NITROBENZENE

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

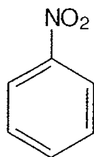
Chem. Abstr. Serv. Reg. No.: 98-95-3

Chem. Abstr. Name: Nitrobenzene

IUPAC Systematic Name: Nitrobenzene

Synonyms: Essence of mirbane; essence of myrbane; mirbane oil; nitrobenzol; oil of mirbane; oil of myrbane

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_6H_5NO_2$

Relative molecular mass: 123.11

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Greenish yellow crystals or yellow oily liquid with an odour of bitter almonds (Booth, 1991; Lewis, 1993)

(*b*) *Boiling-point*: 210.8 °C (Lide, 1993)

(*c*) *Melting-point*: 5.8 °C (Dunlap, 1981)

(*d*) *Density*: 1.2037 at 20 °C/4 °C (Lide, 1993)

(*e*) *Spectroscopy data*: Infrared (prism [12], grating [10]), ultraviolet [8], nuclear magnetic resonance (proton [4], C-13 [1401]) and mass spectral data have been reported (Sadtler Research Laboratories, 1980).

(*f*) *Solubility*: Moderately soluble in water (1.9 g/L at 20 °C); soluble in acetone, benzene, diethyl ether and ethanol (Booth, 1991; Lide, 1993)

(*g*) *Volatility*: Vapour pressure, 0.15 mm Hg [20 Pa] at 20 °C; relative vapour density (air = 1), 4.1 (Verschueren, 1983; Booth, 1991; Lide, 1993)

(*h*) *Stability*: Moderate explosion hazard when exposed to heat or flame; explosive reaction with solid or concentrated alkali (e.g. sodium hydroxide or potassium hydroxide) and heat, with aluminium chloride and phenol (at 120 °C), with aniline, glycerol and sulfuric acid, and with nitric and sulfuric acids and heat;

forms explosive mixtures with aluminium chloride, oxidants, phosphorous pentachloride, potassium and sulfuric acid (Sax & Lewis, 1989)

(*i*) *Octanol/water partition coefficient (P)*: log P, 1.85 (Hansch *et al.*, 1995)

(*j*) *Conversion factor*: $mg/m^3 = 5.04 \times ppm$[1]

1.1.4 *Technical products and impurities*

Nitrobenzene is available commercially at a purity of 99.9% (First Chemical Corp., 1993).

1.1.5 *Analysis*

Selected methods for the analysis of nitrobenzene in various media are identified in Table 1.

The physicochemical properties (volatility, water solubility and partition coefficient) of nitrobenzene determine the manner in which it is analysed in biological samples. Typically, the routine determination of nitrobenzene in the urine, at concentrations in the range of 5–50 mg/L, is based on colorimetric analysis. This is achieved through acidification of the urine and zinc reduction of the nitro group of nitrobenzene. Subsequent diazotization and coupling to 1-amino-8-naphthol-2,4-disulfonic acid (Chicago acid) allows spectrophotometric determination of the primary aromatic amine as a red azo dye. An alternative method, in which reduction of the aromatic nitro compounds is carried out under alkaline conditions through the use of formamidine sulfinic acid (thiourea dioxide), has been described (Koniecki & Linch, 1958).

The difficulty of analysis of nitrobenzene and its metabolite aniline in animals has been discussed (Albrecht & Neumann, 1985). Excretion of the parent compound and metabolites in urine has been determined, but, for practical reasons, this type of biological monitoring has not yet produced satisfactory results. Nitrobenzene metabolites are bound to blood proteins, both in haemoglobin and in plasma. Acute poisoning by nitrobenzene is usually monitored by measuring levels of methaemoglobin, which is produced by the metabolic products of nitrobenzene, but this is a relatively non-specific method since many toxicants produce methaemoglobin. Determination of total 4-nitrophenol in urine specimens collected at the end of the work week has also been recommended for monitoring nitrobenzene exposure (Albrecht & Neumann, 1985; Agency for Toxic Substances and Disease Registry, 1990; American Conference of Governmental Industrial Hygienists, 1995).

Pendergrass (1994) reported an approach for estimating workplace exposure to nitrobenzene. A qualitative estimate of potential dermal exposure to nitrobenzene in the workplace was obtained using gauze surface wipes, a dermal badge sampler was developed to estimate potential worker dermal exposure to nitrobenzene via splashes, spills and aerosol vapours, and an air sampling train, consisting of an acid-treated glass fibre filter in series with a large silica gel tube, allowed airborne workplace exposures to nitro-

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

benzene to be quantified. All samples were desorbed with ethanol followed by analysis using capillary gas chromatography with flame ionization detection.

Table 1. Methods for the analysis of nitrobenzene

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Air	Draw air through solid sorbent tube; desorb with methanol	GC/FID	0.02 mg	Eller (1994) [Method 2005]
	Trap in ethanol or isopropanol; reduce to aniline with zinc/hydrochloric acid; couple with 1,2-naphthoquinone-4-sulfonic acid, disodium salt; extract with carbon tetrachloride; read at 450 nm	Colorimetry	10 μg	Dangwal & Jethani (1980)
Water	Extract sample with dichloromethane or adsorb on Amberlite XAD resin and elute with dichloromethane	GC/ECD	NR	Feltes *et al.* (1990)
	Purge sample with helium; trap on solid sorbent; desorb thermally (capillary column method)	GC/MS	1.2 μg/L	Munch & Eichelberger (1992)
	Solid-phase microextraction with a polydimethylsiloxane-coated fibre; desorb thermally	GC/FID	9 μg/L	Horng & Huang (1994)
Municipal and industrial discharges	Add sodium oxalate/EDTA/perchloric acid solutions; filter; adjust to pH 3.0 with perchloric acid	LC/UV	NR	Nielen *et al.* (1985)
	Extract with dichloromethane; dry; exchange to hexane	GC/ECD GC/FID	13.7 μg/L 3.6 μg/L	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	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	GC/MS	10 μg/L	US Environmental Protection Agency (1994) [Method 1625B]
Water, soil, sediment, waste	Extract with dichloromethane (capillary column method)	GC/MS	PQL[a]	US Environmental Protection Agency (1986c) [Method 8270]

Table 1 (contd)

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Soil	Extract with methanol; clean-up with solid-phase extraction	HPLC/UV	NR	Grob & Cao (1990)
Urine	Reduce to aniline with zinc/hydrochloric acid; couple with 1,2-naphthoquinone-4-sulfonic acid, disodium salt; extract with carbon tetrachloride; read at 450 nm	Colorimetry	0.8 mg/L	Dangwal & Jethani (1980)
Blood	Extract from separated plasma and concentrate with 2,2,4-trimethylpentane	GC/ECD	10 μg/L	Lewalter & Ellrich (1991)

GC, gas chromatography; FID, flame ionization detection; ECD, electron capture detection; NR, not reported; MS, mass spectrometry; EDTA, ethylenediaminotetraacetic acid; LC, liquid chromatography; UV, ultraviolet detection; HPLC, high-performance liquid chromatography

[a]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 mg/kg

1.2 Production and use

1.2.1 *Production*

Nitrobenzene was first synthesized in 1834 by treating benzene with fuming nitric acid, and was first produced commercially in England in 1856 (Dunlap, 1981).

Nitrobenzene is manufactured commercially by the direct nitration of benzene using what is known as 'mixed acid' or 'nitrating acid' (27–32% HNO_3, 56–60% H_2SO_4, 8–17% H_2O). Historically, it was produced by a batch process. With a typical batch process, the reactor was charged with benzene at a temperature of 50–55 °C. The mixed acid was then added slowly below the surface of the benzene and the temperature raised to 80–90 °C. The reaction mixture was fed into a separator where the spent acid settled to the bottom and was drawn off to be refortified. The crude nitrobenzene was drawn from the top of the separator and was washed in several steps with dilute sodium carbonate and then water. Depending on the desired purity of the nitrobenzene, the product was then distilled. Today, nitrobenzene is made by a continuous process, but the sequence of operations is basically the same as for batch processing; however, for a given rate of production, the size of the reactors is much smaller in a continuous process. A 120-L continuous reactor has been reported to give the same output of nitrobenzene as a 6000-L batch reactor (Dunlap, 1981; Booth 1991).

The annual world production capacity for nitrobenzene in 1985 was approximately 1.7 million tonnes, with about one-third of this production located in western Europe and one-third in the United States of America (Booth, 1991). The increasing production/demand for nitrobenzene in the United States since 1960 is presented in Table 2.

