2-NITROTOLUENE, 3-NITROTOLUENE AND 4-NITROTOLUENE

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

2-Nitrotoluene

Chem. Abstr. Serv. Reg. No.: 88-72-2

Deleted CAS Reg. No.: 57158-05-1

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

IUPAC Systematic Name: *ortho*-Nitrotoluene

Synonyms: 2-Methylnitrobenzene; *ortho*-methylnitrobenzene; 2-methyl-1-nitrobenzene; *ortho*-mononitrotoluene; 2-nitrotoluol; *ortho*-nitrotoluol

3-Nitrotoluene

Chem. Abstr. Serv. Reg. No.: 99-08-1

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

IUPAC Systematic Name: *meta*-Nitrotoluene

Synonyms: 3-Methylnitrobenzene; *meta*-methylnitrobenzene; 3-methyl-1-nitrobenzene; *meta*-mononitrotoluene; 3-nitrotoluol; *meta*-nitrotoluol

4-Nitrotoluene

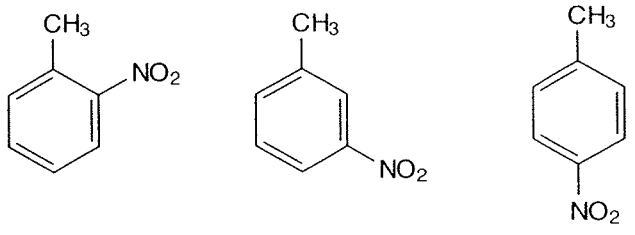
Chem. Abstr. Serv. Reg. No.: 99-99-0

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

IUPAC Systematic Name: *para*-Nitrotoluene

Synonyms: 4-Methylnitrobenzene; *para*-methylnitrobenzene; 4-methyl-1-nitrobenzene; *para*-nitrotoluene; 4-nitrotoluol; *para*-nitrotoluol

1.1.2 *Structural and molecular formulae and relative molecular mass*



2-Nitrotoluene 3-Nitrotoluene 4-Nitrotoluene

$C_7H_7NO_2$ Relative molecular mass: 137.15

1.1.3 *Chemical and physical properties of the pure substance*

2-Nitrotoluene

(*a*) *Description*: Pale yellowish liquid which crystallizes at lower temperatures to solid α- and β-forms (Budavari, 1989; Lide, 1993)

(*b*) *Boiling-point*: 221.7 °C (Lide, 1993)

(*c*) *Melting-point*: –9.5 °C (needles; α-form), –2.9 °C (crystals, β-form) (Lide, 1993)

(*d*) *Density*: 1.1629 at 20 °C/4 °C (Lide, 1993)

(*e*) *Spectroscopy data*: Infrared (prism [4692], grating [437]), ultraviolet (UV) [1292], nuclear magnetic resonance (proton [676], C-13 [857]) and mass spectral data have been reported (Sadtler Research Laboratories, 1980).

(*f*) *Solubility*: Slightly soluble in water (0.54 g/L at 20 °C); soluble in benzene, diethyl ether, ethanol and petroleum ether (Budavari, 1989; Hoechst Chemicals, 1989; Lide, 1993)

(*g*) *Volatility*: Vapour pressure, 0.1 mm Hg [13 Pa] at 20 °C; relative vapour density (air = 1), 4.72 (Verschueren, 1983; Booth, 1991)

(*h*) *Stability*: Combustible when exposed to heat or open flame; potentially explosive reaction with alkali (Sax & Lewis, 1989)

(*i*) *Octanol/water partition coefficient (P)*: log P, 2.30 (Hansch *et al.*, 1995)

(*j*) *Conversion factor*: $mg/m^3 = 5.6 \times ppm$[1]

3-Nitrotoluene

(*a*) *Description*: Pale yellow liquid (Budavari, 1989; Lide, 1993)

(*b*) *Boiling-point*: 232.6 °C (Lide, 1993)

(*c*) *Melting-point*: 16 °C (Lide, 1993)

(*d*) *Density*: 1.1571 at 20 °C/4 °C (Lide, 1993)

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

(*e*) *Spectroscopy data*: Infrared (prism [183], grating [61]), UV [73], nuclear magnetic resonance (proton [22], C-13 [2002]) and mass spectral data have been reported (Sadtler Research Laboratories, 1980).

(*f*) *Solubility*: Slightly soluble in water (0.5 g/L at 30 °C); soluble in benzene, diethyl ether and ethanol (Budavari, 1989; Booth, 1991; Lewis, 1993)

(*g*) *Volatility*: Vapour pressure, 0.1 mm Hg [13 Pa] at 20 °C; relative vapour density (air = 1), 4.72 (Verschueren, 1983; Booth, 1991)

(*h*) *Stability*: Combustible when exposed to heat, flame or oxidizers (Sax & Lewis, 1989)

(*i*) *Octanol/water partition coefficient (P)*: log P, 2.42 (Hansch *et al.*, 1995)

(*j*) *Conversion factor*: $mg/m^3 = 5.61 \times ppm$[1]

4-Nitrotoluene

(*a*) *Description*: Yellowish orthorhombic crystals (from diethyl ether or ethanol) (Budavari, 1989; Lide, 1993)

(*b*) *Boiling-point*: 238.3 °C (Lide 1993)

(*c*) *Melting-point*: 54.5 °C (Lide, 1993)

(*d*) *Spectroscopy data*: Infrared (prism [4693], grating [438]), UV [1293], nuclear magnetic resonance (proton [677], C-13 [632]) and mass spectral data have been reported (Sadtler Research Laboratories, 1980).

(*e*) *Solubility*: Slightly soluble in water (0.26 g/L at 20 °C); soluble in acetone, benzene, chloroform, diethyl ether and ethanol (Budavari, 1989; Booth, 1991; Lide, 1993)

(*f*) *Volatility*: Vapour pressure, 0.1 mm Hg [13 Pa] at 20 °C; relative vapour density (air = 1), 4.72 (Verschueren, 1983; Booth, 1991)

(*g*) *Stability*: Combustible when exposed to heat or flame; mixtures with tetranitromethane are sensitive high explosives (Sax & Lewis, 1989)

(*h*) *Octanol/water partition coefficient (P)*: log P, 2.37 (Hansch *et al.*, 1995)

(*i*) *Conversion factor*: $mg/m^3 = 5.61 \times ppm$[1]

1.1.4 *Technical products and impurities*

2-Nitrotoluene is available commercially at a purity of 99.2%–99.5% and containing the following typical impurities: 3- and 4-nitrotoluenes, 0.8%; water, 0.2%; toluene, 0.1% (Hoechst Chemicals, 1989; DuPont Chemicals, 1994; First Chemical Corp., 1995a).

