observed in the groups treated with the higher doses of dinitrotoluene. The proportion of total implants per dam that were resorbed in the group receiving the highest dose was 46.0% (standard error, 22.3%) compared with 16.8% (standard error, 5.4%) in the control group receiving the corn oil vehicle only; this difference was not statistically significant. In litters with live fetuses, no statistically significant difference in the proportion of male fetuses per litter, the average fetal body weight per litter, the average fetal crown–rump length per litter or the average placental weight per litter was observed. No statistically significant difference was observed in the proportion of litters with one or more malformed fetuses or in the percentage of malformed fetuses per litter between the groups. Thus, dinitrotoluene was not found to produce malformations even at dose levels which produced significant maternal and embryo/fetal toxicity.

Using an abbreviated assay protocol intended to evaluate previously untested chemicals to help prioritize them for conventional testing of developmental toxicity, Hardin *et al.* (1987) administered by gavage a dose of 390 mg/kg bw per day 2,4-dinitrotoluene to 50 CD-1 mice on gestational days 6–13. This dose represents the LD_{10} predicted on the basis of dose finding. A concurrent control group of 50 mice received

the corn oil vehicle only. Fifteen of the 50 treated mice died, compared with none of the 50 controls. No adverse effect on reproductive indices was observed (live births per litter, percentage survival, birth weight or weight gain). Pups were not examined systematically for malformations.

Groups of 10 adult male Sprague Dawley rats received 0, 60, 180 or 240 mg/kg bw per day 2,4-dinitrotoluene dissolved in corn oil by gavage for five days (Lane *et al.*, 1985). A single oral dose (0.5 mg/kg bw per day) of triethylenemelamine was used as a positive control. Although a range-finding study preceded dose-selection, an unexpected excess of deaths among rats receiving the highest dose resulted in the requirement for another group of rats to receive a similar dose one week after the other groups. Of this group, 53% (8/15) died within two weeks of receiving the first dose. Each male was allowed to mate with two naïve, nulliparous females for five days each week. Mating lasted for seven weeks except for the group receiving the high dose, where the mating period was extended by six weeks to examine possible reversibility of the effects of 2,4-dinitrotoluene. No significant change in any of the indices of reproductive performance was observed in the group receiving 60 mg/kg bw per day dinitrotoluene. In the group mated with male rats receiving 180 mg/kg bw per day 2,4-dinitrotoluene, there was a significant increase in the preimplantation loss index at week 2, a significant decrease in the mating index at week 5, and a significant increase in the corpora lutea index also at week 5. In rats receiving 240 mg/kg bw per day, the mating index was low during weeks 1–6, and this made it difficult to interpret the other data for those weeks. However, by week 13, all reproductive and dominant lethal indices had recovered and were comparable to control values. Triethylenemelamine produced its classical dominant lethal effects for the first four weeks of mating. Slight cyanosis was observed in the group receiving the lowest dose, more severe cyanosis in the group receiving 180 mg/kg bw per day; in addition to the high death rates observed in the group receiving the highest dose, weight loss and cyanosis were observed. In the group receiving the highest dose, the severe reproductive effects persisted for at least eight weeks, which corresponds approximately to one spermatogenic cycle in the rat. Recovery from the toxic effects was complete by the end of two spermatogenic cycles.

Groups of nine to 10 male Sprague-Dawley rats were administered 0, 0.1 or 0.2% 2,4-dinitrotoluene in chow for three weeks (Bloch *et al.*, 1988). An ultrastructural study of the testes was performed, serum was assayed for testosterone and gonadotropins and sperm reserve count (concentration of sperm heads in the cauda epididymides) was determined. No clinical sign of toxicity was observed in the rats. However, the final body weights were significantly reduced in animals treated with 2,4-dinitrotoluene compared with controls. No difference between testes from animals treated with 0.1% dinitrotoluene and those of controls was observed by light microscopy but electron microscopic examination showed focal alterations at this dose level. In animals receiving the higher dose, extensive disruption of spermatogenesis, irregularity of the peritubular tissue and widespread vacuolization of Sertoli cells were observed. Under electron microscopy, vesicles of varying sizes were associated with swollen mitochondria and distended endoplasmic reticula were observed. Circulating levels of follicle-stimulating hormone and luteinizing hormone were increased in the group receiving the higher dose. In this group,

cauda epididymal sperm counts were reduced by 63%. There was no treatment-related effect on the levels of testosterone.

As noted above (see p. 338), Kozuka *et al.* (1979) reported testicular atrophy and Hong *et al.* (1985) reported degeneration of seminiferous tubules in rats.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see also Table 4 and Appendices 1 and 2)

(a) Macromolecular adducts

2,4-Dinitrotoluene was reported to bind covalently to hepatic DNA in Fischer 344 rats after oral administration. Upon intraperitoneal injection, 2,4-dinitrotoluene showed DNA binding in liver, lung, small intestine and large intestine of mice and rats (Dixit *et al.*, 1986). Using ^{32}P-postlabelling after intraperitoneal administration of 2,4-dinitrotoluene to Fischer 344 rats, three distinct adducts were detected in liver, kidney, lung and mammary gland. DNA binding was highest in the liver (La & Froines, 1992a,b).

2,6-Dinitrotoluene was reported to bind covalently to hepatic macromolecules, including DNA, in Fischer 344 rats after oral administration and intraperitoneal injection. Twice as much ^{14}C was found to be bound covalently to hepatic macromolecules in male than in female Fischer 344 rats after oral dosing of 10 mg/kg bw 2,6-dinitro[^{14}C]toluene. The covalent binding to rat liver macromolecules is increased in the presence of gut microflora (Long & Rickert, 1982). Hepatic macromolecular binding was increased by feeding pectin (DeBethizy *et al.*, 1983), Aroclor 1254 (Chadwick *et al.*, 1993) or coal-tar creosote (Chadwick *et al.*, 1995). After intraperitoneal injection into A/J mice, 2,6-dinitrotoluene showed DNA binding in liver but not in extrahepatic tissues such as lung, small intestine and large intestine; in rats, DNA binding was found in liver, lung and large intestine but not in small intestine (Dixit *et al.*, 1986). Using ^{32}P-postlabelling after intraperitoneal administration of 2,6-dinitrotoluene to Fischer 344 rats, four distinct adducts were detected in liver, kidney, lung and mammary gland; DNA binding was highest in the liver, and the 2,6-isomer produced a greater adduct yield than the 2,4-isomer (La & Froines, 1992a, 1993).

(b) Mutation and allied effects

2,4-Dinitrotoluene (technical grade) induced both forward and reverse mutations in *S. typhimurium* in the presence and absence of an exogenous metabolic system. 2,4-Dinitrotoluene (technical grade) did not induce gene mutation to 6-thioguanine resistance in Chinese hamster ovary cells with or without rat liver preparations for metabolic activation. Technical-grade 2,4-dinitrotoluene, both in the presence and absence of S9-mix, caused a dose-related decrease in survival but no induction of mutation in the mouse lymphoma test. Technical-grade 2,4-dinitrotoluene did not induce morphological transformation of Syrian hamster embryo (SHE) cells.

Table 4. Genetic and related effects of dinitrotoluenes

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
2,4-Dinitrotoluene (technical grade)				
SAF, *Salmonella typhimurium* TM677, forward mutation to 8-azaguanine resistance	+	+	500	Couch *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	500	Couch *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	100	Ashby (1986)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	500	Couch *et al.* (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	500	Couch *et al.* (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	+	+	500	Couch *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	250	Couch *et al.* (1981)
URP, Unscheduled DNA synthesis, rat primary hepatocytes	–	0	18	Bermudez *et al.* (1979)
GCO, Gene mutation, Chinese hamster ovary CHO cells *hprt* locus *in vitro*	–	–	364	Abernethy & Couch (1982)
GML, Gene mutation P388 mouse lymphoma cells *tk* locus *in vitro*	–	–	NR	Styles & Cross (1983)
TCS, Cell transformation, Syrian hamster embryo cells, clonal assay	–	0	NR	Holen *et al.* (1990)
UPR, Unscheduled DNA synthesis, rat hepatocytes *in vivo*	+		100 po × 1 with gut flora	Mirsalis *et al.* (1982a)
UPR, Unscheduled DNA synthesis, rat hepatocytes *in vivo*	–		100 po × 1 germ-free	Mirsalis *et al.* (1982a)
UPR, Unscheduled DNA synthesis, rat hepatocytes *in vivo*	+		100 po × 1	Mirsalis & Butterworth (1982)
UPR, Unscheduled DNA synthesis, AP and Fischer 344 rat hepatocytes *in vivo*	+		100 po × 1	Ashby *et al.* (1985)
UPR, Unscheduled DNA synthesis, rat hepatocytes *in vivo*	+		125 po × 1	Mirsalis *et al.* (1989)
MST, Mouse spot test, BL/6xBL/6 mice	–		100 ip × 1	Soares & Lock (1980)
MST, Mouse spot test, TxBL/6 mice	–		100 ip × 1	Soares & Lock (1980)