Table 2. Production/demand levels for nitrobenzene in the United States

Year	Production/demand (thousand tonnes)
1960	73
1965	127
1970	249
1975	259
1980	277
1984	431
1986	435
1987	422[a]
1989	533[a]
1990	533[a]
1992	612[a]
1993	671[a]

From Mannsville Chemical Products Corp. (1984); Anon. (1987, 1990); American Conference of Governmental Industrial Hygienists (1991); Anon. (1993)
[a] Demand

Nitrobenzene is known to be produced by seven companies in China, six companies in the United States, five companies each in Brazil and Japan, four companies each in Germany and India, two companies each in Italy and Russia, and one company each in Argentina, Armenia, Belgium, Czech Republic, France, Hungary, Mexico, Portugal, Republic of Korea, Romania, Spain and the United Kingdom (Chemical Information Services, 1994).

1.2.2 *Use*

Nitrobenzene has a wide variety of uses. Most significantly, and accounting for 95% or more of nitrobenzene use, is the manufacture of aniline (see IARC, 1982, 1987) through the reduction of the nitro group of nitrobenzene. Aniline is a major chemical intermediate in the production of dyestuffs and other products.

Lower-volume industrial uses of nitrobenzene include electrolytic reduction to 4-aminophenol, nitration to give 1,3-dinitrobenzene, chlorination to give 3-chloronitrobenzene (see this volume), sulfonation to give 3-nitrobenzenesulfonic acid and chlorosulfonation to give 3-nitrobenzenesulfonyl chloride. The last three products are consumed mainly as their reduction products, 3-chloroaniline, metanilic acid and 3-aminobenzenesulfonamide, respectively. Nitrobenzene is also used as a solvent for cellulose ethers, in modifying the esterification of cellulose acetate, in the preparation of nitrocellulose (pyroxylin) derivatives and in refining lubricating oils (Parmeggiani, 1983;

Budavari, 1989; Booth, 1991). It is also used in the production of various pharmaceutical products, in rubber industry applications and as an industrial solvent (Mannsville Chemical Products Corp., 1984).

Nitrobenzene is also used as a constituent of soap and polishes, as a solvent for some paints and as a preservative in spray paints. Owing to its musk-like odour, nitrobenzene can be used to mask unpleasant smells (Parmeggiani, 1983; Budavari, 1989; Booth, 1991; Lewis, 1993). In addition, it is reportedly used as a substitute for almond essence in the perfume industry. It is registered as an insecticide for use on cadavers (United States Environmental Protection Agency, 1988).

Nigrosin (CI Solvent Black 5), where it is still produced and used (e.g. in certain inks), is the crude mixture obtained by reacting nitrobenzene with aniline and aniline hydrochloride at 200 °C in the presence of iron or copper (Booth, 1991).

1.3 Occurrence

1.3.1 *Natural occurrence*

Nitrobenzene is not known to occur as a natural product.

1.3.2 *Occupational exposure*

No data on occupational exposures to nitrobenzene were available to the Working Group.

The National Occupational Exposure Survey conducted between 1981 and 1983 indicated that 5080 employees in the United States were potentially exposed to nitrobenzene. The estimate is based on a survey of United States companies and did not involve measurements of actual exposure (United States National Institute for Occupational Safety and Health, 1995).

1.3.3 *Environmental occurrence*

(*a*) *Air*

In 1981, sites in three industrialized cities in New Jersey, United States, were monitored continuously for six weeks for a number of airborne toxic substances. The results for nitrobenzene were as follows: Newark, 81% of 37 samples positive; geometric mean concentration, 0.07 ppb [0.35 μg/m^3]; Elizabeth, 86% of 36 samples positive; geometric mean concentration, 0.10 ppb [0.5 μg/m^3]; and Camden, 86% of 37 samples positive; geometric mean concentration, 0.07 ppb [0.35 μg/m^3] (Harkov *et al.*, 1983). Trace levels of nitrobenzene were found in two of 13 air samples from the Lipari and BFI landfills in New Jersey (Howard, 1989). Mean air concentrations of nitrobenzene at five abandoned hazardous landfill sites in New Jersey ranged from 0.01 to 1.32 ppb [0.05–6.65 μg/m^3], with a maximum concentration of 3.46 ppb [17.4 μg/m^3] (Harkov *et al.*, 1985).

The United States Environmental Protection Agency assessed volatile organic compounds in the atmosphere at 595 urban/suburban sites using available data. Nitro-

benzene was found to have had a maximal concentration of 2.8 ppb [14 μg/m^3] and a mean concentration of 0.17 ppb [0.86 μg/m^3]; 75% of the samples contained less than 0.09 ppb [0.45 μg/m^3] (Brodzinsky & Singh, 1982).

(*b*) *Water*

Among United States water supplies, nitrobenzene was detected but not quantified in finished water from the Carrollton Water Plant in Louisiana, and in drinking-water in Cincinnati, OH. Also, in a survey of 14 treated drinking-water supplies of varied sources in the United Kingdom, nitrobenzene was detected in one supply which came from an upland reservoir (Howard, 1989).

Ambient surface water and industrial effluents have been monitored for nitrobenzene at 836 and 1245 stations, respectively, in the United States for the United States Environmental Protection Agency STORET database. Of these, 0.4% and 1.8% reported detectable levels (< 10 μg/L) of nitrobenzene, respectively (Staples *et al.*, 1985). In the Netherlands, average and maximal levels of nitrobenzene were 1.7 and 13.8 μg/L in the River Wall and < 0.1 and 0.3 μg/L in the River Maas (Meijers & van der Leer, 1976); in another study in the Netherlands, water in the River Rhine contained 0.5 μg/L nitrobenzene (Zoeteman *et al.*, 1980). In water samples collected in 1986 from the Scheldt Estuary, located in the South-west Netherlands and North-west Belgium, the dissolved concentration of nitrobenzene was 0.13 μg/L (van Zoest & van Eck, 1991). A two-week composite water sample taken in 1984 from the River Rhine near Dusseldorf, Germany, contained a mean nitrobenzene concentration of 0.42 g/L (Sontheimer *et al.*, 1985). In the late 1980s, the concentrations of nitrobenzene in the River Elbe, Germany, were 0.1 μg/L in a sample collected near Lauenberg, 0.03 μg/L in a sample collected near Brokdorf and 0.02 μg/L in the sample collected near Brunsbüttel (Feltes *et al.*, 1990). Samples of river water and seawater from various locations in Japan contained 0.16–0.99 ppb [μg/L] nitrobenzene (Sugiyama *et al.*, 1978).

In groundwater samples collected from January to March 1987 in Degrémont, France, nitrobenzene was identified as a pollutant at concentrations ranging from 3 to 12 μg/L (Duguet *et al.*, 1988).

A comprehensive survey of wastewater from 4000 industrial and publicly owned treatment works, carried out by the United States Environmental Protection Agency, identified nitrobenzene in discharges from the following industrial categories (frequency of occurrence, median concentration in μg/L): organic chemicals (36, 43.7); organics and plastics (13, 3876.7); explosives (8, 51.7); inorganic chemicals (3, 1995.3); leather tanning (1, 3.7); petroleum refining (1, 7.7); nonferrous metals (1, 47.7); pulp and paper (1, 124.3); auto and other laundries (1, 40.4); and pesticides manufacture (1, 16.3). The highest effluent concentration for a single sample was 100 mg/L in the organics and plastics industry (Howard, 1989). Nitrobenzene was also detected in the final effluent of three wastewater treatment works and an oil refinery in Illinois (Ellis *et al.*, 1982), and two samplings of the final effluent of the Los Angeles County Municipal Wastewater Treatment Plant collected in 1978 and 1980 contained mean concentrations of 20 and < 10 μg/L nitrobenzene, respectively (Young *et al.*, 1983).

Wastewaters discharged from a nitrobenzene manufacturing plant in India were found to contain 55–138 mg/L (mean, 107 mg/L) nitrobenzene in an acidic stream and 52–93 mg/L (mean, 67 mg/L) nitrobenzene in an alkaline stream. Wastewaters discharged from a chloronitrobenzene manufacturing plant in India were found to contain 4–17 mg/L (mean, 9 mg/L) nitrobenzene (Swaminathan *et al.*, 1987).

(*c*) *Soil and sediments*

Nitrobenzene was detected at a concentration of 8 mg/kg (ppm) in one out of two soil samples along the Buffalo River in Buffalo, NY, United States, but was not detected in three samples of bottom sediment from the river (Nelson & Hites, 1980). None of the 349 stations monitoring for nitrobenzene in sediment in the United States Environmental Protection Agency STORET database reported detectable levels of nitrobenzene (Staples *et al.*, 1985).

1.3.4 *Food*

None of the 122 monitoring stations analysing for nitrobenzene in fish in the United States Environmental Protection Agency STORET database reported detectable levels in any sample (Staples *et al.*, 1985)

1.4 Regulations and guidelines

Occupational exposure limits and guidelines for nitrobenzene in several countries are presented in Table 3.