3-Nitrotoluene is available commercially at a purity of 99.0% with the following typical impurities: 2- and 4-nitrotoluenes, 1.0%; and water, 0.1% (First Chemical Corp., 1995b).

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

4-Nitrotoluene is available commercially at a purity of 99.5% with the following typical impurities (max.): 2- and 3-nitrotoluenes, 0.5%; dinitrotoluene, 0.1%; and water, 0.1% (First Chemical Corp., 1995c).

1.1.5 *Analysis*

The routine determination of urinary nitrotoluenes (2- and 4-isomers) at concentrations of 5–50 mg/L is achieved by colorimetric analysis. In acidified urine, the nitro group of the nitrotoluenes is zinc reduced and, after diazotization and coupling to 1-amino-8-naphthol-2,4-disulfonic acid (Chicago acid), the primary aromatic amine is determined as a red azo dye. An alternative method, which uses formamidine sulfinic acid (thiourea dioxide) to reduce the aromatic nitro compounds under alkaline conditions, has been described (Koniecki & Linch, 1958).

Selected methods for the analysis of 2-, 3- and 4-nitrotoluenes in various media are identified in Table 1.

Table 1. Methods for the analysis of nitrotoluenes[a,b,c]

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Air	Draw air through solid sorbent tube; desorb with methanol	GC/FID	0.8 µg/sample[a,b,c]	Eller (1994) [Method 2005]
Water	Extract with dichloromethane or adsorb on Amberlite XAD resin and elute with dichloromethane	GC/ECD	NR[b]	Feltes *et al.* (1990)
	Extract with diethyl ether; dry over anhydrous magnesium sulfate; filter	GC/FID	NR[a,c]	Spanggord *et al.* (1982a)
	Liquid–liquid extraction with dichloromethane; dry with anhydrous sodium sulfate; evaporate to dryness; redissolve in methanol	SFC/FID	20 ppm[c] (mg/L)	Ong *et al.* (1992)
Blood	Extract from separated plasma and concentrate using 2,2,4-trimethylpentane	GC/ECD	15 µg/L[c]	Lewalter & Ellrich (1991)

GC, gas chromatography; FID, flame ionization detection; ECD, electron capture detection; NR, not reported; SFC, capillary supercritical fluid chromatography

[a] 2-Nitrotoluene

[b] 3-Nitrotoluene

[c] 4-Nitrotoluene

1.2 Production and use

1.2.1 *Production*

The nitrotoluenes are produced commercially by the nitration of toluene with mixed acids: nitric acid (HNO_3)/sulfuric acid, HNO_3/aromatic sulfonic acid or HNO_3/phosphoric acid. The resultant isomer ratio depends on the catalyst and conditions but generally is in the range of 45–62% 2-nitrotoluene, 2–5% 3-nitrotoluene and 33-50% 4-nitrotoluene (Booth, 1991).

Large-scale nitration is carried out under typical mixed acid conditions at 25–40 °C and with a nitric acid:toluene molar ratio close to one. A 2-nitrotoluene : 4-nitrotoluene ratio of 1.6 and an isolated yield of 96% total nitrotoluenes are typical in a continuous reactor, similar to that described for nitrobenzene (see the monograph on p. 384), but operating at as low a temperature as possible to avoid the more-readily formed by-products. 2-, 3- and 4-Nitrotoluenes are separated and purified by fractional distillation and crystallization (Booth, 1991).

It has been estimated that 13 000 tonnes of 2-nitrotoluene, very small quantities of 3-nitrotoluene and 6800 tonnes of 4-nitrotoluene are used annually in the United States of America (American Conference of Governmental Industrial Hygienists, 1991). In 1984, the yearly production capacity for mononitrotoluene (all isomers) in the western world was approximately 200 000 tonnes (Booth, 1991).

2-Nitrotoluene and 4-nitrotoluene are produced by six companies in China, three companies in Japan, two companies each in Germany, India, the Republic of Korea and the United States, and one company each in the Czech Republic, Italy, Romania, Sweden and the United Kingdom. 3-Nitrotoluene is produced by five companies in China, and one company each in Germany, India, Italy, Japan, the Republic of Korea, Sweden, the United Kingdom and the United States (Chemical Information Services, 1994).

1.2.2 *Use*

2-Nitrotoluene is used for the production of derivatives that are principally colourant intermediates. For example, the following derivatives are all intermediates in the preparation of various azo dyes: *ortho*-toluidine (see IARC, 1982, 1987), 2-amino-4-chlorotoluene (Fast Scarlet TR Base, by reduction of 4-chloro-2-nitrotoluene), 2-amino-6-chlorotoluene (Fast Red KB Base, by reduction of 2-chloro-6-nitrotoluene) and *ortho*-toluidine-4-sulfonic acid (by reduction of 2-nitrotoluene-4-sulfonic acid). A more recent use for *ortho*-toluidine is its conversion to 2-ethyl-6-methylaniline, an intermediate in the manufacture of agricultural chemicals. This is an important outlet for the typical surplus of 2-nitrotoluene. 2-Nitrotoluene is also used in the manufacture of rubber chemicals and in various azo and sulfur dyes for cotton, wool, silk, leather and paper (American Conference of Governmental Industrial Hygienists, 1991; Booth, 1991).

3-Nitrotoluene is used in the manufacture of *meta*-toluidine and nitrobenzoic acids, and in the manufacture of agricultural, pharmaceutical, photographic and rubber chemicals (Budavari, 1989; American Conference of Governmental Industrial Hygienists, 1991; Booth, 1991; First Chemical Corp., 1995b).

4-Nitrotoluene is used for the production of derivatives that are used principally as colourant intermediates. Important derivatives include *para*-toluidine, 4-nitrobenzoic acid (by oxidation of 4-nitrotoluene), 4-amino-2-chlorotoluene (by reduction of 2-chloro-4-nitrotoluene) and 4-nitrotoluene-2-sulfonic acid, the last of which is of importance in forming stilbene intermediates for fluorescent whitening agents. 4-Nitrotoluene is also used in the manufacture of agricultural, pharmaceutical and rubber chemicals, and in various azo and sulfur dyes for cotton, wool, silk, leather and paper (American Conference of Governmental Industrial Hygienists, 1991; Booth, 1991).

1.3 Occurrence

1.3.1 *Natural occurrence*

Nitrotoluenes are not known to occur as natural products.