Table 4 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
2,4-Dinitrotoluene (technical grade) (contd)				
SVA, Sister chromatid exchange, rat hepatocytes *in vivo*	+		100 po × 1	Kligerman *et al.* (1982)
MVM, Micronucleus test, mice *in vivo*	–		400 ip × 1	Ashby *et al.* (1985)
DLM, Dominant lethal test, DBA/2J mice	–		250 po × 2	Soares & Lock (1980)
DLM, Dominant lethal test, DBA/2J mice	–		250 ip × 2	Soares & Lock (1980)
ICR, Inhibition of intercellular communication, Syrian hamster embryo cell line BPNi *in vitro*	+	0	100	Holen *et al.* (1990)
ICR, Inhibition of intercellular communication, Chinese hamster lung V79 cells *in vitro*	–	0	182	Dorman & Boreiko (1983)
2,4-Dinitrotoluene (high purity)				
PRB, Prophage induction, SOS repair test, DNA strand breaks, cross-links or related damage in *Salmonella typhimurium* TA1535/pSK1002	–	–	100	Nakamura *et al.* (1987)
PRB, Prophage induction, SOS repair test, DNA strand breaks, cross-links or related damage in *Salmonella typhimurium* TA1535/pSK1002	+	0	50	Oda *et al.* (1992)
PRB, Prophage induction, SOS repair test, DNA strand breaks, cross-links or related damage in *Salmonella typhimurium* TA1535/pSK1002	+	0	50.5	Oda *et al.* (1993)
PRB, Prophage induction, SOS repair test, DNA strand breaks, cross-links or related damage in *Salmonella typhimurium* NM1000	–	0	100	Oda *et al.* (1992)
PRB, Prophage induction, SOS repair test, DNA strand breaks, cross-links or related damage in *Salmonella typhimurium* NM1000	–	0	NR	Oda *et al.* (1993)
PRB, Prophage induction, SOS repair test, DNA strand breaks, cross-links or related damage in *Salmonella typhimurium* NM1011	+	0	25	Oda *et al.* (1992)
PRB, Prophage induction, SOS repair test, DNA strand breaks, cross-links or related damage in *Salmonella typhimurium* NM1011	+	0	2.7	Oda *et al.* (1993)

Table 4 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
2,4-Dinitrotoluene (high purity) (contd)				
PRB, Prophage induction, SOS repair test, DNA strand breaks, cross-links or related damage in *Salmonella typhimurium* NM2000	+	0	91	Oda *et al.* (1993)
PRB, Prophage induction, SOS repair test, DNA strand breaks, cross-links or related damage in *Salmonella typhimurium* NM2009	+	0	19	Oda *et al.* (1993)
PRB, Prophage induction, SOS repair test, DNA strand breaks, cross-links or related damage in *Salmonella typhimurium* NM3009	+	0	7	Oda *et al.* (1993)
SAF, *Salmonella typhimurium* TM677, forward mutation to 8-azaguanine resistance	+	+	500	Couch *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	0	910	Chiu *et al.* (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	100	Couch *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	(+)	250	Tokiwa *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	0	50	Mori *et al.* (1982)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	–	250	Spanggord *et al.* (1982b)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	385	Haworth *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	125	Dunkel *et al.* (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	100	Ashby (1986)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	250	Kawai *et al.* (1987)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	210	Dellarco & Prival (1989)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	(+)	–	100	Couch *et al.* (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	2500	Spanggord *et al.* (1982b)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	385	Haworth *et al.* (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	5000	Dunkel *et al.* (1985)

Table 4 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
2,4-Dinitrotoluene (high purity) (contd)				
SA7, *Salmonella typhimurium* TA1537, reverse mutation	(+)	–	100	Couch *et al.* (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	2500	Spanggord *et al.* (1982b)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	1280	Haworth *et al.* (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	3333	Dunkel *et al.* (1985)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	+	–	100	Couch *et al.* (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	2500	Spanggord *et al.* (1982b)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	+	–	250	Dunkel *et al.* (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	0	910	Chiu *et al.* (1978)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	100	Couch *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	(+)	–	250	Tokiwa *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	(+)	0	50	Mori *et al.* (1982)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	2500	Spanggord *et al.* (1982b)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	1280	Haworth *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	(+)	(+)	125	Dunkel *et al.* (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	1250	Kawai *et al.* (1987)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–[c]	210	Dellarco & Prival (1989)
SAS, *Salmonella typhimurium*, TA100NR3, reverse mutation	–	+	NR	Spanggord *et al.* (1982b)
ECW, *Escherichia coli* WP2 *uvrA*, reverse mutation	–	–	5000	Dunkel *et al.* (1985)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		10 000 feeding	Woodruff *et al.* (1985)

Table 4 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
2,6-Dinitrotoluene (contd)				
GML, Gene mutation, P388 mouse lymphoma cells, *tk* locus *in vitro*	–	–	NR	Styles & Cross (1983)
UIA, Unscheduled DNA synthesis, rat spermatocytes *in vitro*	–	0	18	Working & Butterworth (1984)
TCS, Cell transformation, Syrian hamster embryo cells, clonal assay	–	0	NR	Holen *et al.* (1990)
UIH, Unscheduled DNA synthesis, human hepatocytes *in vitro*	–	0	182	Butterworth *et al.* (1989)
BFA, Body fluids (urine) from CD-1 mice, reverse mutation in TA98	+	0	75	George *et al.* (1991)
UPR, Unscheduled DNA synthesis, rat hepatocytes *in vivo*	+		20 po × 1	Mirsalis *et al.* (1982b)
UPR, Unscheduled DNA synthesis, rat hepatocytes *in vivo*	+		20 po × 1	Mirsalis & Butterworth (1982)
UVR, Unscheduled DNA synthesis, rat spermatocytes *in vivo*	–		20 po × 1	Working & Butterworth (1984)
BID, Binding (covalent) to DNA, A/J mouse or Fischer 344 rat hepatocytes *in vitro*	–	+	44	Dixit *et al.* (1986)
BVD, Binding (covalent) to DNA, Fischer 344 rat liver *in vivo*	+		10 po × 1	Long & Rickert (1982)
BVD, Binding (covalent) to DNA, Fischer 344 rat liver *in vivo*	+		10 po × 1	Rickert *et al.* (1983)
BVD, Binding (covalent) to DNA, BALB/c mouse liver *in vivo*	+		9.0 topical × 4	Reddy *et al.* (1984)
BVD, Binding (covalent) to DNA, Fischer 344 rat liver	+		28 po × 1	Kedderis *et al.* (1984)
BVD, Binding (covalent) to DNA, A/J mouse liver, lung, small intestine and large intestine	+		150 ip × 1	Dixit *et al.* (1986)
BVD, Binding (covalent) to DNA, Fischer 344 rat liver, lung, small intestine and large intestine	+		150 ip × 1	Dixit *et al.* (1986)

Table 4 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
2,4-Dinitrotoluene (high purity) (contd)				
BVD, Binding (covalent) to DNA, Fischer 344 rat liver, lung, small intestine and large intestine	+		150 ip × 1	Dixit *et al.* (1986)
BVD, Binding (covalent) to DNA, Fischer 344 rat liver, kidney, lung and mammary gland	+		150 ip × 1	La & Froines (1992a)
BVP, Binding (covalent) to RNA or protein, Fischer 344 rat liver *in vivo*	+		10 po × 1	Rickert *et al.* (1983)
BVP, Binding (covalent) to RNA or protein, animals *in vivo*	+		28 po × 1	Kedderis *et al.* (1984)
ICR, Inhibition of intercellular communication, Chinese hamster lung V79 cells, *in vitro*	–	0	182	Dorman & Boreiko (1983)
ICR, Inhibition of intercellular communication, Syrian hamster embryo cell line BPNi *in vitro*	+	0	100	Holen *et al.*(1990)
SPM, Sperm morphology, DBA/2J mice *in vivo*	–		250 po × 2	Soares & Lock (1980)
SPM, Sperm morphology, DBA/2J mice *in vivo*	–		250 ip × 2	Soares & Lock (1980)
2,6-Dinitrotoluene				
SAF, *Salmonella typhimurium* TM677, forward mutation to 8-azaguanine resistance	+	+	500	Couch *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	–	500	Couch *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	–	500	Tokiwa *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	250	Spanggord *et al.* (1982b)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	250	Kawai *et al.* (1987)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	690	Dellarco & Prival (1989)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	475	Sayama *et al.* (1989a)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	500	George *et al.* (1991)

Table 4 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
2,6-Dinitrotoluene (contd)				
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	500	Couch *et al.* (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	2500	Spanggord *et al.* (1982b)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	+	500	Couch *et al.* (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	2500	Spanggord *et al.* (1982b)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	+	–	500	Couch *et al.* (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	2500	Spanggord *et al.* (1982b)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	250	Couch *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	(+)	–	500	Tokiwa *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	2500	Spanggord *et al.* (1982b)
SA9, *Salmonella typhimurium* TA98, reverse mutation	(+)	+	500	Kawai *et al.* (1987)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–[c]	690	Dellarco & Prival (1989)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	475	Sayama *et al.* (1989a)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	+	250	George *et al.* (1991)
SAS, *Salmonella typhimurium*, TA100NR3, reverse mutation	–	–	2500	Spanggord *et al.* (1982b)
DIA, DNA strand breaks, cross-links or related damage, rat hepatocytes *in vitro*	(+)	0	546	Sina *et al.* (1983)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	0	182	Bermudez *et al.* (1979)
GCO, Gene mutation, Chinese hamster ovary CHO cells, *hprt* locus *in vitro*	–	–	455	Abernethy & Couch (1982)