Table 3. Occupational exposure limits and guidelines for nitrobenzene

Country	Year	Concentration (mg/m^3)	Interpretation
Argentina	1991	5 (Sk)	TWA
Australia	1993	5 (Sk)	TWA
Belgium	1993	5 (Sk)	TWA
Bulgaria[a]	1995	5 (Sk)	TWA
Canada	1991	5 (Sk)	TWA
Colombia[a]	1995	5 (Sk)	TWA
Czech Republic	1993	5 (Sk)	TWA
		25	STEL
Denmark	1993	5 (Sk)	TWA
Egypt	1993	5 (Sk)	TWA
Finland	1993	5 (Sk)	TWA
		15	STEL (15 min)
France	1993	5 (Sk)	TWA
Germany	1995	5 (Sk)[b]	MAK
Hungary	1993	3 (Sk)	TWA
		6	STEL
Japan	1993	5 (Sk)	TWA

Table 3 (contd)

Country	Year	Concentration (mg/m^3)	Interpretation
Jordan[a]	1995	5 (Sk)	TWA
Mexico	1991	5 (Sk)	TWA
		10	STEL
Netherlands	1994	5 (Sk)	TWA
New Zealand[a]	1995	5 (Sk)	TWA
Republic of Korea[a]	1995	5 (Sk)	TWA
Poland	1993	3	TWA
Russia	1993	3 (Sk)	STEL
Singapore[a]	1995	5 (Sk)	TWA
Sweden	1993	5 (Sk)	TWA
		10	STEL
Switzerland	1993	5 (Sk)	TWA
		10	STEL
Turkey	1993	5 (Sk)	TWA
United Kingdom	1995	5 (Sk)	TWA
		10	STEL (15 min)
USA			
ACGIH (TLV)	1995	5 (Sk)[b]	TWA
OSHA (PEL)	1994	5 (Sk)	TWA
NIOSH (REL)	1994	5 (Sk)	TWA
Viet Nam[a]	1993	5 (Sk)	TWA

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

TWA, time-weighted average; Sk, absorption through the skin may be a significant source of exposure; STEL, short-term exposure limit; MAK, maximal workplace concentration; TLV, threshold limit value; PEL, permissible exposure limit; REL, recommanded exposure limit

[a] Follows ACGIH TLVs

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

Two methods for biological monitoring of nitrobenzene exposures have been adopted. Total 4-nitrophenol in urine is measured as a biological marker for exposure to nitrobenzene with a Biological Exposure Index of 5 mg/g creatinine (Lauwerys, 1991;

American Conference of Governmental Industrial Hygienists, 1995). A less specific biological marker for exposure to nitrobenzene is methaemoglobin level in the blood, with a maximal permissible value of 1.5% of haemoglobin (American Conference of Governmental Industrial Hygienists, 1995).

In Germany, a BAT (Biological Tolerance Value) at the workplace of 100 μg aniline/L blood has been established (aniline released from aniline-haemoglobin conjugate) (Deutsche Forschungsgemeinschaft, 1995).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

3.1 Inhalation exposure

3.1.1 *Mouse*

Groups of 70 male and 70 female B6C3F1 mice, 63 days of age, were exposed by inhalation to air containing target concentrations of 0, 5, 25 or 50 ppm [0, 25, 125 or 250 mg/m^3] nitrobenzene (> 99.8% pure) for 6 h per day on five days per week for 24 months. Body weights of high-dose male mice were approximately 5–8% lower than those of controls throughout the study. Probability of survival at 24 months was 60% for males and 45% for females and was not affected by exposure to nitrobenzene, except that mid-dose females had better survival than controls (70%). The incidence of alveolar–bronchiolar neoplasms was increased in treated males (alveolar–bronchiolar adenomas and carcinomas: 9/68 in controls, 21/67 at the low dose, 21/65 at the mid dose and 23/66 at the high dose; $p < 0.05$, Cochran-Armitage trend test). The incidence of alveolar–bronchiolar hyperplasia was also increased in mid- and high-dose males and in mid-dose females. The incidence of thyroid follicular-cell adenomas was increased in treated males (0/65 in controls, 4/65 at the low dose, 1/65 at the mid dose, 7/64 at the high dose; $p < 0.05$ trend test) and that of thyroid follicular-cell hyperplasia was increased in mid- and high-dose males. The incidence of hepatocellular adenomas was increased in treated females (6/51 in controls, 5/61 at the low dose, 5/64 at the mid dose, 13/62 at the high dose; $p < 0.05$ trend test), although the incidence of hepatocellular adenomas and carcinomas combined was not increased (7/51, 7/61, 7/64, 14/62, respectively). Mammary gland adenocarcinomas were found in 5/60 ($p < 0.05$) high-dose females compared to 0/48 controls (Cattley *et al.*, 1994).

3.1.2 *Rat*

Groups of 70 male and 70 female Fischer 344 rats, 62 days of age, were exposed by inhalation to air containing target concentrations of 0, 1, 5 or 25 ppm [0, 5, 25 or

125 mg/m³] nitrobenzene (> 99.8% pure) for 6 h per day on five days per week for 24 months. Groups of 10 rats per sex and per group were killed for an interim evaluation at 15 months. Body weights of high-dose males were slightly lower than those of controls during the study. Probability of survival at 24 months was 75% for males and 80% for females and was not affected by exposure to nitrobenzene. Increased incidences were noted for hepatic eosinophilic foci in mid- and high-dose males and in high-dose females, and for hepatocellular neoplasms in both treated males (adenomas and carcinomas: 1/69 in controls, 4/69 at the low dose, 5/70 at the mid dose, 16/70 at the high dose; $p < 0.05$, Cochran-Armitage trend test) and treated females (0/70 in controls, 2/66 at the low dose, 0/66 at the mid dose, 4/70 at the high dose; $p < 0.05$ trend test). Thyroid follicular-cell hyperplasia occurred with a positive exposure-related trend in males and the incidences of thyroid follicular-cell adenomas and adenocarcinomas were increased in exposed males (2/69 in controls, 1/69 at the low dose, 5/70 at the mid dose, 8/70 at the high dose; $p < 0.05$ trend test). The incidence of endometrial stromal polyps was increased in exposed females (11/69 in controls, 17/65 at the low dose, 15/65 at the mid dose, 25/69 at the high dose; $p < 0.05$); that of renal tubular-cell adenomas was increased in exposed males (0/69 in controls, 0/68 at the low dose, 0/70 at the mid dose, 5/70 at the high dose; $p < 0.05$, Fisher exact test) and one renal tubular-cell carcinoma occurred in another high-dose male. There was an increased severity of nephropathy in exposed males and females (Cattley *et al.*, 1994).

Groups of 70 male Charles River CD rats, 62 days of age, were exposed by inhalation to air containing target concentrations of 0, 1, 5 or 25 ppm [0, 5, 25 or 125 mg/m³] nitrobenzene (> 99.8% pure) for 6 h per day on five days per week for 24 months. Groups of 10 rats per sex and per group were killed for an interim evaluation at 15 months. Body weights and survival were not affected by exposure to nitrobenzene during the study. The incidence of hepatocellular neoplasms was increased in treated groups (adenomas and carcinomas: 2/63 in controls, 1/67 at the low dose, 4/70 at the mid dose, 9/65 at the high dose; $p < 0.05$, Cochran-Armatage trend test). The incidence of spongiosis hepatis was increased in high-dose rats, and that of centrilobular hepatocytomegaly was increased in mid- and high-dose groups. The incidence of Kupffer-cell pigmentation was increased in all treated groups (Cattley *et al.*, 1994, 1995).

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 nitrobenzene has been reviewed (Beauchamp *et al.*, 1982; Rickert, 1987).

Salmowa *et al.* (1963) exposed seven volunteers to nitrobenzene vapours (5–30 µg/L) [5–30 mg/m³, 1–6 ppm] for 6 h. Nitrobenzene was readily absorbed, initially at an

average of approximately 87% in the first hour to 73% in the sixth hour, probably because of saturation. 4-Nitrophenol rapidly appeared in the urine, and its maximal excretion, at a concentration of 5 mg/L urine, occurred approximately 2 h after the end of exposure. Of the absorbed dose, 6–37% were recovered as 4-nitrophenol, and the level of this metabolite could be used as an index of exposure. 4-Aminophenol was not found in the urine. [However, the method used to analyse 4-aminophenol was fairly insensitive and low concentrations may have occurred.] After the end of exposure, there was a biphasic decline in 4-nitrophenol concentration in the urine. The initial half-life was about 5 h and the terminal half-life was about 60 h, which indicates that, potentially, accumulation could occur during a working week.

In vitro, nitrobenzene is rapidly absorbed through excised human skin in a diffusion cell (Bronaugh & Maibach, 1985). Feldmann and Maibach (1970) applied [^{14}C]-nitrobenzene on the forearm of volunteers, who did not wash the area of skin for 24 h. Over five days, excretion in urine was only 1.5% of the applied dose. Piotrowski (1967) used a technique to expose the whole skin surface to nitrobenzene vapour without inhalation of the compound. During the first day, at an air level of 1 ppm [5 mg/m^3], about 7 mg nitrobenzene were absorbed through the skin, of which about 20% were excreted into the urine.