1.3.2 *Occupational exposure*

The most probable routes of human exposure to nitrotoluenes are inhalation and dermal contact of workers involved in the production and use of nitrotoluenes, dinitrotoluenes and trinitrotoluene. Very few data on occupational exposures to nitrotoluene exist. Ahlborg *et al.* (1985) reported a maximal air concentration of 2.0 mg/m^3 2-nitrotoluene in the nitrotoluene production area of a chemical plant producing pharmaceuticals and explosives.

1.3.3 *Environmental occurrence*

(*a*) *Air*

2-Nitrotoluene was detected in the ambient air at a plant in Deepwater, NJ, United States, at a concentration of 47 ng/m^3; 4-nitrotoluene was detected at concentrations ranging from 59 to 89 ng/m^3 (Pellizzari, 1978).

(*b*) *Water*

2-Nitrotoluene has been identified qualitatively in German drinking-water (Kool *et al.*, 1982).

In the Netherlands, during 1974, 2- and 4-nitrotoluenes were detected in the River Waal at an average concentration of 4.5 µg/L (max., 18.1 mg/L) and in the River Maas at a maximal concentration of 0.3 µg/L (Meijers & van der Leer, 1976). Also in the Netherlands, 2- and 4-nitrotoluenes were detected in the River Rhine at a concentration of 10 µg/L (Piet & Morra, 1983).

2-Nitrotoluene concentrations of 0.4 and 7.4 µg/L were detected in surface-water samples collected from two brooks near Hischagen/Waldhof, Germany, in the vicinity of a former munitions manufacturing plant closed after the Second World War; the brooks fed into the River Losse, which had a concentration of 1.2 µg/L. Two ponds in the Clausthal-Zellerfeld region of Germany, again near previous munitions manufacturing plants, had levels of 0.4 and 22.0 µg/L; these ponds fed into the River Oder, which had a level of < 0.01 µg/L. Concentrations at three locations (Brunsbüttel, Brokdorf, Lauenburg) along the River Elbe ranged from 0.05 to 0.4 µg/L. Corresponding levels of

3-nitrotoluene were 0.1 and 0.9 μg/L in the brooks, 0.1 μg/L in the River Losse, 0.1 and 1.5 μg/L in the ponds and < 0.01 μg/L in the River Oder. Corresponding levels of 4-nitrotoluene were 0.3 and 4.5 μg/L in the brooks, 0.4 μg/L in the River Losse, 0.2 and 4.8 μg/L in the ponds and < 0.01 μg/L in the River Oder (Feltes *et al.*, 1990).

3-Nitrotoluene was measured at a concentration of 1 μg/L in water from the River Rhine and detected qualitatively in the River Rhine on several other occasions (United States National Library of Medicine, 1995).

2- and 4-Nitrotoluenes were detected in the effluent from a plant manufacturing trinitrotoluene in Radford, VA, United States, at concentrations ranging from 0.32 to 16 mg/L and from 0.12 to 9.2 mg/L, respectively (Howard, 1989). These compounds were also detected in the wastewater resulting from the production and purification of 2,4,6-trinitrotoluene at concentrations ranging from 0.02 to 0.14 mg/L (in 21/54 samples) and from 0.01 to 0.17 mg/L (in 23/54 samples), respectively (Spanggord *et al.*, 1982a), and in the raw effluent from a plant manufacturing dinitrotoluene at 7.8 mg/L and 8.8 mg/L, respectively. 2-Nitrotoluene was detected in a waste-treatment lagoon of a paper mill and 4-nitrotoluene in a waste-treatment lagoon of a chemical company at 0.04 mg/L (Howard, 1989). An acidic stream of wastewater discharged from a nitrotoluene-manufacturing plant in India was found to contain 87–102 mg/L, 0.4–3.1 mg/L and 8–67 mg/L 2-, 3- and 4-nitrotoluenes, respectively; corresponding levels in an alkaline stream were 53–80 mg/L, 6–11 mg/L and 84–145 mg/L (Swaminathan *et al.*, 1987).

Nitrotoluenes were identified as pollutants in groundwater samples collected from January to March 1987 in Degrémont, France. Concentrations ranged from 90 to 165 μg/L for 2-nitrotoluene, 9 to 19 μg/L for 3-nitrotoluene and from 3 to 20 μg/L for 4-nitrotoluene (Duguet *et al.*, 1988).

1.4 Regulations and guidelines

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

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals[1]

There have been no reports of long-term studies of the carcinogenicity of 2-, 3- or 4-nitrotoluene in experimental animals.

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[1] The Working Group noted that additional studies, including short-term and carcinogenicity studies, are currently being conducted on 2-nitrotoluene (IARC, 1994).

Table 2. Occupational exposure limits and guidelines for nitrotoluenes (all isomers)

Country	Year	Concentration (mg/m^3)	Interpretation
Australia	1993	11 (Sk)	TWA
Belgium	1993	11 (Sk)	TWA
Bulgaria[a]	1995	11 (Sk)	TWA
Colombia[a]	1995	11 (Sk)	TWA
Czech Republic[b]	1993	5	TWA
		20	STEL (15 min)
Denmark	1993	12 (Sk)	TWA
Finland	1993	30 (Sk)	TWA
		60	STEL (15 min)
France[b]	1991	11 (Sk)	TWA
Germany	1995	30 (Sk) (3- and 4-isomers)	MAK
		None (Sk), IIIA2 (2-isomer)	
Jordan[a]	1995	11 (Sk)	TWA
Netherlands[b]	1994	11 (Sk)	TWA
New Zealand[a]	1995	11 (Sk)	TWA
Poland	1991	3	TWA
Republic of Korea[a]	1995	11 (Sk)	TWA
Russia	1991	3 (Sk)	STEL
Singapore[a]	1995	11 (Sk)	TWA
Switzerland	1991	11 (Sk)	TWA
		22	STEL (15 min)
United Kingdom	1995	30 (Sk)	TWA
		60	STEL (15 min)
USA			
ACGIH (TLV)	1995	11 (Sk)[c]	TWA
OSHA (PEL)	1994	30 (Sk)	TWA
NIOSH (REL)	1994	11 (Sk)	TWA
Viet Nam[a]	1995	11 (Sk)	TWA

From International Labour Office (1991); Työministeriö (1993); Arbeitsinspectie (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)