Table 4 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
2,4-Dinitrotoluene (high purity) (contd)				
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		10 000 inj.	Woodruff *et al.* (1985)
DMH, *Drosophila melanogaster*, heritable translocation test	–		10 000 inj.	Woodruff *et al.* (1985)
DIA, DNA strand breaks, cross-links or related damage, rat hepatocytes *in vitro*	(+)	0	546	Sina *et al.* (1983)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	0	182	Bermudez *et al.* (1979)
UIA, Unscheduled DNA synthesis, rat spermatocytes *in vitro*	–	0	18	Working & Butterworth (1984)
GCO, Gene mutation, Chinese hamster ovary CHO cells, *hprt* locus *in vitro*	–	–	546	Abernethy & Couch (1982)
GML, Gene mutation, P388 mouse lymphoma cells, *tk* locus *in vitro*	+	–	160	Styles & Cross (1983)
SIC, Sister chromatid exchange, Chinese hamster CHO cells *in vitro*	–	+	1010	Loveday *et al.* (1989)
CIC, Chromosomal aberrations, Chinese hamster CHO cells *in vitro*	–	–	1000	Loveday *et al.* (1989)
TCS, Cell transformation, Syrian hamster embryo cells, clonal assay	–	0	10	Holen *et al.* (1990)
UIH, Unscheduled DNA synthesis, human hepatocytes *in vitro*	–	0	182	Butterworth *et al.* (1989)
UPR, Unscheduled DNA synthesis, rat hepatocytes *in vivo*	+		200 po × 1	Mirsalis *et al.* (1982b)
UPR, Unscheduled DNA synthesis, rat hepatocytes *in vivo*	(+)		100 po × 1	Mirsalis & Butterworth (1982)
DLM, Dominant lethal test, DBA/2J mice	–		250 po × 2	Soares & Lock (1980)
DLM, Dominant lethal test, DBA/2J mice	–		250 ip × 2	Soares & Lock (1980)
BVD, Binding (covalent) to DNA, Fischer 344 rat liver	+		10 po × 1	Rickert *et al.* (1983)
BVD, Binding (covalent) to DNA, Fischer 344 rat liver	+		28 po × 1	Kedderis *et al.* (1984)
BVD, Binding (covalent) to DNA, A/J mouse liver, lung, small intestine and large intestine	+		150 ip × 1	Dixit *et al.* (1986)

Table 4 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
2,6-Dinitrotoluene (contd)				
BVD, Binding (covalent) to DNA, Fischer 344 rat liver, kidney, lung and mammary gland	+		150 ip × 1	La & Froines (1992a)
BVD, Binding (covalent) to DNA, Fischer 344 rat liver	+		220 ip × 1	La & Froines (1993)
BVP, Binding (covalent) to RNA or protein, Fischer 344 rat liver *in vivo*	+		10 po × 1	Rickert *et al.* (1983)
BVP, Binding (covalent) to RNA or protein, animals *in vivo*	+		10 po × 1	DeBethizy *et al.* (1983)
BVP, Binding (covalent) to RNA or protein, animals *in vivo*	+		28 po × 1	Kedderis *et al.* (1984)
ICR, Inhibition of intercellular communication, Chinese hamster lung V79 cells *in vitro*	–	0	182	Dorman & Boreiko (1983)
ICR, Inhibition of intercellular communication, Syrian hamster embryo cell line BPNi *in vitro*	+	0	100	Holen *et al.* (1990)
3,5-Dinitrotoluene				
SAF, *Salmonella typhimurium* TM677, forward mutation to 8-azaguanine resistance	+	+	50	Couch *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	–	20	Couch *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	125	Spanggord *et al.* (1982b)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	20	Couch *et al.* (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	550	Spanggord *et al.* (1982b)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	+	–	20	Couch *et al.* (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	+	+	NR	Spanggord *et al.* (1982b)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	+	+	20	Couch *et al.* (1981)

Table 4 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
3,5-Dinitrotoluene				
SA8, *Salmonella typhimurium* TA1538, reverse mutation	+	+	NR	Spanggord *et al.* (1982b)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	25	Couch *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	NR	Spanggord *et al.* (1982b)
SAS, *Salmonella typhimurium*, TA100NR3, reverse mutation	–	–	550	Spanggord *et al.* (1982b)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	0	182	Bermudez *et al.* (1979)
GCO, Gene mutation, Chinese hamster ovary CHO cells, *hprt* locus *in vitro*	–	–	364	Abernethy & Couch (1982)
DLM, Dominant lethal test, DBA/2j mice	–		250 po × 2	Soares & Lock (1980)
DLM, Dominant lethal test, DBA/2j mice	–		250 ip × 2	Soares & Lock (1980)

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

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

[c] Positive in preincubation assay with 2 mμ flavin mononucleotide (FMN)

When administered *in vivo* to rats, 2,4-dinitrotoluene (technical grade) induced a dose-related increase in unscheduled DNA synthesis in hepatocytes. Treated female rats showed a much lower level than males. Unscheduled DNA synthesis was observed in rats with a normal complement of gut flora, but not in rats lacking a gut flora, which indicates that metabolism by gut flora is a necessary step in the genotoxicity of this nitroaromatic compound. After in-vivo treatment with 2,4-dinitrotoluene (technical grade) by gavage, cultured rat lymphocytes showed a positive sister chromatid exchange response but no cell cycle inhibition or mitotic depression (Kligerman *et al.*, 1982).

Technical-grade 2,4-dinitrotoluene inhibited intercellular communication at toxic concentrations in the Syrian hamster embryo cell line BPNi, but not in the V79 Chinese hamster lung cell assay.

Technical-grade 2,4-dinitrotoluene was negative in the mouse bone-marrow micronucleus test, the mouse dominant lethal test and the mouse spot test.

High-purity 2,4-dinitrotoluene was genotoxic in the *S. typhimurium umu* test. In the *S. typhimurium* reverse mutation assay, a number of different studies gave a heterogeneous picture. 2,4-Dinitrotoluene was negative in mutagenicity tests with the *E.* WP2 *uvrA* strain. It had little or no mutagenic activity in *S. typhimurium* TA98 using the standard plate test with or without rat liver S9, but was found to have a clear flavin mononucleotide-dependent mutagenic activity in a modified preincubation assay with hamster S9 (Dellarco & Prival, 1989).

In *Drosophila melanogaster*, high-purity 2,4-dinitrotoluene induced sex-linked recessive lethal mutations after injection, but failed to induce lethal mutations after feeding and translocations after injection.

High-purity 2,4-dinitrotoluene, at the highest dose tested, induced a small number of DNA strand breaks in rat hepatocytes *in vitro*.

High-purity 2,4-dinitrotoluene did not induce gene mutation to 6-thioguanine resistance in Chinese hamster ovary cells with or without rat liver preparations for metabolic activation. The induction of gene mutations was reported for mouse lymphoma cells in the absence of a metabolic activation system. 2,4-Dinitrotoluene produced a small but reproducible increase in sister chromatid exchange in Chinese hamster cells *in vitro* with S9, but not without S9; chromosomal aberrations were not induced with or without S9.

When high-purity 2,4-dinitrotoluene was incubated anaerobically with whole cells from rabbit intestine, culture extracts were mutagenic to *S. typhimurium* strain TA98NR (nitroreductase-deficient) (Combes & Walters, 1986).

High-purity 2,4-dinitrotoluene has been reported to be negative in a number of other mammalian genotoxicity assays: unscheduled DNA synthesis in primary rat hepatocytes and in human hepatocytes *in vitro*, the dominant lethal assay and the sperm morphology test in mice.

When administered to rats *in vivo*, high-purity 2,4-dinitrotoluene induced a weak response in unscheduled DNA synthesis in hepatocytes.

High-purity 2,4-dinitrotoluene inhibited intercellular communication at toxic concentrations in the Syrian hamster embryo cell line BPNi, but not in the V79 Chinese

hamster lung cell assay. 2,4-Dinitrotoluene did not induce morphological transformation of Syrian hamster embryo (SHE) cells.

2,6-Dinitrotoluene is weakly mutagenic in the *S. typhimurium* reverse mutation test without metabolic activation and in TA1535 and TA1537 with rat liver S9. 2,6-Dinitrotoluene is metabolized by *S. typhimurium* strains TA98, TA98/1,8-DNP_6 and TA98NR. Results indicate that the low mutagenic activity of 2,6-dinitrotoluene is not due to low reductive metabolism by bacteria, but due to the lack of mutagenic activity of the bacterial reductive products (Sayama *et al.*, 1992). 2,6-Dinitrotoluene had little or no mutagenic activity in *S. typhimurium* TA98 using the standard plate test with or without rat liver S9, but was found to have a clear flavin mononucleotide-dependent mutagenic activity in a modified preincubation assay with hamster S9 (Dellarco & Prival, 1989).

2,6-Dinitrotoluene did not induce morphological transformation of Syrian hamster embryo (SHE) cells.

2,6-Dinitrotoluene induced DNA strand breaks in rat hepatocytes *in vitro*.

2,6-Dinitrotoluene did not induce gene mutation to 6-thioguanine resistance in Chinese hamster ovary cells with or without rat liver preparations for metabolic activation.

Oral administration of 2,6-dinitrotoluene by gavage led to excretion of mutagenic 2,6-dinitrotoluene-derived metabolites detectable in hydrolysed urines in a microsuspension bioassay (DeMarini *et al.*, 1989) using *S. typhimurium* strain TA98 without rat liver S9 activation. In a similar urinary assay, a positive response was observed with Fischer 344 rats as well as with CD-1 mice.

When administered *in vivo* to rats, 2,6-dinitrotoluene induced a strong response in unscheduled DNA synthesis in hepatocytes.

2,6-Dinitrotoluene inhibited intercellular communication at toxic concentrations in the Syrian hamster embryo cell line BPNi, but not in the V79 Chinese hamster lung cell assay.

3,5-Dinitrotoluene was mutagenic in the *S. typhimurium* reverse mutation test without metabolic activation and also in some tester strains with rat liver S9.

3,5-Dinitrotoluene did not induce gene mutation to 6-thioguanine resistance in Chinese hamster ovary cells with or without rat liver preparations for metabolic activation. It did not induce unscheduled DNA synthesis in primary rat hepatocytes *in vitro*.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

2,4-, 2,6- and 3,5-Dinitrotoluenes are produced by nitration of toluene or nitrotoluenes. Dinitrotoluenes are used primarily as chemical intermediates in the production of toluene diamines and diisocyanates (mainly as the mixture of 2,4- and 2,6-isomers), while smaller amounts of the three isomers are also used to produce dyes, explosives and propellants. Human exposure to dinitrotoluenes can occur by inhalation or skin

absorption during their production and use as intermediates. They have been detected in wastewater from dinitrotoluene production and use, and in surface and groundwater in the vicinity of these manufacturing facilities.