Feldmann and Maibach (1970) injected [^{14}C]nitrobenzene intravenously into volunteers. Excretion in the urine was 60.5% of the dose over five days. The elimination half-life was 20 h.

In a case of nitrobenzene poisoning in a woman using a paint containing nitrobenzene as solvent (99.7% nitrobenzene, 0.27% benzene in distillate), the urinary level of 4-nitrophenol was 1056 nmol/mL [142 mg/L] one day after the end of exposure. Simultaneously, a concentration of 400 nmol/mL [39.6 mg/L] 4-aminophenol was detected. The levels decreased with an estimated half-life of a few days (Ikeda & Kita, 1964).

4.1.2 *Experimental systems*

Bronaugh and Maibach (1985) studied the percutaneous absorption of 4 μg/cm^2 nitrobenzene in an acetone vehicle in monkeys. *In vitro*, 6.2 ± 1.0% of the applied dose was absorbed percutaneously, and *in vivo*, 4.2 ± 0.5% of the applied dose was excreted in urine after five days. Loss of nitrobenzene due to volatilization could have affected the amount of nitrobenzene absorbed.

Schmieder and Henry (1988) studied the equilibrium binding of nitrobenzene to plasma proteins *in vitro* in pooled plasma samples from Sprague-Dawley rats [sex unspecified]. Aliquots of pooled plasma samples spiked with 0.32–933 mg/L nitrobenzene were allowed to equilibrate for 30–90 min at 25 °C. Of the nitrobenzene, 72.0 ± 4.5% were bound to the rat plasma proteins.

Parke (1956) administered 250 mg/kg bw [^{14}C]nitrobenzene by stomach tube to rabbits and measured metabolites in expired air, urine and faeces. Nearly 70% of the administered radioactivity was excreted within five days. Major metabolic products were 3- and 4-nitrophenols and 4-aminophenol. Minor metabolites included aniline, 2-aminophenol, 3-aminophenol, 4-nitrocatechol and 4-nitrophenyl mercapturic acid.

Rickert *et al.* (1983) administered [^{14}C]nitrobenzene to male Fischer 344 rats (22.5 or 225 mg/kg bw, 20 μCi, in corn oil orally or 225 mg/kg bw intraperitoneally), male CD rats (22.5 or 225 mg/kg bw orally), male B6C3F1 mice (225 mg/kg bw orally) and germ-free male Fischer 344 rats (225 mg/kg bw orally). No significant effect of route of administration or strain was observed for the excretion of radioactivity in urine, faeces or expired air following administration of 225 mg/kg bw nitrobenzene. Following oral administration of 225 mg/kg bw to Fischer 344 rats, excretion of radioactivity was distributed as follows: urine, 63.2%; faeces, 14.2%; expired air, 1.6%. At this same dose, but following intraperitoneal administration, the distribution of excretion of radioactivity was very similar: urine, 56.8%; faeces, 13.7%; expired air, 1.4%. A smaller dose of 22.5 mg/kg bw administered orally to Fischer 344 rats, resulted in a significantly higher proportion of radioactivity excreted in faeces (21.4%). Following a similar treatment pattern, B6C3F1 mice excreted a smaller percentage of the dose in urine (34.7%) than did rats, but similar percentages in faeces (18.8%) and expired air (0.8%). Four major metabolites were found in the urine of Fischer 344 rats: 4-hydroxyacetanilide sulfate; 4-nitrophenol sulfate; 3-nitrophenol sulfate; and an unidentified metabolite. 4-Hydroxyacetanilide sulfate and 4-nitrophenol sulfate were excreted in approximately equal proportions (20% of dose). 3-Nitrophenol sulfate and the unidentified metabolite each made up 10% of the dose. 4-Hydroxyacetanilide, 4-nitrophenol and 3-nitrophenol were found in the urine of B6C3F1 mice and CD rats but not of Fischer 344 rats. B6C3F1 mice and CD rats also excreted each of the above metabolites as glucuronides (except 3-nitrophenol in mice) and sulfates. Mice excreted nearly 10% of the dose as 4-aminophenol sulfate, whereas rats did not excrete this metabolite.

Bile collected from Fischer 344 and CD rats over the first 12 h after oral administration of 225 mg/kg bw nitrobenzene contained 1.8% and 3.8% of the dose, respectively. Of six peaks detected, three co-eluted with 4-hydroxy-3-methylthioacetanilide, 2-acetamido-3-(5′-acetanido-2′-hydroxyphenylthio)propanoic acid and *S*-(5′-acetamido-2′-hydroxyphenyl)glutathione. Another co-eluted with glutathione sulfinanilide. None of the metabolites recovered in bile of conventional Fischer 344 rats was found in bile of germ-free Fischer 344 rats (Rickert *et al.*, 1983).

In-vivo experiments determined the role of microflora in nitrobenzene metabolism in control and animals treated with antibiotics (Levin & Dent, 1982). Antibiotic treatment totally inhibited in-vitro metabolism of nitrobenzene by caecal contents and decreased the expected level of methaemoglobin formation after a single oral dose of 300 mg/kg bw nitrobenzene. The excretion of ^{14}C was not altered by antibiotic treatment; however, the pattern of urinary metabolites was changed. Antibiotic treatment decreased the urinary excretion of the reduced metabolite, 4-hydroxyacetanilide, to 6% of control values and that of an unidentified metabolite to 14% of control values; excretion of 3-nitrophenol was increased over control values.

Nitrobenzene is reduced to aniline in in-vitro hepatic microsome systems via the intermediate products nitrosobenzene and phenylhydroxylamine (Harada & Omura, 1980). Blaauboer and Van Holsteijn (1983) investigated the formation and disposition of *N*-hydroxylated metabolites of nitrobenzene (phenylhydroxylamine and nitrosobenzene) by isolated rat hepatocytes. Apparent kinetic parameters for nitrobenzene reduction by

hepatocytes, as measured by secretion of *N*-oxygenated products into the incubation medium, were V_{max} 1.44 ± 0.21 nmol/min/mL and K_m 4.2 ± 1.4 mM. Phenobarbital pre-treatment stimulated the secretion of hydroxylated metabolites 2.8-fold.

Levin and Dent (1982) studied the metabolism of nitrobenzene using hepatic microsomes and caecal microflora from male Fischer 344 rats *in vitro*. Oxidative metabolism of 100 μM [^{14}C]nitrobenzene occurred at a rate of 0.008 ± 0.003 nmol/mg protein/min. The major product was unidentified and accounted for nearly 40% of the metabolites formed. Metabolism of nitrobenzene was also studied under anaerobic conditions, in which microsomal reduction occurred much more rapidly than did oxidation (0.33 versus 0.022 nmol/mg protein/min). The rate of reduction by caecal contents was 150-fold that in microsomes.

Protein binding

Albrecht and Neumann (1985) measured tissue dosimetry and haemoglobin binding in Wistar rats following a 0.20 mmol/kg bw [24.6 mg/kg bw] oral dose of [^{14}C]nitrobenzene. Radioactivity in tissues (pmol/mg/dose [mmol/kg]) after one day was as follows: blood, 229 ± 48; liver, 129 ± 9.5; kidney, 204 ± 27; and lung, 62 ± 14. The binding index (mmol/mol haemoglobin/dose [mmol/kg]) was 72.8 ± 10. Specific binding (pmol/mg/dose) was 1030 ± 137 for haemoglobin and 136 ± 34 for plasma proteins.

Goldstein and Rickert (1984) determined species differences in the covalent binding of [^{14}C]nitrobenzene to erythrocytes and spleen of male Fischer 344 and male B6C3F1 mice following an oral dose of 75, 150, 200 or 300 mg/kg bw nitrobenzene in corn oil. Total radioactivity in erythrocytes, as a percentage of dose, averaged 0.57 ± 0.11% and 0.08 ± 0.01% in rats and mice, respectively, following treatment with 200 mg/kg nitrobenzene. In both species, total and bound concentrations of ^{14}C were four to six times greater in erythrocytes than in spleen. All of the covalently bound nitrobenzene-related material in haemoblogin was recovered in the protein fraction, suggesting that nitrobenzene or its metabolites bind specifically to the globin moiety.