Sk, absorption through the skin may be a significant source of exposure; TWA, time-weighted average; STEL, short-term exposure limit; IIIA2, substances shown to be clearly carcinogenic only in animal studies but under conditions indicative of carcinogenic potential at the workplace; TLV, threshold limit value; PEL, permissible exposure limit; REL, recommanded exposure limit

[a] Follows ACGIH TLVs

[b] 3-Nitrotoluene only

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

Oral administration

Rat: Groups of 10 male Fischer 344/N rats, six to eight weeks of age, received diets containing 0, 625, 1250, 2500, 5000 or 10 000 mg/kg diet (ppm) 2-nitrotoluene (> 96% pure) for 13 weeks. Animals consumed an estimated average of 0, 45, 89, 179, 353 or 694 mg/kg bw 2-nitrotoluene daily. All animals survived to the end of the 13-week study, at which time they were killed for complete histopathological evaluation. Body-weight gains were reduced by 12, 28 and 44% relative to controls in the 2500-, 5000- and 10 000-ppm dose groups, respectively. Two rats receiving 10 000 ppm had mesothelial-cell hyperplasia of the tunica vaginalis on the surface of the epididymis and three receiving 5000 ppm had mesotheliomas at the same location. Mesotheliomas had not been observed previously in treated or control male rats in approximately 435 other 13-week studies (United States National Toxicology Program, 1992; Dunnick *et al.*, 1994). The historical incidence of mesotheliomas of all organs in control male rats in two-year studies of the United States National Toxicology Program is 2.7% (Haseman *et al.*, 1990).

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

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

In the chemical-processing department of a plant producing pharmaceuticals and explosives, Ahlborg *et al.* (1988) analysed diazo-positive metabolites in the hydrolysed urine from workers exposed to aromatic nitroamino compounds, including nitrotoluenes, 2,4,6-trinitrotoluene (see monograph, p. 460) and nitro- and aminobenzoic acids. In the post-shift samples of 45 exposed workers, levels were higher than in 25 unexposed workers. After a holiday, the levels in the exposed workers were lower than in their own post-shift samples. In the unexposed group, no such difference was noted. [The workers were also exposed to compounds other than nitrotoluenes that may have given rise to diazo-positive urinary excretion.]

4.1.2 *Experimental systems*

(*a*) *Metabolites*

Following the administration of a single oral dose of 200 mg/kg bw nitro[^{14}C]toluene isomers to male Fischer 344 rats, a total of 80–90% was eliminated within 72 h in urine, faeces and expired air. A higher percentage of 2-nitrotoluene (85.8%) than 3-nitrotoluene (67.8%) or 4-nitrotoluene (76.7%) was excreted in urine. Nine metabolites were found in the urine of rats given 2-nitrotoluene. For three rats 72 h after treatment, the mean percentage of the dose administered plus or minus the standard error of the mean for each metabolite was: nitrobenzoic acid (28.6 ± 0.3); 2-nitrobenzyl glucuronide (14.1 ± 0.7);

S-(2-nitrobenzyl)-*N*-acetylcysteine (11.6 ± 0.2); *S*-(2-nitrobenzyl)glutathione (3.9 ± 0.4); 2-aminobenzoic acid (1.8 ± 0.2); 2-nitrobenzyl sulfate (0.5 ± 0.2); 2-nitrobenzyl alcohol (0.4 ± 0.3); and two unidentified metabolites (15.9 ± 1.5 and 6.0 ± 0.6). Eight metabolites were found in the urine of rats given 3-nitrotoluene. For three rats 72 h after treatment, the mean percentage of the dose administered plus or minus the standard error of the mean for each metabolite was: 3-nitrohippuric acid (23.6 ± 2.0); 3-nitrobenzoic acid (21.1 ± 1.1); 3-acetamidobenzoic acid (11.6 ± 0.4); 3-nitrobenzyl glucuronide (2.0 ± 0.1); *S*-(3-nitrobenzyl)glutathione (1.3 ± 0.1); 3-aminobenzoic acid (1.2 ± 0.3); and two unidentified metabolites (2.6 ± 0.3 and 2.2 ± 0.3). Eight metabolites were found in urine following administration of 4-nitrotoluene. Again, for three rats 72 h after treatment, the mean percentage of the dose administered plus or minus the standard error the mean for each metabolite was: 4-nitrobenzoic acid (28.0 ± 2.6); 4-acetamidobenzoic acid (27.1 ± 3.0); 4-nitrohippuric acid (13.0 ± 0.7); *S*-(4-nitrobenzyl)-*N*-acetylcysteine (3.7 ± 0.1); 4-nitrobenzyl glucuronide (1.4 ± 0.1); 4-aminobenzoic acid (0.8 ± 0.1); *S*-methyl-2-nitrophenyl glucuronide (0.3 ± 0.0); and 5-methyl-2-nitrophenyl sulfate (0.2 ± 0.0) (Chism *et al.*, 1984).

In bile duct-cannulated male Fischer 344 rats administered 200 mg/kg bw nitro[^{14}C] toluene isomers, 28.6% of the 2-nitrotoluene dose was excreted in bile in males, compared to 9.6% in females. The major metabolite in bile following 2-nitrotoluene administration was 2-nitrobenzyl glucuronide. Males excreted 22% of the dose as this metabolite and females excreted 8.3%. Following 3-nitrotoluene administration, 10.8% of the dose was excreted in the bile of male rats, compared to 4.3% in female rats. 3-Nitrobenzoic acid was the most abundant biliary metabolite of 3-nitrotoluene (3.4 and 1.7% of the dose in males and females, respectively), followed by 3-nitrobenzyl glucuronide (2.8 and 0.7% of the dose in males and females, respectively). In male rats, 9.8% of the 4-nitrotoluene dose was excreted in bile, compared to 1.3% in females. The most abundant biliary metabolite following 4-nitrotoluene administration was 4-nitrobenzoic acid (2.8 and 0.8% of the dose in males and females, respectively) (Chism & Rickert, 1985). Compared to controls, sham operation decreased the urinary excretion of 2-nitro-[^{14}C]toluene (200 mg/kg) by 20–30% and bile duct cannulation decreased urinary excretion of radioactivity by 52–59%.