5.2 Human carcinogenicity data

A cohort study of workers from a munitions factory in the United States found an increased risk for cancer of the liver and gall-bladder among workers exposed to a mixture of 2,4- and 2,6-dinitrotoluenes, based on six cases. No such increase was detected in a previous study based on a smaller group of workers from the same and another munitions factory in the United States. These findings were not considered to be strong or consistent enough to permit a conclusion on the carcinogenicity of dinitrotoluenes in humans.

5.3 Animal carcinogenicity data

2,4-Dinitrotoluene was tested by oral administration in two adequate studies in mice and two adequate studies in rats. In one study in mice, no tumorigenic effect was reported. In the second study in mice, using higher doses, tumours of the renal tubular epithelium were observed in males. In both studies in rats, the incidence of various tumours of the integumentary system was increased in males. The incidence of hepatocellular carcinomas was increased in treated males and females in one study. The incidence of fibroadenomas of the mammary gland was increased in females in both studies.

2,6-Dinitrotoluene was tested for carcinogenicity by oral administration in two studies in male rats and increased the incidence of hepatocellular neoplastic nodules and carcinomas.

3,5-Dinitrotoluene has not been tested for carcinogenicity in experimental animals.

Technical-grade dinitrotoluene (approximately 80/20 2,4/2,6-isomers) was tested for carcinogenicity in two studies in rats by oral administration producing hepatocellular neoplastic nodules and hepatocellular carcinomas in male rats in one study and in both sexes in a second study.

5.4 Other relevant data

Dinitrotoluenes are absorbed following dermal and inhalation exposure of workers.

The most abundant metabolites of dinitrotoluenes found in urine from exposed workers were dinitrobenzoic acids. In addition, amino metabolites have been reported. The appearance of reduced metabolites suggests either that human hepatic enzymes are capable of reduction of the nitro group of dinitrotoluene or that dinitrotoluene (or its metabolites) gains access to the intestinal microflora which is capable of reduction, after which the metabolites are reabsorbed and excreted into urine. Limited data indicate a sex difference in humans as regards the urinary metabolite pattern. In humans, the elimination half-life for the urinary metabolites is 1–2.7 h.

The metabolism and excretion of dinitrotoluenes by rats seem to be qualitatively similar to those in humans. However, there are quantitative differences as regards prevalence of different metabolites. Thus, the major urinary metabolites of 2,4-dinitrotoluene are 2,4-dinitrobenzoic acid in humans and 2,4-dinitrobenzyl alcohol in rats.

Heavy human exposure to technical-grade dinitrotoluene may cause a variety of symptoms and signs, including cyanosis — presumably because of methaemoglobinaemia — anaemia and toxic hepatitis. Further, dinitrotoluenes may give rise to allergic contact dermatitis.

A variety of toxic effects are observed in animals following acute administration of various dinitrotoluene isomers. Certain dinitrotoluene isomers, most notably 2,6-dinitrotoluene, produce extensive centrilobular hepatic necrosis following administration *in vivo*.

In laboratory animals, the chronic toxic effects following exposure to dinitrotoluene include various neurotoxic effects (including paralysis), hepatotoxicity, including dysplasia, hyperplastic foci and hepatic megalocytosis, anaemia and methaemoglobinaemia.

No association was found between exposure to the compounds of male workers in a dinitrotoluene facility and the results of semen analysis, the levels of follicle-stimulating hormone or the occurrence of miscarriages or delayed conception in their partners.

In female rats, administration of technical-grade dinitrotoluene by gavage did not produce teratogenic effects even at dose levels which produce significant maternal and embryo/fetal toxicity. In studies in male rats, 2,4-dinitrotoluene induced adverse reproductive effects and anti-spermatogenic activity.

No data on the metabolism or toxicity of 3,5-dinitrotoluene were available to the Working Group.

2,4-Dinitrotoluene (technical grade) is weakly mutagenic in bacteria. It was inactive in mammalian cells *in vitro* in tests for gene mutation, unscheduled DNA synthesis and transformation, but inhibited intercellular communication at toxic concentrations. In rats *in vivo*, it induced unscheduled DNA synthesis in hepatocytes, provided the normal gut flora was present. It induced sister chromatid exchange in rat lymphocytes exposed *in vivo*. In mice, it was negative in the bone-marrow micronucleus test, the dominant lethal test and the spot test.

Purified 2,4-dinitrotoluene showed DNA binding in rats *in vivo* in several organs, the binding being highest in the liver. Three distinct adducts were identified. In bacteria, it induced DNA damage and gene mutation. In insects, it induced sex-linked recessive lethal mutations but not dominant lethal mutations or translocations. In mammalian cells *in vitro*, it induced DNA strand breaks, gene mutations in mouse lymphoma cells (without activation) but not in Chinese hamster ovary cells and a low frequency of sister chromatid exchange but not of chromosomal aberrations in Chinese hamster ovary cells. It inhibited intercellular communication but did not induce cell transformation. In mammals *in vivo*, 2,4-dinitrotoluene induced a weak response in unscheduled DNA synthesis in rat hepatocytes but was negative in the dominant lethal assay and the sperm morphology test in mice.

2,6-Dinitrotoluene is weakly mutagenic in bacteria. In mammalian cells *in vitro*, it induced DNA strand breaks but no gene mutation or cell transformation. Studies of the inhibition of intercellular communication gave equivocal results. With 2,6-dinitrotoluene, DNA adducts were found after in-vivo exposure of rats. *In vivo*, it induced unscheduled DNA synthesis in rat hepatocytes. In the urine of exposed rats, mutagenic metabolites could be detected.

Experiments indicate the following steps in the metabolic activation leading to the formation of adducts: (1) 2,6-dinitrotoluene is metabolized in the liver; (2) metabolites are excreted in the bile; (3) the biliary metabolites are hydrolysed and further metabolized in the intestine; and (4) after enterohepatic transportation of the metabolites back to the liver, the metabolites are activated further and bound to macromolecules.

3,5-Dinitrotoluene is mutagenic in bacteria but did not induce DNA damage or mutations in mammalian cells in culture.

5.5 Evaluation[1]

There is *inadequate evidence* in humans for the carcinogenicity of 2,4-, 2,6- and 3,5-dinitrotoluenes.

There is *sufficient evidence* in experimental animals for the carcinogenicity of 2,4-dinitrotoluene and 2,6-dinitrotoluene.

There is *inadequate evidence* in experimental animals for the carcinogenicity of 3,5-dinitrotoluene.

Overall evaluation

2,4- and 2,6-Dinitrotoluenes are *possibly carcinogenic to humans (Group 2B).*

3,5-Dinitrotoluene is *not classifiable as to its carcinogenicity to humans (Group 3).*

6. References

Abernethy, D.J. & Couch, D.B. (1982) Cytotoxicity and mutagenicity of dinitrotoluenes in Chinese hamster ovary cells. *Mutat. Res.*, **103**, 53–59

Acros Organics (1995) *Catalog of Fine Chemicals 95–96*, Pittsburgh, PA, Fisher Scientific, p. 754

Air Products and Chemicals (1995) *Material Safety Data Sheet: Dinitrotoluene*, Allentown, PA

American Conference of Governmental Industrial Hygienists (1995) *1995–1996 Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices*, Cincinnati, OH, p. 20

Arbeidsinspectie [Labour Inspection] (1994) *De Nationale MAC-Lijst 1994* [National MAC list 1994], The Hague, p. 25

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

Ashby, J. (1986) Carcinogen/mutagen screening strategies (Letter to the Editor). *Mutagenesis*, **1**, 309–317

Ashby, J., Burlinson, B., Lefevre, P.A. & Topham, J. (1985) Non-genotoxicity of 2,4,6-trinitrotoluene (TNT) to the mouse bone marrow and the rat liver: implications for its carcinogenicity. *Arch. Toxicol.*, **58**, 14–19

Bermudez, E., Tillery, D. & Butterworth, B.E. (1979) The effect of 2,4-diaminotoluene and isomers of dinitrotoluene on unscheduled DNA synthesis in primary rat hepatocytes. *Environ. Mutag.*, **1**, 391–398

Bloch, E., Gondos, B., Gatz, M., Varma, S.K. & Thysen, B. (1988) Reproductive toxicity of 2,4-dinitrotoluene in the rat. *Toxicol. appl. Pharmacol.*, **94**, 466–472

Bond, J.A. & Rickert, D.E. (1981) Metabolism of 2,4-dinitro[^{14}C]toluene by freshly isolated Fischer-344 rat primary hepatocytes. *Drug Metab. Dispos.*, **9**, 10–14

Bond, J.A., Medinsky, M.E., Dent, J.G. & Rickert, D.E. (1981) Sex-dependent metabolism and biliary excretion of [2,4-^{14}C]dinitrotoluene in isolated perfused rat livers. *J. Pharmacol. exp. Ther.*, **219**, 598–603

Booth, G. (1991) Nitro compounds, aromatic. In: Elvers, B., Hawkins, S. & Schulz, G., eds, *Ullmann's Encyclopedia of Industrial Chemistry*, 5th rev. Ed., Vol. A17, New York, NY, VCH Publishers, pp. 411–455