Reddy *et al.* (1976) administered nitrobenzene to normal and germ-free rats to determine the role of gut flora and tissues with regard to nitrobenzene reduction and formation of methaemoglobin. When nitrobenzene (200 mg/kg bw) was administered intraperitoneally to normal male Sprague-Dawley rats, 30–40% of the blood haemoglobin was converted to methaemoglobin. No measurable formation was observed 7 h after administration to germ-free rats. Suzuki *et al.* (1989) administered orally 0.5 mmol/kg bw nitrobenzene in a corn oil solution to male Sprague-Dawley rats; 48 h after administration, haemoglobin binding was 657.0 ± 36.7 nmol/g haemoglobin. Pre-treatment with antibiotics decreased haemoglobin binding to 88.2 ± 10.5 nmol/g haemoglobin. The effect of dietary pectin on methaemogolobin formation from nitrobenzene was studied in male CDF rats (Goldstein *et al.*, 1984). Rats were held on one of three dietary regimens (0%, 5% or 8.4% pectin) for 28 days, after which they received 600 mg/kg bw nitrobenzne orally in corn oil. Animals fed the 8.4% pectin diet had the highest methaemoglobin content (64 ± 1%) and those fed the pectin-free diet had the lowest (20 ± 5%). The total number of caecal anaerobes was elevated (2–2.5 fold) and

the metabolism of nitrobenzene by the caecal contents was also greater in animals fed the diets containing pectin.

4.2 Toxic effects

4.2.1 *Humans*

The toxic effects of nitrobenzene have been reviewed (Agency for Toxic Substances and Disease Registry, 1990).

Cases of severe poisoning were reported as early as 1886 in infants exposed to dye-stamped diapers and persons wearing freshly dyed shoes. The condition was often referred to as 'nitrobenzene poisoning', although exposure to nitrobenzene had not necessarily occurred; the conditions may have been caused by aniline (Agency for Toxic Substances and Disease Registry, 1990).

Methaemoglobinaemia, with cyanosis, headache, dyspnoea, weakness and ultimately coma and death, is the main characteristic of acute nitrobenzene poisoning. Nitrobenzene may also induce haemolysis, which is, however, usually mild (Hunter, 1943).

Methaemoglobinaemia was reported in three-week-old twins (Stevens, 1928) and in a 12-month-old girl (Stevenson & Forbes, 1942) exposed to nitrobenzene from insect-exterminator sprays for several hours. Moreover, a woman who worked under bad hygienic conditions in a cable insulation factory for three months developed serious poisoning. Her methaemoglobin level in the blood was 29.5% (37 g/L) up to 36 h after the end of exposure. [Lethal at about 80%; 'normally' about 1% or 1 g/L; 'normal' half-life 15–20 h.] She also developed haemolysis, as well as slight toxic hepatitis and peripheral neuropathy. It was discovered that she had a hereditary deficiency of NADH-methaemoglobin reductase, which may have made her particularly sensitive, and which also probably explained the high methaemoglobin level a long time after exposure (Kokal *et al.*, 1984). Development of toxic hepatitis after acute episodes of methaemoglobinaemia has been reported repeatedly (Ajmani *et al.*, 1986).

There is little quantitative information on the relationship between toxic effects and exposure. A man who had ingested about 7 g nitrobenzene developed methaemoglobinaemia (78% of methaemoglobin; Schimelman *et al.*, 1978). Pacséri *et al.* (1958) found air concentrations of nitrobenzene averaging 6 ppm [30 mg/m^3] in a plant producing nitroaromatic compounds. There was no obvious case of poisoning, although 'one or two' cases of headache and vertigo were mentioned. Examination of the blood revealed low concentrations of methaemoglobin. Previously, concentrations of nearly 40 ppm [200 mg/m^3] were stated to have caused poisonings.

Ikeda and Kita (1964) reported methaemoglobinaemia (about 30 g/L) two days after the end of exposure in a woman who for 17 months had used a paint containing nitrobenzene as solvent. The urinary level of 4-nitrophenol was 1056 μmol/mL [142 mg/mL] and that of *para*-aminophenol was about 400 μmol/mL [39.6 mg/mL] one day after the end of exposure. The patient also had clinical and laboratory signs of haemolytic anaemia and toxic hepatitis. Salmowa *et al.* (1963) found no increase of methaemoglobin

concentration in blood in seven volunteers exposed for 6 h to air levels of up to 30 μg/L [6 ppm] and excreting up to 5 mg/L 4-nitrophenol in urine.

4.2.2 *Experimental systems*

(*a*) *Single dose studies*

The single oral LD_{50} for nitrobenzene in rats was 600 mg/kg bw (Agency for Toxic Substances and Disease Registry, 1990). Single acute exposures of male Fischer 344 rats to ≥ 200 mg/kg bw nitrobenzene resulted in significantly elevated (> 20%) methaemoglobin (Goldstein *et al.*, 1984), while higher single oral exposures (550 mg/kg bw) resulted in encephalomalacia and haemorrhage of the brainstem and cerebellum in male Fischer 344 rats (Morgan *et al.*, 1985). Necrosis of seminiferous tubules and hepatocellular nucleolar enlargement in male Fischer 344 rats following single oral exposure have also been reported (Bond *et al.*, 1981). The latter liver lesions were observed at doses as low as 110 mg/kg bw whereas the testicular lesions occurred at doses ≥ 300 mg/kg bw. Acute exposure by injection of nitrobenzene has been reported to cause methaemoglobinaemia, neurotoxicity and death in a variety of animal species (reviewed in Beauchamp *et al.*, 1982).

(*b*) *Repeated-dose studies*

Male and female Fischer 344 rats, Sprague-Dawley (CD) rats and B6C3F1 mice (9–10 weeks old) were exposed by inhalation to 10, 35 or 125 ppm [50, 175 or 625 mg/m^3] nitrobenzene vapours for 6 h per day on five days per week for up to two weeks (Medinsky & Irons, 1985). Animals were sacrificed at three or 14 days following the last exposure. Early morbidity among male and female mice exposed to 125 ppm necessitated euthanasia between two and four days of exposure. Some male and female Sprague-Dawley rats were found dead after the fourth day of exposure, but the remaining animals in the group exhibited rapid shallow breathing, wheezing and an orange discoloration around the urogenital orifice. In contrast, Fischer 344 rats exposed to 125 ppm exhibited no adverse clinical signs over the entire two-week period. The presumptive cause of death of the Sprague-Dawley rats exposed to 125 ppm nitrobenzene was perivascular haemorrhage in the cerebellar peduncle. Species and sex-related differences in liver pathology were also observed in animals exposed to 125 ppm nitrobenzene. Male mice exhibited centrilobular necrosis, superimposed on severe central lobular hydropic degeneration. In contrast, no necrosis was observed in livers from female mice at the same concentration. Liver pathology observed in Sprague-Dawley rats was similar but not as severe as that described for the mice. Livers from Sprague-Dawley rats that died early exhibited centrilobular hydropic degeneration and basophilic hepatocytic degeneration in periportal areas. No significant histological findings was observed in the livers from male and female Fischer 344 rats.

Moderate bronchiolar hyperplasia was observed in male and female mice exposed to 125 ppm nitrobenzene; mild hyperplasia was present in animals examined three days after the last exposure to 35 ppm. Perivascular oedema and vascular congestion were found in lungs taken from dead or moribund Sprague-Dawley rats after three to five days

of exposure to 125 ppm nitrobenzene. No histopathology was found in the lungs from Fischer 344 rats exposed to 125 ppm nitrobenzene. Sprague-Dawley rats also exhibited moderate-to-severe hydropic degeneration of cortical tubular cells. Minimal degenerative changes were noted in the kidneys of some mice. The only renal lesion in Fischer 344 rats was a moderate to severe hyaline nephrosis in males that regressed in animals allowed to recover for 14 days. Splenic lesions were evident in all rats and mice in all groups exposed to nitrobenzene. Lesions consisted of increased extramedullary haematopoiesis and acute congestion. Thus for nitrobenzene, the most sensitive organ after 14-day inhalation exposure was the spleen (Medinsky & Irons, 1985).

The effects of chronic (two-year) inhalation exposure to nitrobenzene in B6C3F1 mice and Fischer 344 and Charles River (CD) rats have been described (Cattley *et al.*, 1994). Methaemoglobinaemia and anaemia were observed in both species at ≥ 25 ppm [100 mg/m^3] exposure concentrations. Other effects included lesions of the nose, liver, testis and lung. In mice, degeneration and loss of olfactory epithelium were observed at ≥ 5 ppm; the incidence of pigment deposition in olfactory epithelium was increased in mice and rats. Cytomegaly of centrilobular hepatocytes was induced in mice and rats, particularly males, at ≥ 5 ppm; in male mice multinucleation of hepatocytes was also induced. An increased incidence of testicular atrophy and epididymal hypospermia was observed in male CD (but not Fischer 344) rats at 25 ppm. In mice, an unusual pulmonary lesion, alveolar bronchialization, was frequently induced by exposure to ≥ 5 ppm nitrobenzene.