(*b*) *Macromolecular binding*

Following an oral dose (200 mg/kg bw) to male and female Fischer 344 rats, more 2-nitrotoluene than 3-nitrotoluene or 4-nitrotoluene was bound covalently to hepatic macromolecules in both males (2-, 3- and 4-nitrotoluenes: 36.6, 6.9 and 10.1 nmol nitrotoluene equivalents/g protein, respectively) and females (2-, 3- and 4-nitrotoluenes: 11.2, 7.9 and 8.5 nmol nitrotoluene equivalents/g protein, respectively). Three times more 2-nitrotoluene was bound to hepatic macromolecules in males than in females. Interruption of enterohepatic circulation by bile duct cannulation decreased hepatic macromolecular covalent binding of 2-nitrotoluene in male rats by 93% when compared to sham-operated controls and by 98% when compared to intact controls. In female rats, after bile duct cannulation, binding of 2-nitrotoluene was decreased by 85% compared to intact controls and by 78% compared to sham-operated controls (Chism & Rickert,

1985). In-vitro studies suggest that the metabolite of 2-nitrotoluene responsible for covalent binding to DNA is 2-aminobenzyl sulfate (Chism & Rickert, 1989).

4.2 Toxic effects

4.1.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

(*a*) *Single-dose studies*

The single oral LD_{50}s determined in male Sprague-Dawley rats, male and female Wistar rats and male CF-1 mice were: 2-nitrotoluene — 891, 2100, 2100 and 2463 mg/kg bw, respectively; 3-nitrotoluene — 1072, 2200, 2000 and 330 mg/kg bw, respectively; and 4-nitrotoluene — 2144, 4700, 3200 and 1231 mg/kg bw, respectively (Ciss *et al.*, 1980a; Registry of Toxic Effects of Chemical Substances, 1991).

(*b*) *Repeated-dose studies*

In 14-day studies, 2-, 3- and 4-nitrotoluenes were administered to male and female Fischer 344/N rats at concentrations ranging from 625 to 20 000 mg/kg diet (ppm). Estimated consumption based on food intake was 56–696 mg/kg bw 2-nitrotoluene, 61–881 mg/kg bw 3-nitrotoluene and 106–869 mg/kg bw 4-nitrotoluene for males, and 55–779 mg/kg bw 2-nitrotoluene, 58–754 mg/kg bw 3-nitrotoluene and 105–611 mg/kg bw 4-nitrotoluene for females. Body-weight gains in male rats were decreased in groups receiving the isomers at concentrations of 5000 ppm and above; male rats receiving 3-nitrotoluene at 2500 ppm (259 mg/kg bw) gained less weight than controls and male rats receiving 4-nitrotoluene at 20 000 ppm (849 mg/kg bw) lost weight. Body weights of female rats were affected at higher concentrations than those of males. No gross lesion related to 2-nitrotoluene treatment was observed, but in the livers of 4/5 male rats in the 10 000-ppm (696 mg/kg bw) group minimal oval-cell hyperplasias, which consisted of proliferations of small cells with pale-staining oval-shaped nuclei, were found. These cells were dispersed between hepatocytes in the portal areas. No lesion was observed in the livers of female rats. Following administration of 4-nitrotoluene, no treatment-related gross lesion was observed either. However, increased congestion and extramedullary haematopoiesis were seen in the spleen of one male rat at 5000 ppm (446 mg/kg bw) and in most males and females at 10 000 (431 and 420 mg/kg bw) and 20 000 ppm (869 and 611 mg/kg bw). Lymphoid depletion occurred in the thymus and spleen of a few rats in the 10 000- and 20 000-ppm groups; this was attributed to the marked reduction in body-weight gain or body-weight loss during the study (United States National Toxicology Program, 1992).

In 14-day studies, the three isomers were given in the diet to male and female B6C3F1 mice at doses of 388–10 000 mg/kg (ppm). Estimated consumption was 63–854 mg/kg bw 2-nitrotoluene, 66–779 mg/kg bw 3-nitrotoluene and 202–1548 mg/kg bw 4-nitrotoluene for males, and 134–1224 mg/kg bw 2-nitrotoluene, 92–901 mg/kg bw

3-nitrotoluene and 388–2010 mg/kg bw 4-nitrotoluene for females. In male mice, liver weights were increased in the group administered the highest dose of 2-nitrotoluene. Relative liver weights were also increased in female mice that received all but the lowest dose of 3-nitrotoluene and in males that received diets containing 2500 ppm (409 mg/kg bw) and 5000 ppm (779 mg/kg bw) 3-nitrotoluene. Following 4-nitrotoluene administration, relative liver weights were increased in a dose-related manner in all males and all but the low-dose females (United States National Toxicology Program, 1992).

In a 13-week feeding study in male and female Fischer 344 rats (625–10 000 ppm in the diet; about 40–700 mg/kg bw per day), all three nitrotoluene isomers caused an increase in the incidence of hyaline-droplet nephropathy in male rats, with 2-nitrotoluene being the most toxic. Hyaline droplets were associated with accumulation of $\alpha_{2\mu}$-globulin in the kidney and were characterized by an accumulation of eosinophilic crystalline-like inclusions and globular droplets within the cytoplasm and lumen of the renal tubules. Other kidney lesions included minimal to mild enlargement (karomegaly) of the proximal tubule epithelium in male and female rats receiving 4-nitrotoluene, and yellow- to-brown pigment (possibly lipofuscin) in the cytoplasm of renal tubule epithelial cells of male and female rats receiving 2- or 4-nitrotoluene. A treatment-related increase in extramedullary haematopoiesis, haemosiderin pigment and/or congestion occurred in the splenic pulp of male and female rats treated with either isomer. At higher doses, there was minimal to mild thickening of the splenic capsule accompanied by mesothelial-cell hypertrophy. Some decreases in haematocrit, erythrocyte counts and haemoglobin concentrations and small increases in platelet and lymphocyte counts were observed at the highest-dose level (United States National Toxicology Program, 1992; Dunnick *et al.*, 1994).

In the livers of male rats given 2500 mg/kg diet (179 mg/kg bw) 2-nitrotoluene or more for 14 weeks, various non-neoplastic lesions were observed, including cytoplasmic vacuolization, oval-cell hyperplasia and inflammation. Cytoplasmic vacuolization was characterized by multiple round clear spaces of varying size in hepatocytes throughout the liver lobule, most prominantly in the portal area. Oval-cell hyperplasia consisted of increased numbers of small cells with pale staining cytoplasm and round-to-oval nuclei. These cells were generally interspersed between hepatocytes in single or double rows, but sometimes formed small nodules or ductular structures in the portal area of the liver lobule (United States National Toxicology Program, 1992; Dunnick *et al.*, 1994).

In a 13-week feeding study of the three isomers in male and female B6C3F1 mice (625–10 000 ppm in the diet; about 100–1700 mg/kg bw per day), the only evidence of toxicity was degeneration and metaplasia of the olfactory epithelium following administration of 2-nitrotoluene. No hepatic toxicity was noted in mice, although all three isomers caused increased liver weights (United States National Toxicology Program, 1992; Dunnick *et al.*, 1994).