Butterworth, B.E., Smith-Oliver, T., Earle, L., Loury, D.J., White, R.D., Doolittle, D.J., Working, P.K., Cattley, R.C., Jirtle, R., Michalopoulos, G. & Strom, S. (1989) Use of primary cultures of human hepatocytes in toxicology studies. *Cancer Res.*, **49**, 1075–1084

Chadwick, R.W., George, S.E., Chang, J., Kohan, M.J., Dekker, J.P., Long, J.E. & Duffy, M.C. (1990) Comparative gastrointestinal enzyme activity and activation of the promutagen 2,6-dinitrotoluene in male CD-1 mice and male Fischer 344 rats. *Cancer Lett.*, **52**, 13–19

Chadwick, R.W., George, S.E., Kohan, M.J., Williams, R.W., Allison, J.C., Hayes, Y.O. & Chang, J. (1993) Potentiation of 2,6-dinitrotoluene genotoxicity in Fischer-344 rats by pretreatment with Aroclor 1254. *Toxicology*, **80**, 153–171

Chadwick, R.W., George, S.E., Kohan, M.J., Williams, R.W., Allison, J.C., Talley, D.L., Hayes, Y.O. & Chang, J. (1995) Potentiation of 2,6-dinitrotoluene genotoxicity in Fischer 344 rats by pretreatment with coal tar creosote. *J. Toxicol. environ. Health*, **44**, 319–336

Chapman, D.E., Michener, S.R. & Powis, G. (1992) Metabolism of 2,6-dinitro[3-^{3}H]toluene by human and rat liver microsomal and cytosolic fractions. *Xenobiotica*, **22**, 1015–1028

Chemical Information Services (1994) *Directory of World Chemical Producers 1995/96 Standard Edition*, Dallas, TX, p. 306

Chism, J.P. & Rickert, D.E. (1985) Isomer- and sex-specific bioactivation of mononitrotoluenes. Role of enterohepatic circulation. *Drub Metab. Disp.*, **13**, 651–657

Chism, J.P. & Rickert, D.E. (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

Chiu, C.W., Lee, L.H., Wang, C.Y. & Bryan, G.T. (1978) Mutagenicity of some commercially available nitro compounds for *Salmonella typhimurium*. *Mutat. Res.*, **58**, 11–22

Combes, R.D. & Walters, J.M. (1986) The contribution of the intestinal microflora and bacterial tester strains to metabolic activation (Abstract no. 7). *Mutagenesis*, **1**, 68

Couch, D.B., Allen, P.F. & Abernethy, D.J. (1981) The mutagenicity of dinitrotoluenes in *Salmonella typhimurium*. *Mutat. Res.*, **90**, 373–383

Davis, W.M., Coates, J.A., Garcia, K.L., Signorella, L.L. & Delfino, J.J. (1993) Efficient screening method for determining base/neutral and acidic semi-volatile organic priority pollutants in sediments. *J. Chromatogr.*, **643**, 341–350

DeBethizy, J.D., Sherrill, J.M., Rickert, D.E. & Hamm, T.E., Jr (1983) Effects of pectin-containing diets on the hepatic macromolecular covalent binding of 2,6-dinitro[^{3}H]toluene in Fischer-344 rats. *Toxicol. appl. Pharmacol.*, **69**, 369–376

Decad, G.M., Graichen, M.E. & Dent, J.G. (1982) Hepatic microsomal metabolism and covalent binding of 2,4-dinitrotoluene. *Toxicol. appl. Pharmacol.*, **62**, 325–334

Dellarco, V.L. & Prival, M.J. (1989) Mutagenicity of nitro compounds in *Salmonella typhimurium* in the presence of flavin mononucleotide in a preincubation assay. *Environ. Mutag.*, **13**, 116–127

DeMarini, D., Dallas, M.M. & Lewtas, J. (1989) Cytotoxicity and effect on mutagenicity of buffers in a microsuspension assay. *Teratog. Carcinog. Mutag.*, **9**, 287–295

Dent, J.G., Schnell, S.R. & Guest, D. (1981) Metabolism of 2,4-dinitrotoluene by rat hepatic microsomes and cecal flora. *Adv. exp. Med. Biol.*, **136**, 431–436

Deutsche Forschungsgemeinschaft (1995) *MAK and BAT Values 1995* (Report No. 31), Weinheim, VCH Verlagsgesellschaft, p. 48

Dixit, R., Schut, H.A.J., Klaunig, J.E. & Stoner, G.D. (1986) Metabolism and DNA binding of 2,6-dinitrotoluene in Fischer-344 rats and A/J mice. *Toxicol. appl. Pharmacol.*, **82**, 53–61

Dorman, B.H. & Boreiko, C.J. (1983) Limiting factors of the V79 cell metabolic cooperation assay for tumor promoters. *Carcinogenesis*, **4**, 873–877

Dunkel, V.C., Zeiger, E., Brusick, D., McCoy, E., McGregor, D., Mortelmans, K., Rosenkranz, H.S. & Simmon, V.F. (1985) Reproducibility of microbial mutagenicity assays: II. Testing of carcinogens and noncarcinogens in *Salmonella typhimurium* and *Escherichia coli*. *Environ. Mutag.*, **7** (Suppl. 5), 1–248

Ellis, H.V., III, Hong, C.B., Lee, C.C., Dacre, J.C. & Glennon, J.P. (1985) Subchronic and chronic toxicity studies of 2,4-dinitrotoluene. Part I. Beagle dogs. *J. Am. Coll. Toxicol.*, **4**, 233–242

Emtestam, L. & Forsbeck, M. (1985) Occupational photosensitivity to dinitrotoluene. *Photodermatology*, **2**, 120–121

Feltes, J., Levsen, K., Volmer, D. & Spiekermann, M. (1990) Gas chromatographic and mass spectrometric determination of nitroaromatics in water. *J. Chromatogr.*, **518**, 21–40

George, S.E., Chadwick, R.W., Creason, J.P., Kohan, M.J. & Dekker, J.P. (1991) Effect of pentachlorophenol on the activation of 2,6-dinitrotoluene to genotoxic urinary metabolites in CD-1 mice: a comparison of G1 enzyme activities and urine mutagenicity. *Environ. mol. Mutag.*, **18**, 92–101

Girindus Corp. (1994) *Data Sheets: Girstrat® —2,4-DNT HM (2,4-Dinitrotoluene High Melting Grade) and Girstrat® — 2,4-DNT SHM (2,4-Dinitrotoluene Super High Melting Grade)*, Palm Harbor, FL

Goldsworthy, T.L., Hamm, T.E., Jr, Rickert, D.E. & Popp, J.A. (1986) The effect of diet on 2,6-dinitrotoluene hepatocarcinogenesis. *Carcinogenesis*, **7**, 1909–1915

Guest, D., Schnell, S.R., Rickert, D.E. & Dent, J.G. (1982) Metabolism of 2,4-dinitrotoluene by intestinal microorganisms from rat, mouse, and man. *Toxicol. appl. Pharmacol.*, **64**, 160–168

Hable, M., Stern, C., Asowata, C. & Williams, K. (1991) The determination of nitroaromatics and nitramines in ground and drinking water by wide-bore capillary gas chromatography. *J. chromatogr. Sci.*, **29**, 131–135

Hall, L.W., Jr, Hall, W.S., Bushong, S.J. & Herman, R.L. (1987) In situ striped bass (*Morone saxatilis*) contaminant and water quality studies in the Potomac River. *Aquat. Toxicol.*, **10**, 73–99

Hamill, P.V.V., Steinberger, E., Levine, R.J., Rodriguez-Rigau, L.J., Lemeshow, S. & Avrunin, J.S. (1982) The epidemiologic assessment of male reproductive hazard from occupational exposure to TDA and DNT. *J. occup. Med.*, **24**, 985–993

Hansch, C., Leo, A. & Hoekman, D.H. (1995) *Exploring QSAR*, Washington DC, American Chemical Society, p. 28

Hardin, B.D., Schuler, R.L., Burg, J.R., Booth, G.M., Hazelden, K.P., MacKenzie, K.M., Piccirillo, V.J. & Smith, K.N. (1987) Evaluation of 60 chemicals in a preliminary developmental toxicity test. *Teratog. Carcinog. Mutag.*, **7**, 29–48

Hashimoto, A., Sakino, H., Yamagami, E. & Tateishi, S. (1980) Determination of dinitrotoluene isomers in sea water and industrial effluents by high-resolution electron capture gas chromatography with a glass capillary column. *Analyst*, **105**, 787–793

Hashimoto, A., Sakino, H., Kojima, T., Yamagami, E., Tateishi, S. & Akiyama, T. (1982) Sources and behaviour of dinitrotoluene isomers in sea-water. *Water Res.*, **16**, 891–897

Haworth, S., Lawlor, T., Mortelmans, K., Speck, W. & Zeiger, E. (1983) Salmonella mutagenicity test results for 250 chemicals. *Environ. Mutag.*, **Suppl. 1**, 3–142

Health and Safety Executive (1995) *Occupational Exposure Limits 1995* (Guidance Note EH 40/95), Sudbury, Suffolk, HSE Books

Holen, I., Mikalsen, S.-O. & Sanner, T. (1990) Effects of dinitrotoluenes on morphological cell transformation and intracellular communication in Syrian hamster embryo cells. *J. Toxicol. environ. Health*, **29**, 89–98

Hong, C.B., Ellis, H.V., III, Lee, C.C., Sprinz, H., Dacre, J.C. & Glennon, J.P. (1985) Subchronic and chronic toxicity studies of 2,4-dinitrotoluene. Part III. CD-1® mice. *J. Am. Coll. Toxicol.*, **4**, 257–269

Horng, J.-Y. & Huang, S.-D. (1994) Determination of the semi-volatile compounds nitrobenzene, isophorone, 2,4-dinitrotoluene and 2,6-dinitrotoluene in water using solid-phase microextraction with a polydimethylsiloxane-coated fibre. *J. Chromatogr. A*, **678**, 313–318