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 26 pregnant Sprague-Dawley rats were exposed by inhalation to 0, 1, 10 and 40 ppm [5, 50 and 200 mg/m^3] nitrobenzene vapour for 6 h per day on gestational days 6–15. Maternal weight gain was reduced during exposure to 40 ppm, with full recovery by gestational day 21. Absolute and relative spleen weights were increased at 10 and 40 ppm. There was no effect of treatment on resorptions or dead fetuses, on the sex ratio of live fetuses or on fetal body weights per litter. No treatment-related effect on the incidence of fetal malformations or variations was observed (Tyl *et al.*, 1987).

In an accompanying paper, a two-generation reproduction study was described, again involving exposure of Sprague-Dawley rats to nitrobenzene vapour (Dodd *et al.*, 1987). Groups of 30 male and 30 female rats were exposed to concentrations of 0, 1, 10 or 40 ppm nitrobenzene vapour for 6 h per day on five days per week for 10 weeks. F_1 rats were produced from the F_0 rats and at least one male and one female were picked randomly from each litter to form a group size of 30 per sex. F_1 rats remained in the same exposure group as their F_0 parents. Additional female rats were used for a second mating with the recovery group high-dose and control F_1 males. No effect on reproduction was

observed at doses of 1 or 10 ppm nitrobenzene. At 40 ppm, a decrease in the fertility index of the F_0 and F_1 generations occurred, and this was associated with reduced testicular and epididymal weight, atrophy of the seminiferous tubules, spermatocytic degeneration and the presence of giant syncytial spermatocytes. The only significant observation in the litter derived from rats exposed to 40 ppm was an approximate 12% decrease in the mean body weights of F_1 rats on postnatal day 21. Survival indices were unaltered. In the F_1 rats, males of the high-dose and control groups were allowed a nine-week nonexposure recovery period. At the end of this period, the F_1 males were mated with virgin females, which had never been exposed to nitrobenzene. An almost five-fold increase in the fertility index was observed, indicating at least partial functional reversibility upon removal from nitrobenzene exposure. In addition, the numbers of giant syncytial spermatocytes and degenerated spermatocytes were greatly reduced; testicular seminiferous tubule atrophy persisted.

In a study reported as an abstract, groups of 22 pregnant rabbits were exposed by inhalation to 0, 9.9, 41 and 101 ppm [50, 207 and 509 mg/m^3] nitrobenzene for 6 h per day on gestational days 7–19. The dams were sacrificed on gestational day 30 and the fetuses were evaluated for external, visceral and skeletal malformations. No adverse effect was associated with the lowest dose. At the two higher doses (41 and 101 ppm), liver weights were slightly higher and methaemoglobin levels were significantly increased compared with controls. At the highest dose, a slight increase in fetal resorption was observed. No teratogenic effect was apparent at any of the exposure levels investigated (Schroeder *et al.*, 1986).

Necrosis of seminiferous tubules has been described in Fischer 344 rats after exposure to nitrobenzene (see Section 4.2.2).

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)

No standard reverse mutation test with *Salmonella typhimurium* showed mutagenic activity of nitrobenzene. Only a few *Salmonella* tests in the presence of S9 and norharman were positive.

In cultures of primary human hepatocytes *in vitro*, no unscheduled DNA synthesis was observed.

In Fischer 344 rats, no significant increase in sister chromatid exchange frequency or chromosomal aberrations was found in peripheral blood lymphocytes. No significant increase in sister chromatid exchange was observed in the isolated splenic lymphocytes after in-vivo exposure to up to 50 ppm nitrobenzene for 6 h per day for 21 days during a 29-day period; the toxicity of the dosing regimen was demonstrated by cell cycle inhibition and mitotic depression in the lymphocytes.

Table 4. Genetic and related effects of nitrobenzene

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	–	1250	Anderson & Styles (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	0	615	Chiu *et al.* (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	385	Haworth *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	2355	Shimizu *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	0	50	Suzuki *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	NR	Nohmi *et al.* (1984)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	0	500	Vance & Levin (1984)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	NR	Kawai *et al.* (1987)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–[c]	465	Dellarco & Prival (1989)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	0	–	1250	Anderson & Styles (1978)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	128	Haworth *et al.* (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	2355	Shimizu *et al.* (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	0	500	Vance & Levin (1984)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	128	Haworth *et al.* (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	2355	Shimizu *et al.* (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	0	500	Vance & Levin (1984)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	0	–	1250	Anderson & Styles (1978)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	2355	Shimizu *et al.* (1983)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	0	500	Vance & Levin (1984)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	–	1250	Anderson & Styles (1978)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	0	615	Chiu *et al.* (1978)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	385	Haworth *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	2355	Shimizu *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–[d]	50	Suzuki *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	NR	Nohmi *et al.* (1984)

Table 4 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	0	500	Vance & Levin (1984)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	NR	Kawai *et al.* (1987)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	–[d]	100	Suzuki *et al.* (1987)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–[c]	465	Dellarco & Prival (1989)
SAS, *Salmonella typhimurium* TA100NR, reverse mutation	–	0	500	Vance & Levin (1984)
SAS, *Salmonella typhimurium* TA1537NR, reverse mutation	–	0	500	Vance & Levin (1984)
SAS, *Salmonella typhimurium* TA98NR, reverse mutation	–	0	500	Vance & Levin (1984)
SAS, *Salmonella typhimurium* TA98a, reverse mutation	–	0	500	Vance & Levin (1984)
SAS, *Salmonella typhimurium* TA98NR reverse mutation	0	–[d]	500	Suzuki *et al.* (1987)
SAS, *Salmonella typhimurium* TA98/1,8-DNP$_6$, reverse mutation	0	–[d]	100	Suzuki *et al.* (1987)
UIH, Unscheduled DNA synthesis, human hepatocytes *in vitro*	–	0	123	Butterworth *et al.* (1989)
UPR, Unscheduled DNA synthesis, rat hepatocytes *in vivo*	–		500 po × 1	Mirsalis *et al.* (1982)
SVA, Sister chromatid exchange, peripheral blood lymphocytes, male F344 rats *in vivo*	–		53 inh 6h/d × 21	Kligerman *et al.* (1983)
SVA, Sister chromatid exchange, splenic lymphocytes, male F344 rats *in vivo*	–		53 inh 6h/d × 21	Kligerman *et al.* (1983)
CVA, Chromosomal aberrations, peripheral blood lymphocytes, male F344 rats *in vivo*	–		53 inh 6h/d × 21	Kligerman *et al.* (1983)

[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] Negative with or without 2mM flavin mononucleotide (FMN) in preincubation mix
[d] Positive in the presence of 200 μg/plate norharman

Oral administration of 500 mg/kg bw nitrobenzene to rats did not induce unscheduled DNA synthesis in hepatocytes cultured from the exposed animals.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Nitrobenzene has been produced commercially since the early nineteenth century by nitration of benzene. It is a major chemical intermediate used mainly in the production of aniline, itself a major chemical intermediate in the production of dyes. Human exposure may occur both by inhalation and by skin absorption during its production and use. Nitrobenzene has been detected in surface and groundwater.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Nitrobenzene was tested by inhalation exposure in one study in mice and in two studies in rats. In mice, the incidences of alveolar–bronchiolar neoplasms and thyroid follicular-cell adenomas were increased in males. In one study in rats, the incidences of hepatocellular neoplasms, thyroid follicular-cell adenomas and adenocarcinomas and renal tubular-cell adenomas were increased in treated males. In treated females, the incidences of hepatocellular neoplasms and endometrial stromal polyps were increased. In a study using male rats only, the incidence of hepatocellular neoplasms was increased.

5.4 Other relevant data

In humans, nitrobenzene is readily absorbed by inhalation. Penetration through the skin also occurs. A major part of the absorbed dose is excreted into the urine: 10–20% of the dose is excreted as 4-nitrophenol, the concentration of which may be used for biological monitoring. A smaller fraction is excreted as 4-aminophenol. The elimination kinetics contains at least two compartments, the first with a half-life of hours and the second with a half-life of days.

In rodents and rabbits, 4-nitrophenol and 4-aminophenol are major urinary metabolites.

There is limited information on the toxic effects of exposure to nitrobenzene in humans. However, it is clear that both accidental ingestion and occupational exposure may cause methaemoglobinaemia, haemolytic anaemia and toxic hepatitis.

Following inhalation of nitrobenzene, liver, lung and splenic toxicity is observed in both rats and mice, although mice appear to be more sensitive than rats to the toxic effects of this chemical. Methaemoglobinaemia and anaemia are also observed in both rats and mice.

In female rats, no teratogenic or reproductive effect of exposure to nitrobenzene was observed. Testicular atrophy has been observed in rats. In a two-generation reproduction study in rats, a decrease in the fertility index of the F_0 and F_1 generations occurred. No teratogenic effect has been observed in rabbits.

Nitrobenzene was non-genotoxic in bacteria and mammalian cells *in vitro*. In mammals *in vivo*, it was inactive.