An increase in liver foci (placental glutathione-*S*-transferase-positive) was observed in male Fischer 344 rats fed 5000 mg/kg diet 2-nitrotoluene for 13 or 26 weeks (Ton *et al.*, 1995).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Groups of 10 male and 10 female Wistar rats were administered daily by gavage 0.2 g/kg bw 2-nitrotoluene in olive oil, 0.3 g/kg bw 3-nitrotoluene in olive oil, 0.4 g/kg bw 4-nitrotoluene suspended in 1% methylcellulose, olive oil alone or methylcellulose alone on five days per week for three months. At the end of this period, the rats were paired in four groups: (i) five exposed males with five exposed females; (ii) five exposed males with five unexposed females; (iii) five unexposed males with five unexposed females; (iv) five unexposed males and five exposed females. The treatment was continued for three more months. Two deaths were observed in the exposed groups; these were due to errors in performing the intubation procedure. Splenic enlargement was observed in males exposed to each of the three isomers. With the 2-isomer, renal tubules were dilated and contained hyaline droplets. These were more frequent in females (7/9) than in males (3/8). With the 4-isomer, all nine exposed males had testicular atrophy and, in five of these, this was associated with necrosis of the seminiferous tubules. No adverse effect on reproduction or on the offspring was observed for any of the isomers (Ciss *et al.*, 1980b). [The Working Group noted the absence of descriptions of animal randomization and husbandry.]

Male and female Fischer 344 rats were administered 3-nitrotoluene at doses of 625–10 000 mg/kg diet in a 14-day study (see Section 4.2). At necropsy, chemically related gross lesions were described as a reduction in size of the testis and uterus in rats from the 10 000-ppm (881 and 754 mg/kg bw for males and females, respectively) groups. The testis, epididymis, uterus and liver from all rats were examined microscopically. All males in the highest-dose group (881 mg/kg bw) had mild to moderate degeneration of the testis characterized by a loss of germinal epithelium and the presence of abnormal (syncytia) spermatids in the lumen of the seminiferous tubules and ducts of the epididymis. One of the males in the 5000-ppm group (431 mg/kg bw) had moderate testicular degeneration, but the lesion was unilateral and the relationship to treatment was uncertain. Compared to controls and lower-dose groups, the uteri of female rats in the highest-dose group (754 mg/kg bw) had thinner muscular walls and less developed endometrium (United States National Toxicology Program, 1992).

Groups of 10 male and 10 female Fischer 344/N and B6C3F1 mice were administered doses of 0, 2500, 5000 or 10 000 ppm 2-, 3,- or 4-nitrotoluene in their feed for 13 weeks (United States National Toxicology Program, 1992). [The concentration of 10 000 ppm corresponds to an estimated dose, based on measures of food consumption, of about 700 mg/kg bw per day for rats and about 1500 mg/kg bw per day for mice.] Treatment had no effect on survival, and clinical signs of toxicity were limited to decreases in food consumption. Decreased body-weight gain was observed in both species for each of the isomers at the higher-dose levels and was most pronounced in rats receiving

4-nitrotoluene. All three isomers impaired testicular function in rats, as shown microscopically and by measurement of sperm density, motility and number. The three isomers also increased the length of the oestrus cycle in rats. The 2-isomer appeared generally to be more toxic than the other isomers. Degeneration of the testes occurred in all male rats receiving 5000 or 10 000 ppm of the 2-isomer. Virtually no sperm was present in the epididymides of rats receiving 2-nitrotoluene at 10 000 ppm. Sperm counts were also diminished in the group of rats receiving 5000 ppm of this isomer. Only 4/10 female rats receiving the highest dose had a measurable oestrus cycle. With the 3- and 4-isomers, degeneration of the testes occurred only in rats receiving doses of 10 000 ppm. The severity of this lesion was less than that observed with the 2-isomer at this level of dose. Among females receiving 3-nitrotoluene, there was a dose-related increase in the length of the oestrus cycle; concurrently, the number of cycling animals diminished. With the 4-isomer, 9/10 females in the group receiving 10 000 ppm had no discernible oestrus cycle. No growth or histopathological change in the uterus or ovaries was associated with treatment with any of the three isomers. Except for a significant decrease in sperm motility in mice receiving 10 000 ppm 2-nitrotoluene, no change was noted in the reproductive system evaluations in male or female mice for any of the three isomers (United States National Toxicology Program, 1992).

Administration of single intraperitoneal doses of 30 and 100 mg/kg bw 4-nitrotoluene to female Sprague-Dawley rats increased uterine weights without producing overt toxicity. Doses of 1000 mg/kg were toxic (Smith & Quinn, 1992).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

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

2-Nitrotoluene did not induce unscheduled DNA synthesis in primary cultures of human hepatocytes treated *in vitro* or in rat spermatogonic cells exposed *in vitro*. It did not induce chromosomal aberrations, but induced sister chromatid exchange in the presence of S9 in Chinese hamster ovary cells *in vitro*.

In vivo in rats, 2-nitrotoluene bound covalently to hepatic macromolecules, including DNA.

In hepatocytes of male Fischer 344 rats, 2-nitrotoluene induced unscheduled DNA-synthesis after dosing by gavage *in vivo*, but not after treatment of the hepatocytes *in vitro*. That 2-nitrotoluene did not induce unscheduled DNA synthesis in germ-free animals suggests an obligatory role of intestinal bacteria in the metabolic activation (Butterworth *et al.*, 1982; Doolittle *et al.*, 1983). The in-vivo activity is dependent upon the sex — in male and female Fischer 344 rats having similar populations of intestinal bacteria, 2-nitrotoluene induced unscheduled DNA synthesis only in males. This difference might be explained (Doolittle *et al.*, 1983) by sex differences in biliary excretion (Chism & Rickert, 1985).