Howard, P.H. (1989) *Handbook of Environmental Fate and Exposure Data for Organic Chemicals*, Vol. 1, Chelsea, MI, Lewis Publishers, pp. 305–318

IARC (1978) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man*, Vol. 16, *Some Aromatic Amines and Related Nitro Compounds — Hair Dyes, Colouring Agents and Miscellaneous Industrial Chemicals*, Lyon, pp. 83–95

IARC (1979) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 19, *Some Monomers, Plastics and Synthetic Elastomers, and Acrolein*, Lyon, pp. 303–340

IARC (1986) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 39, *Some Chemicals Used in Plastics and Elastomers*, Lyon, pp. 287–323

IARC (1987a) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Suppl. 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, p. 61

IARC (1987b) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Suppl. 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, p. 72

Jenkins, T.F. & Walsh, M.E. (1992) Development of field screening methods for TNT, 2,4-DNT and RDX in soil. *Talanta*, **39**, 419–428

Jenkins, T.F., Leggett, D.C., Grant, C.L. & Bauer, C.F. (1986) Reversed-phase high-performance liquid chromatographic determination of nitroorganics in munitions wastewater. *Anal. Chem.*, **58**, 170–175

Kanerva, L., Laine, R., Jolanki, R., Tarvainen, K., Estlander, T. & Helander, I. (1991) Occupational allergic contact dermatitis caused by nitroglycerin. *Contact Derm.*, **24**, 356–362

Kawai, A., Goto, S., Matsumoto, Y. & Matsushita, H. (1987) Mutagenicity of aliphatic and aromatic nitro compounds. Industrial materials and related compounds. *Jpn. J. ind. Health*, **29**, 34–54

Kedderis, G.L. & Rickert, D.E. (1985) Characterization of the oxidation of amine metabolites of nitrotoluenes by rat hepatic microsomes. *Mol. Pharmacol.*, **28**, 207–214

Kedderis, G.L., Dyroff, M.C. & Rickert, D.E. (1984) Hepatic macromolecular covalent binding of the hepatocarcinogen 2,6-dinitrotoluene and its 2,4-isomer *in vivo*: modulation by the sulfotransferase inhibitors pentachlorophenol and 2,6-dichloro-4-nitrophenol. *Carcinogenesis*, **5**, 1199–1204

Kligerman, A.D., Wilmer, J.L. & Erexson, G.L. (1982) The use of rat and mouse lymphocytes to study cytogenetic damage after in vivo exposure to genotoxic agents. In: Bridges, B.A., Butterworth, B.E. & Weinstein, I.B., eds, *Indicators of Genotoxic Exposure* (Banbury Report 13), Cold Spring Harbor, CSH Press, pp. 277–291

Koniecki, W.B. & Linch, A.L. (1958) Determination of aromatic nitro compounds. *Anal. Chem.*, **30**, 1134–1137

Kozuka, H., Mori, M.-A. & Naruse, Y. (1979) Studies on the metabolism and toxicity of dinitrotoluenes; toxicological study of 2,4-dinitrotoluene (2,4-DNT) in rats in long term feeding. *J. toxicol. Sci.*, **4**, 221–228

La, D.K. & Froines, J.R. (1992a) Comparison of DNA adduct formation between 2,4- and 2,6-dinitrotoluene by ^{32}P-postlabelling analysis. *Arch. Toxicol.*, **66**, 633–640

La, D.K. & Froines, J.R. (1992b) ^{32}P-Postlabelling analysis of DNA adducts from Fischer-344 rats administered 2,4-diaminotoluene. *Chem.-biol. Interactions*, **83**, 121–134

La, D.K. & Froines, J.R. (1993) Comparison of DNA binding between the carcinogen 2,6-dinitrotoluene and its noncarcinogenic analog 2,6-diaminotoluene. *Mutat Res.*, **301**, 79–85

Lancaster Synthesis (1995) *Lancaster Chemicals Catalog 1995/96*, Windham, NH, p. 635

Lane, R.W., Simon, G.S., Dougherty, R.W., Egle, J.L., Jr & Borzelleca, J.F. (1985) Reproductive toxicity and lack of dominant lethal effects of 2,4-dinitrotoluene in the male rat. *Drug chem. Toxicol.*, **8**, 265–280

Lee, C.C., Dilley, J.V., Hodgson, J.R., Helton, D.D., Wiegand, W.J., Roberts, D.N., Anderson, B.S., Halfpap, L.M., Jurtz, L.D. & West, N. (1975) *Mammalian Toxicity of Munition Compounds: Phase I. Acute Oral Toxicity, Primary Skin and Eye Irritation, Dermal Sensitization and Disposition and Metabolism* (Report No. 1, MRI Project No. 3900-B), Kansas City, MI, Midwest Research Institute

Lee, C.C., Hong, C.B., Ellis, H.V., III, Dacre, J.C. & Glennon, J.P. (1985) Subchronic and chronic toxicity studies of 2,4-dinitrotoluene. Part II. CD® rats. *J. Am. Coll. Toxicol.*, **4**, 243–256

Leonard, T.B., Lyght, O. & Popp, J.A. (1983) Dinitrotoluene structure-dependent initiation of hepatocytes *in vivo*. *Carcinogenesis*, **4**, 1059–1061

Leonard, T.B., Adams, T. & Popp, J.A. (1986) Dinitrotoluene isomer-specific enhancement of the expression of diethylnitrosamine-initiated hepatocyte foci. *Carcinogenesis*, **7**, 1797–1803

Leonard, T.B., Graichen, M.E. & Popp, J.A. (1987) Dinitrotoluene isomer-specific hepatocarcinogenesis in F344 Rats. *J. natl Cancer Inst.*, **79**, 1313–1319

Levine, R.J., Turner, M.J, Crume, Y.S., Dale, M.E., Starr, T.B. & Rickert, D.E. (1985) Assessing exposure to dinitrotoluene using a biological monitor. *J. occup. Med.*, **27**, 627–638

Levine, R.J., Andjelkovich, D.A., Kersteter, S.L., Arp, E.W., Balogh, S.A., Blunden, P.B. & Stanley, J.M. (1986) Heart disease in workers exposed to dinitrotoluene. *J. occup. Med.*, **28**, 811–816

Lewalter, J. & Ellrich, D. (1991) Nitroaromatic compounds (nitrobenzene, p-nitrotoluene, p-nitrochlorobenzene, 2,6-dinitrotoluene, o-dinitrobenzene, 1-nitronaphthalene, 2-nitronaphthalene, 4-nitrobiphenyl). In: Angerer, J. & Schaller, K.H., eds, *Analyses of Hazardous Substances in Biological Materials*, Vol. 3, New York, VCH Publishers, pp. 207–229

Lewis, R.J., Sr (1993) *Hawley's Condensed Chemical Dictionary*, 12th Ed., New York, Van Nostrand Reinhold Co., p. 424

Lide, D.R., ed. (1993) *CRC Handbook of Chemistry and Physics*, 74th Ed., Boca Raton, FL, CRC Press, pp. 3-489 — 3-490

Liu, D., Thomson, K. & Anderson, A.C. (1984) Identification of nitroso compounds from biotransformation of 2,4-dinitrotoluene. *Appl. environ. Microbiol.*, **47**, 1295–1298

Lloyd, J.B.F. (1983a) High-performance liquid chromatography of organic explosives components with electrochemical detection at a pendent mercury drop electrode. *J. Chromatogr.*, **257**, 227–236

Lloyd, J.B.F. (1983b) Clean-up procedures for the examination of swabs for explosive traces by high-performance liquid chromatography with electrochemical detection at a pendent mercury drop electrode. *J. Chromatogr.*, **261**, 391–406

Long, R.M. & Rickert, D.E. (1982) Metabolism and excretion of 2,6-dinitro[^{14}C]toluene *in vivo* and in isolated perfused rat livers. *Drug Metab. Dispos.*, **10**, 455–458

Loveday, K.S., Lugo, M.H., Resnick, M.A., Anderson, B.E. & Zeiger, E. (1989) Chromosome aberration and sister chromatid exchange tests in Chinese hamster ovary cells *in vitro*: II. Results with 20 chemicals. *Environ. mol. Mutag.*, **13**, 60–94

Mabey, W.R., Smith, J.H., Podoll, R.T., Johnson, H.L., Mill, T., Chou, T.W., Gates, J., Waight-Partridge, I. & Vandenberg, D. (1982) *Aquatic Fate Process Data for Organic Priority Pollutants* (US EPA-440/4-81-014; US NTIS PB87-169090), Washington DC, Office of Water Regulations and Standards, pp. 239–243

Matson, C. (1986) Feasibility findings for in situ biodegradation of nitroaromatic compounds with specific gravities greater than that of water. In: *Proceedings of the Conference on Southwestern Ground Water Issues, Oct. 20–22, Tempe, AL*, pp. 256–268

McGee, L.M., McCausland, A., Plume, C.A. & Marlett, N.C. (1942) Metabolic disturbances in workers exposed to dinitrotoluene. *Am. J. digest. Dis.*, **9**, 329–332

Medinsky, M.A. & Dent, J.G. (1983) Biliary excretion and enterohepatic circulation of 2,4-dinitrotoluene metabolites in Fischer-344 rats. *Toxicol. appl. Pharmacol.*, **68**, 359–366

Miles (1994) *Miles Organica, The Basis for Quality*, Pittsburgh, PA, Division of Bayer AG, p. 51

Mirsalis, J.C. & Butterworth, B.E. (1982) Induction of unscheduled DNA synthesis in rat hepatocytes following in vivo treatment with dinitrotoluene. *Carcinogenesis*, **3**, 241–245