5.5 Evaluation[1]

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

There is *sufficient evidence* in experimental animals for the carcinogenicity of nitrobenzene.

Overall evaluation

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

6. References

Agency for Toxic Substances and Disease Registry (1990) *Toxicological Profile for Nitrobenzene* (TP-90-19), Atlanta, GA, United States Public Health Service

Ajmani, A., Prakash, S.K., Jain, S.K. & Shah, P. (1986) Aquired methemoglobinemia following nitrobenzene poisoning. *J. Assoc. Phys. India,* **34**, 891–892

Albrecht, W. & Neumann, H.-G. (1985) Biomonitoring of aniline and nitrobenzene: hemoglobin binding in rats and analysis of adducts. *Arch. Toxicol.*, **57**, 1–5

American Conference of Governmental Industrial Hygienists (1991) *Documentation of the Threshold Limit Values and Biological Exposure Indices*, 6th Ed., Vol. 2, Cincinnati, OH, pp. 1096–1099

American Conference of Governmental Industrial Hygienists (1995) *1995–1996 Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices*, Cincinnati, OH, pp. 28, 65

Anderson, D. & Styles, J.A. (1978) The bacterial mutation test. *Br. J. Cancer*, **37**, 924–930

Anon. (1987) Chemical profile: nitrobenzene. *Chem. Mark. Rep.*, **232**, 50

Anon. (1990) Chemical profile: nitrobenzene. *Chem. Mark. Rep.*, **238**, 50

Anon. (1993) Chemical profile: nitrobenzene. *Chem. Mark. Rep.*, **244**, 57

Arbeidsinspectie [Labour Inspection] (1994) *De Nationale MAC-Lijst 1994* [National MAC list 1994], The Hague, p. 35

Beauchamp, R.O., Jr, Irons, R.D., Rickert, D.E., Couch, D.B. & Hamm, T.E., Jr (1982) A critical review of the literature on nitrobenzene toxicity. *CRC crit. Rev. Toxicol.*, **11**, 33–84

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

Blaauboer, B.J. & Van Holsteijn, C.W. M. (1983) Formation and disposition of *N*-hydroxylated metabolites of aniline and nitrobenzene by isolated rat hepatocytes. *Xenobiotica*, **13**, 295–302

Bond, J.A., Chism, J.P., Rickert, D.E. & Popp, J.A. (1981) Induction of hepatic and testicular lesions in Fischer-344 rats by single oral doses of nitrobenzene. *Fundam. appl. Toxicol.*, **1**, 389–394

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

Brodzinsky, R. & Singh, H.B. (1982) *Volatile Organic Chemicals in the Atmosphere: An Assessment of Available Data* (US EPA Report No. EPA-600/3-83027a; US NTIS PB83-195503), Research Triangle Park, NC, United States Environmental Protection Agency

Bronaugh, R.L. & Maibach, H.I. (1985) Percutaneous absorption of nitroaromatic compounds: in vivo and in vitro studies in the human and monkey. *J. invest. Dermatol.*, **84**, 180–183

Budavari, S., ed. (1989) *The Merck Index*, 11th Ed., Rahway, NJ, Merck & Co., p. 1042

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**, 1079–1084

Cattley, R.C., Everitt, J.I., Gross, E.A., Moss, O.R., Hamm, T.E., Jr & Popp, J.A. (1994) Carcinogenicity and toxicity of inhaled nitrobenzene in B6C3F1 mice and F344 and CD rats. *Fundam. appl. Toxicol.*, **22**, 328–340

Cattley, R.C., Everitt, J.I., Gross, E.A., Moss, O.R., Hamm, T.E., Jr & Popp, J.A. (1995) Erratum. *Fundam. appl. Toxicol.*, **25**, 159

Chemical Information Services (1994) *Directory of World Chemical Producers 1995/96 Standard Edition*, Dallas, TX, pp. 524–525

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

Dangwal, S.K. & Jethani, B.M. (1980) A simple method of determination of nitrobenzene and nitrochlorobenzene in air and urine. *Am. ind. Hyg. Assoc. J.*, **41**, 847–850

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

Deutsche Forschungsgemeinschaft (1995) *MAK and BAT Values 1995* (Report No. 31), Weinheim, VCH Verlagsgesellschaft, pp. 49, 145

Dodd, D.E., Fowler, E.H., Snellings, W.M., Pritts, I.M., Tyl, R.W., Lyon, J.P., O'Neal, F.O. & Kimmerle, G. (1987) Reproduction and fertility evaluations in CD rats following nitrobenzene inhalation. *Fundam. appl. Toxicol.*, **8**, 493–505

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

Dunlap, K.L. (1981) Nitrobenzene and nitrotoluenes. In: Mark, H.F., Othmer, D.F., Overberger, C.G., Seaborg, G.T. & Grayson, N., eds, *Kirk-Othmer Encyclopedia of Chemical Technology*, 3rd Ed., Vol. 15, New York, John Wiley & Sons, pp. 916–932

Eller, P.M., ed. (1994) Nitrobenzenes — Method 2005. In: *NIOSH Manual of Analytical Methods*, 4th Ed., Vol 2 (E-N) (DHHS (NIOSH) Publ. No. 94-113), Washington DC, United States Government Printing Office

Ellis, D.D., Jone, C.M., Larson, R.A. & Schaeffer, D.J. (1982) Organic constitutents of mutagenic secondary effluents from wastewater treatment plants. *Arch. environ. Contam. Toxicol.*, **11**, 373–382

Feldmann, R.J. & Maibach, H.I. (1970) Absorption of some organic compounds through the skin in man. *J. invest. Dermatol.*, **54**, 399–404

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

First Chemical Corp. (1993) *Material Safety Data Sheet: Nitrobenzene*, Pascagoula, MS

Goldstein, R.S. & Rickert, D.E. (1984) Macromolecular covalent binding of [^{14}C]nitrobenzene in the erythrocyte and spleen of rats and mice. *Chem.-biol. Interactions,* **50**, 27–37

Goldstein, R.S., Chism, J.P., Sherrill, J.M. & Hamm, T.E., Jr (1984) Influence of dietary pectin on intestinal microfloral metabolism and toxicity of nitrobenzene. *Toxicol. appl. Pharmacol.,* **75**, 547–553

Grob, R.L. & Cao, K.B. (1990) High performance liquid chromatographic study of the recovery of aromatic amine and nitro compounds from soil. *J. environ. Sci. Health*, **A25**, 117–136

Hansch, C., Leo, A. & Hoekman, D.H. (1995) *Exploring QSAR*, Washington DC, American Chemical Society, p. 18

Harada, N. & Omura, T. (1980) Participation of cytochrome P450 in the reduction of nitro compounds by rat liver microsomes. *J. Biochem. Tokyo,* **87**, 1539–1544

Harkov, R., Kebbekus, B., Bozzelli, J.W. & Lioy, P. (1983) Measurement of selected volatile compounds at three locations in New Jersey during the summer season. *J. Air Pollut. Control Assoc.*, **33**, 1177–1183

Harkov, R., Gianti, S.J., Jr, Bozzelli, J.W. & LaRegina, J.E. (1985) Monitoring volatile organic compounds at hazardous and sanitary landfills in New Jersey. *J. environ. Sci. Health*, **A20**, 491–501

Haworth, S., Lawlor, T., Mortelsmans, 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, p. 35

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, Michigan, Lewis Publishers, pp. 421–430

Hunter, D. (1943) Industrial toxicology. *Q. J. Med.,* **12**, 185–258

IARC (1982) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans,* Vol. 27, *Some Aromatic Amines, Anthraquinones and Nitroso Compounds, and Inorganic Fluorides Used in Drinking Water and Dental Preparations*, Lyon, pp. 39–61

IARC (1987) *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, pp. 99–100

Ikeda, M. & Kita, A. (1964) Excretion of *p*-nitrophenol and *p*-aminophenol in the urine of a patient exposed to nitrobenzene. *Br. J. ind. Med.,* **21**, 210–213

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

Kligerman, A.D., Erexson, G.L., Wilmer, J.L. & Phelps, M.C. (1983) Analysis of cytogenetic damage in rat lymphocytes following in vivo exposure to nitrobenzene. *Toxicol. Lett.*, **18**, 219–226

Kokal, K.C., Khanna, S.S., Retnam, V.J. & Dastur, F.D. (1984) Methemoglobinemia: an unusual presentaion. *J. Assoc. Phys. India,* **32**, 833–834

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

Lauwerys, R.R. (1991) Occupational toxicology. In: Amdur, M.O., Doull, J. & Klaassen, C.D., eds, *Casarett and Doull's Toxicology — The Basic Science of Poisons*, 4th Ed., New York, Pergamon Press, pp. 947–969