Table 3. Genetic and related effects of nitrotoluenes

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
2-Nitrotoluene				
BSD, *Bacillus subtilis rec* strains, differential toxicity	–	0	NR	Shimizu & Yano (1986)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	0	685	Chiu *et al.* (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	500	Miyata *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	500	Tokiwa *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	2500	Spanggord *et al.* (1982b)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	256	Haworth *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	0	50	Suzuki *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	450	Shimizu & Yano (1986)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	500	Miyata *et al.* (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	2500	Spanggord *et al.* (1982b)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	256	Haworth *et al.* (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	450	Shimizu & Yano (1986)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	150	Miyata *et al.* (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	2500	Spanggord *et al.* (1982b)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	256	Haworth *et al.* (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	450	Shimizu & Yano (1986)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	2500	Spanggord *et al.* (1982b)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	450	Shimizu & Yano (1986)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	0	685	Chiu *et al.* (1978)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	150	Miyata *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	500	Tokiwa *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	2500	Spanggord *et al.* (1982a)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	256	Haworth *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	50	Suzuki *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	450	Shimizu & Yano (1986)

Table 3 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
2-Nitrotoluene (contd)				
SAS, *Salmonella typhimurium* TA92, reverse mutation	–	–	150	Miyata *et al.* (1981)
SAS, *Salmonella typhimurium* TA94, reverse mutation	–	–	500	Miyata *et al.* (1981)
SIC, Sister chromatid exchange, Chinese hamster CHO cells *in vitro*	?	+	355	Galloway *et al.* (1987)
CIC, Chromosomal aberrations, Chinese hamster CHO cells *in vitro*	–	–	420	Galloway *et al.* (1987)
CIC, Chromosomal aberrations, Chinese hamster CHL cells *in vitro*	–	0	250	Ishidate *et al.* (1988)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	0	13.7	Doolittle *et al.* (1983)
UIA, Unscheduled DNA synthesis, rat pachytene spermatocytes and round spermatids *in vitro*	–	0	13.7	Working & Butterworth (1984)
UIH, Unscheduled DNA synthesis, human hepatocytes *in vitro*	–	0	137	Butterworth *et al.* (1989)
UPR, Unscheduled DNA synthesis, male rat hepatocytes *in vivo*	+		200 po × 1	Doolittle *et al.* (1983)
UPR, Unscheduled DNA synthesis, male germ-free rat hepatocytes *in vivo*	–		500 po × 1	Doolittle *et al.* (1983)
UPR, Unscheduled DNA synthesis, female rat hepatocytes *in vivo*	–		200 po × 1	Doolittle *et al.* (1983)
UPR, Unscheduled DNA synthesis, male rat hepatocytes *in vivo*	+		200 po × 1	US National Toxicology Program (1992)
UPR, Unscheduled DNA synthesis, female rat hepatocytes *in vivo*	+		750 po × 1	US National Toxicology Program (1992)
UVM, Unscheduled DNA synthesis, male mouse hepatocytes *in vivo*	–		750 po × 1	US National Toxicology Program (1992)
UVM, Unscheduled DNA synthesis, female mouse hepatocytes *in vivo*	+		750 po × 1	US National Toxicology Program (1992)
BVD, Binding (covalent) to DNA, male rat liver *in vivo*	+		200 po × 1	Rickert *et al.* (1984)
BVP, Binding (covalent) to RNA or protein, male rat liver *in vivo*	+		200 po × 1	Rickert *et al.* (1984, 1986)

Table 3 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
3-Nitrotoluene				
BSD, *Bacillus subtilis rec* strains, differential toxicity	–	0	NR	Shimizu & Yano (1986)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	150	Miyata *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	50	Tokiwa *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	2500	Spanggord *et al.* (1982b)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	0	50	Suzuki *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	38	Haworth *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	445	Shimizu & Yano (1986)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	150	Miyata *et al.* (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	2500	Spanggord *et al.* (1982b)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	128	Haworth *et al.* (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	225	Shimizu & Yano (1986)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	150	Miyata *et al.* (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	2500	Spanggord *et al.* (1982b)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	128	Haworth *et al.* (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	225	Shimizu & Yano (1986)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	2500	Spanggord *et al.* (1982b)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	445	Shimizu & Yano (1986)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	2500	Spanggord *et al.* (1982b)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	128	Haworth *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	150	Miyata *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	50	Tokiwa *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–[d]	50	Suzuki *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	445	Shimizu & Yano (1986)
SAS, *Salmonella typhimurium* TA92, reverse mutation	–	–	500	Miyata *et al.* (1981)
SAS, *Salmonella typhimurium* TA94, reverse mutation	–	–	500	Miyata *et al.* (1981)

Table 3 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
3-Nitrotoluene (contd)				
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	0	13.7	Doolittle *et al.* (1983)
UIA, Unscheduled DNA synthesis, rat pachytene spermatocytes and round spermatids *in vitro*	–	0	13.7	Working & Butterworth (1984)
SIC, Sister chromatid exchange, Chinese hamster CHO cells *in vitro*	+	–	150	Galloway *et al.* (1987)
CIC, Chromosomal aberrations, Chinese hamster CHO cells *in vitro*	–	–	483	Galloway *et al.* (1987)
CIC, Chromosomal aberrations, Chinese hamster CHL cells *in vitro*	–	0	250	Ishidate *et al.* (1988)
UPR, Unscheduled DNA synthesis, male rat hepatocytes *in vivo*	–		500 po × 1	Doolittle *et al.* (1983)
UPR, Unscheduled DNA synthesis, male rat hepatocytes *in vivo*	–		500 po × 1	US National Toxicology Program (1983)
BVD, Binding (covalent) to DNA, male rat liver *in vivo*	–		200 po × 1	Rickert *et al.* (1984)
BVP, Binding (covalent) to RNA or protein, male rat liver *in vivo*	+		200 po × 1	Rickert *et al.* (1984, 1986)
4-Nitrotoluene				
BSD, *Bacillus subtilis rec* strains, differential toxicity	+	0	NR	Shimizu & Yano (1986)
SA0, *Salmonella typhimurium* TA 100, reverse mutation	–	0	685	Chiu *et al.* (1978)
SA0, *Salmonella typhimurium* TA 100, reverse mutation	–	–	500	Miyata *et al.* (1981)
SA0, *Salmonella typhimurium* TA 100, reverse mutation	–	–	250	Tokiwa *et al.* (1981)
SA0, *Salmonella typhimurium* TA 100, reverse mutation	+	+	NR	Spanggord *et al.* (1982b)
SA0, *Salmonella typhimurium* TA 100, reverse mutation	–	–	128	Haworth *et al.* (1983)
SA0, *Salmonella typhimurium* TA 100, reverse mutation	–	0	50	Suzuki *et al.* (1983)
SA0, *Salmonella typhimurium* TA 100, reverse mutation	+	0	385	Shimizu & Yano (1986)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	500	Miyata *et al.* (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	2500	Spanggord *et al.* (1982b)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	128	Haworth *et al.* (1983)