Mirsalis, J.C., Hamm, T.E., Jr & Butterworth, B.E. (1981) The role of gut flora in the induction of DNA repair in rats treated *in vivo* with dinitrotoluene (Abstract no. 307). *Proc. Am. Assoc. Cancer Res.*, **22**, 78

Mirsalis, J.C., Tyson, C.K. & Butterworth, B.E. (1982a) Detection of genotoxic carcinogens in the in vivo-in vitro hepatocyte DNA repair assay. *Environ. Mutag.*, **4**, 553–562

Mirsalis, J.C., Hamm, T.E., Jr, Sherrill, J.M. & Butterworth, B.E. (1982b) The role of gut flora in the genotoxicity of dinitrotoluene. *Nature*, **295**, 322–323

Mirsalis, J.C., Tyson, C.K., Steinmetz, K.L., Loh, E.K., Hamilton, C.M., Bakke, J.P. & Spalding, J.W. (1989) Measurement of unscheduled DNA synthesis and S-phase synthesis in rodent hepatocytes following in vivo treatment: testing of 24 compounds. *Environ. mol. Mutag.*, **14**, 155–164

Mori, M.-A., Naruse, Y. & Kozuka, H. (1977) Studies on the metabolism and toxicity of dinitrotoluenes; on the excretion and distribution of tritium-labelled 2,4-dinitrotoluene (^{3}H-2,4-DNT) in the rat. *Radioisotopes*, **26**, 780–783

Mori, M.-A., Naruse, Y. & Kozuka, H. (1978) Studies on the metabolism and toxicity of dinitrotoluenes — on the absorption and excretion of tritium-labelled 2,4-dinitrotoluene (^{3}H-2,4-DNT) in the rat. *Radioisotopes*, **27**, 715–718

Mori, M.-A., Miyahara, T., Taniguchi, K., Hasegawa, K., Kozuka, H., Miyagoshi, M. & Nagayama, T. (1982) Mutagenicity of 2,4-dinitrotoluene and its metabolites in *Salmonella typhimurium*. *Toxicol. Lett.*, **13**, 1–5

Mori, M.-A., Miyahara, T., Hasegawa, Y., Kudo, Y. & Kozuka, H. (1984a) Metabolism of dinitrotoluene isomers by *Escherichia coli* isolated from human intestine. *Chem. Pharm. Bull.*, **32**, 4070–4075

Mori, M.-A., Matsuhashi, T., Miyahara, T., Shibata, S., Izima, C. & Kozuka, H. (1984b) Reduction of 2,4-dinitrotoluene by Wistar rat liver microsomal and cytosol fractions. *Toxicol. appl. Pharmacol.*, **76**, 105–112

Mori, M.-A., Kudo, Y., Nunozawa, T., Miyahara, T. & Kozuka, H. (1985) Intestinal metabolism of 2,4-dinitrotoluene in rats. *Chem. pharm. Bull.*, **33**, 327–332

Mori, M.-A., Kawajiri, T., Sayama, M., Taniuchi, Y., Miyahara, T. & Kozuka, H. (1989a) Metabolism of 2,6-dinitrotoluene in male Wistar rat. *Xenobiotica*, **19**, 731–741

Mori, M.-A., Kawajiri, T., Sayama, M., Miyahara, T. & Kozuka, H. (1989b) Metabolism of 2,4-dinitrotoluene and 2,6-dinitrotoluene, and their dinitrobenzyl alcohols and dinitrobenzaldehydes by Wistar and Sprague-Dawley rat liver microsomal and cytosol fractions. *Chem. pharm. Bull.*, **37**, 1904–1908

Nakamura, S., Oda, Y., Shimada, T., Oki, I. & Sugimoto, K. (1987) SOS-inducing activity of chemical carcinogens and mutagens in *Salmonella typhimurium* TA1535/pSK1002: examination with 151 chemicals. *Mutat. Res.*, **192**, 239–246

Nelson, C.R. & Hites, R.A. (1980) Aromatic amines in and near the Buffalo River. *Environ. Sci. Technol.*, **14**, 1147–1150

Oda, Y., Shimada, T., Watanabe, M., Ishidate, M., Jr & Nohmi, T. (1992) A sensitive *umu* test system for the detection of mutagenic nitroarenes in *Salmonella typhimurium* NM1011 having a high nitroreductase activity. *Mutat. Res.*, **272**, 91–99

Oda, Y., Yamazaki, H., Watanabe, M., Nohmi, T. & Shimada, T. (1993) Highly sensitive *umu* test system for the detection of mutagenic nitroarenes in *Salmonella typhimurium* NM3009 having high O-acetyltransferase and nitroreductase activities. *Environ. mol. Mutag.*, **21**, 357–364

Ong, C.P., Chin, K.P., Lee, H.K. & Li, S.F.Y. (1992) Analysis of nitroaromatics in aqueous samples by capillary supercritical fluid chromatography. *Int. J. environ. Stud.*, **41**, 17–25

Pereira, W.E., Short, D.L., Manigold, D.B. & Roscio, P.K. (1979) Isolation and characterization of TNT and its metabolites in groundwater by gas chromatograph-mass spectrometer-computer techniques. *Bull. environ. Contam. Toxicol.*, **21**, 554–562

Popp, J.A. & Leonard, T.B. (1982) The use of in vivo hepatic initiation-promotion systems in understanding the hepatocarcinogenesis of technical grade dinitrotoluene. *Toxicol. Pathol.*, **10**, 190–196

Price, C.J., Tyl, R.W., Marks, T.A., Paschke, L.L., Ledoux, T.A. & Reel, J.R. (1985) Teratologic evaluation of dinitrotoluene in the Fischer 344 rat. *Fundam. appl. Toxicol.*, **5**, 948–961

Putnam, H.D., Sullivan, J.H., Jr, Pruitt, B.C., Nichols, J.C., Keirn, M.A. & Swift, D.R. (1981) Impact of trinitrotoluene wastewaters on aquatic biota in Lake Chickamauga, Tennessee. In: Bates, J.M. & Weber, C.I., eds, *Ecological Assessment of Effluent Impacts on Communities of Indigenous Aquatic Organisms* (ASTM Spec. Tech. Publ. 730), Philadelphia, PA, American Society for Testing and Materials, pp. 220-242

Ramos, K.S., McMahon, K.K., Alipui, C. & Demick, D. (1991a) Modulation of aortic smooth muscle cell proliferation by dinitrotoluene. *Adv. exp. Med. Biol.*, **283**, 805–808

Ramos, K.S., McMahon, K.K., Alipui, C. & Demick, D. (1991b) Modulation of DNA synthesis in aortic smooth muscle cells by dinitrotoluenes. *Cell Biol. Toxicol.*, **7**, 111–128

Rawlings, G.D. & Samfield, M. (1979) Toxicity of secondary effluents from textile plants. In: Burns, E.A., ed., *Symposium Proceedings: Process Measurements for Environmental Assessment* (US EPA-600/7-78-168; US NTIS PB-290331), Research Triangle Park, NC, United States Environmental Protection Agency, pp. 153–169

Reddy, M.V., Gupta, R.C., Randerath, E. & Randerath, K. (1984) ^{32}P-Postlabeling test for covalent DNA binding of chemicals *in vivo*: application to a variety of aromatic carcinogens and methylating agents. *Carcinogenesis*, **5**, 231–243

Rickert, D.E. (1987) Metabolism of nitroaromatic compounds. *Drug Metab. Rev.*, **18**, 23–53

Rickert, D.E. & Long, R.M. (1980) Tissue distribution of 2,4-dinitrotoluene and its metabolites in male and female Fischer-344 rats. *Toxicol. appl. Pharmacol.*, **56**, 286–293

Rickert, D.E. & Long, R.M. (1981) Metabolism and excretion of 2,4-dinitrotoluene in male and female Fischer 344 rats after different doses. *Drug Metab. Dispos.*, **9**, 226–232

Rickert, D.E. & Long, R.M. (1982) Metabolism and excretion of 2,6-dinitro[^{14}C]toluene *in vivo* and in isolated perfused rat livers. *Drug Metab. Dispos.*, **10**, 455–458

Rickert, D.E., Long, R.M., Krakowka, S. & Dent, J.G. (1981) Metabolism and excretion of 2,4-[^{14}C]dinitrotoluene in conventional and axenic Fischer-344 rats. *Toxicol. appl. Pharmacol.*, **59**, 574–579

Rickert, D.E., Schnell, S.R. & Long, R.M. (1983) Hepatic macromolecular covalent binding and intestinal disposition of [^{14}C]dinitrotoluenes. *J. Toxicol. environ. Health*, **11**, 555–567

Rickert, D.E., Butterworth, B.E. & Popp, J.A. (1984) Dinitrotoluene: acute toxicity, oncogenicity, genotoxicity, and metabolism. *CRC crit. Rev. Toxicol.*, **13**, 217–234

Sadtler Research Laboratories (1980) *Sadtler Standard Spectra. 1980 Cumulative Index*, Philadelphia, PA, p. 151

Sax, N.I. & Lewis, R.J., Sr (1989) *Dangerous Properties of Industrial Materials*, 7th Ed., Vol. III, New York, Van Nostrand Reinhold, pp. 1461–1462

Sayama, M., Mori, M.-A., Ishida, M., Okumura, K. & Kozuka, H. (1989a) Enterohepatic circulation of 2,4-dinitrobenzaldehyde, a mutagenic metabolite of 2,4-dinitrotoluene, in male Wistar rat. *Xenobiotica*, **19**, 83–92

Sayama, M., Mori, M.-A., Shirokawa, T., Inoue, M., Miyahara T. & Kozuka, H. (1989b) Mutagenicity of 2,6-dinitrotoluene and its metabolites, and their related compounds in *Salmonella typhimurium*. *Mutat. Res.*, **226**, 181–184

Sayama, M., Mori, M.-A., Nakada, Y., Kagamimori, S. & Kozuka, H. (1991) Metabolism of 2,4-dinitrotoluene by *Salmonella typhimurium* strains TA98, TA98NR and TA98/1,8-DNP$_6$, and mutagenicity of the metabolites of 2,4-dinitrotoluene and related compounds to strains TA98 and TA100. *Mutat. Res.*, **264**, 147–153

Sayama, M., Inoue, M., Mori, M.-A., Maruyama, Y. & Kozuka, H. (1992) Bacterial metabolism of 2,6-dinitrotoluene with *Salmonella typhimurium* and mutagenicity of the metabolites of 2,6-dinitrotoluene and related compounds. *Xenobiotica*, **22**, 633–640

Sayama, M., Mori, M.-A., Maruyama, Y., Inoue, M. & Kozuka, H. (1993) Intestinal transformation of 2,6-dinitrotoluene in male Wistar rats. *Xenobiotica*, **23**, 123–131

Schut, H.A., Loeb, T.R. & Stoner, G.D. (1982) Distribution, elimination, and test for carcinogenicity of 2,4-dinitrotoluene in strain A mice. *Toxicol. appl. Pharmacol.*, **64**, 213–220

Schut, H.A.J., Loeb, T.R., Grimes, L.A. & Stoner, C.D. (1983) Distribution, elimination and test for carcinogenicity of 2,6-dinitrotoluene after intraperitoneal and oral administration to strain A mice. *J. Toxicol. environ. Health*, **12**, 659–670

Shafer, K.H. (1982) *Determination of Nitroaromatic Compounds and Isophorone in Industrial and Municipal Wastewaters* (US EPA-600/4-82-024; US NTIS PB82-208398), Cincinnati, OH, United States Environmental Protection Agency

Shah, A.B., Combes, R.D. & Rowland, I.R. (1987) Activation to mutagenicity of dinitrotoluene and some related compounds by bacterial and mammalian enzymes (Abstract no. 12). *Mutagenesis*, **2**, 301

Short, R.D., Dacre, J.C. & Lee, C.C. (1979) A species comparison of 2,4-dinitrotoluene metabolism *in vitro*. *Experientia*, **35**, 1625–1627

Sina, J.F., Bean, C.L., Dysart, G.R., Taylor, V.I. & Bradley, M.O. (1983) Evaluation of the alkaline elution/rat hepatocyte assay as a predictor of carcinogenic/mutagenic potential. *Mutat. Res.*, **113**, 357–391

Soares, E.R. & Lock, L.F. (1980) Lack of an indication of mutagenic effects of dinitrotoluenes and diaminotoluenes in mice. *Environ. Mutag.*, **2**, 111–124

Spanggord, R.J. & Suta, B.E. (1982) Effluent analysis of wastewater generated in the manufacture of 2,4,6-trinitrotoluene. 2. Determination of a representative discharge of ether-extractable components. *Environ. Sci. Technol.*, **16**, 233–236

Spanggord, R.J., Gibson, B.W., Keck, R.G., Thomas, D.W. & Barkley, J.J., Jr (1982a) Effluent analysis of wastewater generated in the manufacture of 2,4,6-trinitrotoluene. 1. Characterization study. *Environ. Sci. Technol.*, **16**, 229–232

Spanggord, R.J., Mortelmans, K.E., Griffin, A.F. & Simmon, V.F. (1982b) Mutagenicity in *Salmonella typhimurium* and structure–activity relationships of wastewater components emanating from the manufacture of trinitrotoluene. *Environ. Mutag.*, **4**, 163–179

Spanggord, R.J., Myers, C.J., LeValley, S.E., Green, C.E. & Tyson, C.A. (1990) Structure–activity relationship for the intrinsic hepatotoxicity of dinitrotoluenes. *Chem. Res. Toxicol.*, **3**, 551–558

Stayner, L.T., Dannenberg, A.L., Thun, M., Reeve, G., Bloom, T., Boeniger, M. & Halperin, W. (1992) Cardiovascular mortality among munitions workers exposed to nitroglycerin and dinitrotoluene. *Scand. J. Work Environ. Health*, **18**, 34–43

Stayner, L.T., Dannenberg, A.L., Bloom, T. & Thun, M. (1993) Excess hepatobiliary cancer mortality among munitions workers exposed to dinitrotoluene. *J. occup. Med.*, **35**, 291–296

Steuckart, C., Berger-Preiss, E. & Levsen, K. (1994) Determination of explosives and their biodegradation products in contaminated soil and water from former ammunition plants by automated multiple development high-performance thin-layer chromatography. *Anal. Chem.*, **66**, 2570–2577

Stoner, G.D., Greisiger, E.A., Schut, H.A.J., Pereira, M.A., Loeb, T.R., Klaunig, J.E. & Branstetter, D.G. (1984) A comparison of the lung adenoma response in strain A/J mice after intraperitoneal and oral administration of carcinogens. *Toxicol. appl. Pharmacol.*, **72**, 313–323

Styles, J.A. & Cross, M.F. (1983) Activity of 2,4,6-trinitrotoluene in an in vitro mammalian gene mutation assay. *Cancer Lett.*, **20**, 103–108

Taylor, D.G. (1978) *NIOSH Manual of Analytical Methods*, 2nd Ed., Vol 4 (DHEW (NIOSH) Publ. No. 78-175), Washington DC, United States Government Printing Office, Method S215

TCI America (1994) *Organic Chemicals 94/95 Catalog*, Portland, OR, p. 587

Tokiwa, H., Nakagawa, R. & Ohnishi, Y. (1981) Mutagenic assay of aromatic nitro compounds with *Salmonella typhimurium*. *Mutat. Res.*, **91**, 321–325

Turner, M.J., Jr, Levine, R.J., Nystrom, D.D., Crume, Y.S. & Rickert, D.E. (1985) Identification and quantification of urinary metabolites of dinitrotoluenes in occupationally exposed humans. *Toxicol. appl. Pharmacol.*, **80**, 166–174

United Nations Environment Programme (1995) *International Register of Potentially Toxic Chemicals. Legal File, Dinitrotoluenes*, Geneva

United States Environmental Protection Agency (1980) *Treatability Manual* (EPA Report No. EPA-600/8-80-042), Washington DC

United States Environmental Protection Agency (1986a) Method 8090. Nitroaromatics and cyclic ketones. In: *Test Methods for Evaluating Solid Waste — Physical/Chemical Methods*, 3rd Ed., Vol. 2 (US EPA No. SW-846), Washington DC, Office of Solid Waste and Emergency Response

United States Environmental Protection Agency (1986b) Method 8250. Gas chromatography/-mass spectrometry for semivolatile organics: packed column technique. In: *Test Methods for Evaluating Solid Waste — Physical/Chemical Methods,* 3rd Ed., Vol. 2 (US EPA No. SW-846), Washington DC, Office of Solid Waste and Emergency Response

United States Environmental Protection Agency (1986c) Method 8270. Gas chromatography/-mass spectrometry for semivolatile organics: capillary column technique. In: *Test Methods for Evaluating Solid Waste — Physical/Chemical Methods*, 3rd Ed., Vol. 2 (US EPA No. SW-846), Washington DC, Office of Solid Waste and Emergency Response

United States Environmental Protection Agency (1994) Nitroaromatics and isophorone. *US Code fed. Regul.*, **Title 40**, Part 136, pp. 503–513 [Method 609], 574–601 [Method 625], 615–634 [Method 1625B]

United States National Cancer Institute (1978) *Bioassay of 2,4-Dinitrotoluene for Possible Carcinogenicity* (Technical Report Series No. 54; DHEW Publication No. (NIH) 78-1360), Bethesda, MD

United States National Institute for Occupational Safety and Health (1994) *Pocket Guide to Chemical Hazards* (DHHS (NIOSH) Publ. No. 94-116), Cincinnati, OH, pp. 118–119, 342

United States National Library of Medicine (1995) *Hazardous Substances Data Bank (HSDB)*, Bethesda, MD

United States Occupational Safety and Health Administration (1990) *OSHA Analytical Methods Manual*, 2nd Ed., Part 1, Vol. 2, Salt Lake City, UT, pp. 44-1–44-26

United States Occupational Safety and Health Administration (1994) Air contaminants. *US Code fed. Regul.*, **Title 29**, Part 1910.1000, p. 11

Vernot, E.H., MacEwen, J.D., Haun, C.C. & Kinkead, E.R. (1977) Acute toxicity and skin corrosion data for some organic and inorganic compounds and aqueous solutions. *Toxicol. appl. Pharmacol.*, **42**, 417–423

Woodruff, R.C., Mason, J.M., Valencia, R. & Zimmering, S. (1985) Chemical mutagenesis testing in *Drosophila*. V. Results of 53 coded compounds tested for the National Toxicology Program. *Environ. Mutag.*, **7**, 677–702

Woollen, B.H., Hall, M.G., Craig, R. & Steel, G.T. (1985) Dinitrotoluene: an assessment of occupational absorption during manufacture of blasting explosives. *Int. Arch. occup. environ. Health*, **55**, 319–330

Working, P.K. & Butterworth, B.E. (1984) An assay to detect chemically induced DNA repair in rat spermatocytes. *Environ. Mutag.*, **6**, 273–286

Zoeteman, B.C.J., Harmsen, K., Linders, J.B.H.J., Morra, C.F.H. & Slooff, W. (1980) Persistent organic pollutants in river water and ground water of the Netherlands. *Chemosphere*, **9**, 231–249