Table 3 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
4-Nitrotoluene (contd)				
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	0	1925	Shimizu & Yano (1986)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	150	Miyata *et al.* (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	2500	Spanggord *et al.* (1982b)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	128	Haworth *et al.* (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	0	1925	Shimizu & Yano (1986)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	2500	Spanggord *et al.* (1982b)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	0	1925	Shimizu & Yano (1986)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	0	685	Chiu *et al.* (1978)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	150	Miyata *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	–	–	128	Haworth *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–[d]	50	Suzuki *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	0	1925	Shimizu & Yano (1986)
SAS, *Salmonella typhimurium* TA92, reverse mutation	–	–	1500	Miyata *et al.* (1981)
SAS, *Salmonella typhimurium* TA92, reverse mutation	–	–	500	Miyata *et al.* (1981)
SCG, *Saccharomyces cerevisiae*, gene conversion	–	0	100	Marquardt *et al.* (1970)
G5T, Gene mutation, mouse L5178Y cells, *tk* locus *in vitro*	–	+	75	US National Toxicology Program (1992)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	+	200	Galloway *et al.* (1987)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–	(+)	550	Galloway *et al.* (1987)
CIC, Chromosomal aberrations, Chinese hamster lung CHL cells *in vitro*	–	0	250	Ishidate *et al.* (1988)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	0	13.7	Doolittle *et al.* (1983)
UIA, Unscheduled DNA synthesis, rat pachytene spermatocytes and round spermatids *in vitro*	–		13.7	Working & Butterworth (1984)

Table 3 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
4-Nitrotoluene (contd)				
UPR, Unscheduled DNA synthesis, male rat hepatocytes *in vivo*	–		500 po × 1	Doolittle *et al.* (1983)
UPR, Unscheduled DNA synthesis, rat hepatocytes *in vivo*	–		1000 po × 1	Mirsalis *et al.* (1989)
UPR, Unscheduled DNA synthesis, male rat hepatocytes *in vivo*	–		500 po × 1	US National Toxicology Program (1992)
MVM, Micronucleus test, mice *in vivo*	–		NR	Ohuchida *et al.* (1989)
BVD, Binding (covalent) to DNA, male rat liver *in vivo*	–		200 po × 1	Rickert *et al.* (1984)
BVP, Binding (covalent) to RNA or protein, male rat liver *in vivo*	+		200 po × 1	Rickert *et al.* (1984, 1986)

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

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

[c] Positive in the presence of 200 μg/plate norharman

[d] Negative also in the presence of 200 μg/plate norharman

In vivo in rats, 3-nitrotoluene bound covalently to hepatic macromolecules, but not to DNA. 3-Nitrotoluene was shown to be a weak inducer of sister chromatid exchange in Chinese hamster ovary cells *in vitro* but did not induce chromosomal aberrations. 3-Nitrotoluene did not induce unscheduled DNA synthesis in Fischer 344 rat hepatocytes, either after in-vitro treatment or after in-vivo treatment. It did not induce unscheduled DNA synthesis in rat spermatogonia exposed *in vitro*.

In the yeast *Saccharomyces cerevisiae*, 4-nitrotoluene did not induce mitotic gene conversion.

In vivo in rats, 4-nitrotoluene bound covalently to hepatic macromolecules, but not to DNA. 4-Nitrotoluene induced sister chromatid exchange and chromosomal aberrations in Chinese hamster ovary cells *in vitro* in one study. 4-Nitrotoluene did not induce unscheduled DNA synthesis in Fischer 344 rat primary hepatocyte cultures after in-vitro or in-vivo treatment. It did not induce unscheduled DNA synthesis in rat spermatogonia exposed *in vitro*.

4-Nitrotoluene did not induce micronuclei in polychromatic erythrocytes after intraperitoneal injection into male BDF1 mice.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

2-, 3- and 4-Nitrotoluenes are produced commercially, as a mixture, by nitration of toluene. 2- and 4-Nitrotoluenes are used mainly to produce intermediates in the production of colourants. All of these isomers are also used in much smaller quantities in the production of agricultural, pharmaceutical and rubber chemicals. Human exposure to nitrotoluenes can occur during their production and use, although few data are available. Nitrotoluenes have been detected in effluents from the manufacture or use nitrotoluenes and in surface and groundwater.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

No long-term study of the carcinogenicity of 2-, 3- or 4-nitrotoluene was available to the Working Group.

Rare mesotheliomas of the tunica vaginalis were reported in male rats receiving 2-nitrotoluene in the diet for 13 weeks.

5.4 Other relevant data

No relevant data on absorption, distribution, metabolism or excretion in humans were available to the Working Group.

Urinary elimination is the major route of excretion in rats exposed to nitrotoluene isomers. Male rats excrete more of an administered dose of nitrotoluene in the bile compared with female rats. All three nitrotoluene isomers cause an increase in the incidence of hyaline droplet nephropathy in male rats: the hyaline droplets were associated with $\alpha_{2\mu}$-globulin. Liver toxicity was observed in rats exposed to 2-nitrotoluene. In mice, the only evidence of toxicity was degeneration and metaplasia of the olfactory epithelium.

In rats, no adverse effect on reproduction or on the offspring was observed following administration of 2-, 3- or 4-nitrotoluene by gavage. All three isomers impaired testicular function and increased the length of the oestrus cycle. The 2-isomer decreased sperm motility in mice.

2-Nitrotoluene was not genotoxic in bacteria, but induced sister chromatid exchange in cultured mammalian cells. *In vivo* in rats, 2-nitrotoluene bound to macromolecules and, in males, induced unscheduled DNA synthesis in liver cells. In-vivo activity depends on the presence of intestinal bacteria.

3-Nitrotoluene produced a weak induction of sister chromatid exchange, but not of chromosomal aberrations or unscheduled DNA synthesis in mammalian cells *in vitro*. *In vivo,* it bound to macromolecules but not to DNA and did not induce unscheduled DNA synthesis.

4-Nitrotoluene was not genotoxic in yeast. In mammalian cells *in vitro*, it induced sister chromatid exchange and chromosomal aberrations. It did not induce unscheduled DNA synthesis in rat cells exposed either *in vitro* or *in vivo*. *In vivo* in rats, it bound to macromolecules, but not to DNA. It did not induce micronuclei in mouse bone marrow *in vivo*.

5.5 Evaluation[1]

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

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

There is *inadequate evidence* in experimental animals for the carcinogenicity of 3- and 4-nitrotoluenes.

Overall evaluation

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

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

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