Levin, A.A. & Dent, J.G. (1982) Comparison of the metabolism of nitrobenzene by hepatic microsomes and cecal microflora from Fischer-344 rats *in vitro* and the relative importance of each *in vivo. Drug Metab. Dispos.,* **10**, 450–454

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. 825

Lide, D.R., ed. (1993) *CRC Handbook of Chemistry and Physics*, 74th Ed., Boca Raton, FL, CRC Press, pp. 3–85, 6–76

Mannsville Chemical Products Corp. (1984) *Chemical Products Synopsis: Nitrobenzene*, Cortland, NY

Medinsky, M.A. & Irons, R.D. (1985) Sex, strain, and species differences in the response of rodents to nitrobenzene vapors. In: Rickert, D.E., ed., *The Toxicity of Nitroaromatic Compounds*, New York, Hemisphere Publishing, pp. 35–51

Meijers, A.P. & van der Leer, R.C. (1976) The occurrence of organic micropollutants in the River Rhine and the River Maas in 1974. *Water Res.*, **10**, 597–604

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

Morgan, K.T., Gross, E.A., Lyght, O. & Bond, J.A. (1985) Morphologic and biochemical studies of a nitrobenzene-induced encephalopathy in rats. *Neurotoxicology*, **6**, 105–116

Munch, J.W. & Eichelberger, J.W. (1992) Evaluation of 48 compounds for possible inclusion in the U.S. EPA Method 524.2, Revision 3.0: expansion of the method analyte list to a total of 83 compounds. *J. chromatogr. Sci.*, **30**, 471–477

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

Nielen, M.W.F., Brinkman, U.A.T. & Frei, R.W. (1985) Industrial wastewater analysis by liquid chromatography with precolumn technology and diode-array detection. *Anal. Chem.*, **57**, 806–810

Nohmi, T., Yoshikawa, K., Nakadate, M., Miyata, R. & Ishidate M., Jr (1984) Mutations in *Salmonella typhimurium* and inactivation of *Bacillus subtilis* transforming DNA induced by phenylhydroxylamine derivatives. *Mutat. Res.*, **136**, 159–168

Pacséri, L., Magos, L. & Batskor, A. (1958) Threshold and toxic limits of some amino and nitro compounds. *Arch. ind. Health*, **18**, 1–18

Parke, D.V. (1956) Detoxication. LXVIII. The metabolism of C^{14}-nitrobenzene in the rabbit and guinea pig. *Biochem. J.*, **62**, 339–346

Parmeggiani, L., ed. (1983) *Encyclopedia of Occupational Health and Safety*, 3rd. Ed., Vol. 2, L-Z, Geneva, International Labour Office, p. 1448

Pendergrass, S.M. (1994) An approach for estimating workplace exposure to o-toluidine, aniline and nitrobenzene. *Am. ind. Hyg. Assoc. J.*, **55**, 733–737

Piotrowski, J. (1967) Further investigations on the evaluation of exposure to nitrobenzene. *Br. J. ind. Med.*, **24**, 60–65

Reddy, B.G., Pohl, T.R. & Krishna, G. (1976) The requirement of gut flora in nitrobenzene-induced methemoglobinemia in rats. *Biochem. Pharmacol.*, **25**, 1119–1122

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

Rickert, D.E., Bond, J.A., Long, R.M. & Chism, J.P. (1983) Metabolism and excretion of nitrobenzene by rats and mice. *Toxicol. appl. Pharmacol.*, **67**, 206–214

Sadtler Research Laboratories (1980) *Sadtler Standard Spectra. 1980 Cumulative Index*, Philadelphia, PA, p. 92

Salmowa, J., Piotrowski, J. & Neuhorn, U. (1963) Evaluation of exposure to nitrobenzene. Absorption of nitrobenzene vapour through lungs and excretion of *p*-nitrophenol in urine. *Br. J. ind. Med.*, **20**, 41–46

Sax, N.I. & Lewis, R.J., Sr (1989) *Dangerous Properties of Industrial Materials*, 7th Ed., Vol. 3, New York, Van Nostrand Reinhold Co., pp. 2504–2505

Schimelman, M.A., Soler, J.M. & Muller, H.A. (1978) Methemoglobinemia: nitrobenzene ingestion. *JACEP*, **7**, 406–408

Schmieder, P.K. & Henry, T.R. (1988) Plasma binding of 1-butanol, phenol, nitrobenzene, and pentachlorophenol in the rainbow trout and rat: a comparative study. *Comp. Biochem. Physiol.*, **91C**, 413–418

Schroeder, R.E., Terrill, J.B., Lyon, J.P., Kaplan, A.M. & Kimmerle, G. (1986) An inhalation teratology study in the rabbit with nitrobenzene. *Toxicologist*, **6**, 93

Shackelford, W.M., Cline, D.M., Faas, L. & Kurth, G. (1983) An evaluation of automated spectrum matching for survey identification of wastewater components by gas chromatography-mass spectrometry. *Anal. chim. Acta*, **146**, 15–27

Shimizu, M., Yasui, Y. & Matsumoto, N. (1983) Structural specificity of aromatic compounds with special reference to mutagenic activity in *Salmonella typhimurium* — a series of chloro- and fluoro-nitrobenzene derivatives. *Mutat. Res.*, **116**, 217–238

Sontheimer, H., Brauch, H.-J. & Kühn, W. (1985) Impact of different types of organic micropollutants present on sources of drinking water on the quality of drinking water. *Sci. total Environ.*, **47**, 27–44

Staples, C.A., Werner, A.F. & Hoogheem, T.J. (1985) Assessment of priority pollutant concentration in the United States using STORET database. *Environ. Toxicol. Chem.*, **4**, 131–142

Stevens, A.M. (1928) Cyanosis in infants from nitrobenzene. *J. Am. med. Assoc.*, **90**, 116

Stevenson, A. & Forbes, R.P. (1942) Nitrobenzene poisoning. *J. Pediat.*, **21**, 224

Sugiyama, H., Tanaka, K., Fukaya, K., Nishiyama, N. & Wada, Y. (1978) Studies on environmental pollution by chemical substances. II. Determination of aromatic nitro compounds in river and sea water. *Eisei Kagaku*, **24**, 11–18

Suzuki, J., Koyama, T. & Suzuki, S. (1983) Mutagenicities of mono-nitrobenzene derivatives in the presence of norharman. *Mutat. Res.*, **120**, 105–110

Suzuki, J., Takahashi, N., Kobayashi, Y., Miyamae, R., Ohsawa, M. & Suzuki, S. (1987) Dependence on *Salmonella typhimurium* enzymes of mutagenicities of nitrobenzene and its derivatives in the presence of rat-liver S9 and norharman. *Mutat. Res.*, **178**, 187–193

Suzuki, J., Meguro, S.-I., Morita, O., Hirayama, S. & Suzuki, S. (1989) Comparison of *in vivo* binding of aromatic nitro and amino compounds to rat hemoglobin. *Biochem. Pharmacol.*, **38**, 3511–3519

Swaminathan, K., Kondawar, V.K., Chakrabarti, T. & Subrahmanyam, P.V.R. (1987) Identification and quantification of organics in nitro aromatic manufacturing wastewaters. *Indian J. environ. Health*, **29**, 32–38

Tyl, R.W., France, K.A., Fisher, L.C., Dodd, D.E., Pritis, I.M., Lyon, J.P., O'Neal, F.O. & Kimmerle, G. (1987) Developmental toxicity evaluation of inhaled nitrobenzene in CD rats. *Fundam. appl. Toxicol.*, **8**, 482–492

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

United States Environmental Protection Agency (1986a) Method 8090. Nitroaromatics and cyclic ketones. In: *Test Methods for Evaluating Solid Wast — 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 (1988) *Extremely Hazardous Substances*, Vol. 2, Park Ridge, NJ, Noyes Data Corp., pp. 1145–1151

United States Environmental Protection Agency (1994) Appendix A to Part 136 — Methods for organic chemical analysis of municipal and industrial wastewater. *US Code fed. Regul.*, **Title 40**, Part. 136, pp. 503–513 [Method 609], 574–601 [Method 625], 615–634 [Method 1625B]

United States National Institute for Occupational Safety and Health (1994a) *Pocket Guide to Chemical Hazards* (DHHS (NIOSH) Publ. No. 94-116), Cincinnati, OH, pp. 226–227

United States National Institute for Occupational Safety and Health (1994b) *RTECs Chem.-Bank*, Cincinnati, OH

United States National Institute for Occupational Safety and Health (1995) *National Occupational Exposure Survey (1981–1983)*, Cincinnati, OH

United States National Library of Medicine (1995) *Registry of Toxic Effects of Chemical Substances (RTECS)*, Bethesda, MD

United States Occupational Safety and Health Administration (1994) Air contaminants. *US Code fed. Regul.*, **Title 29**, Part 1910.1000, p. 14

Vance, W.A. & Levin, D.E. (1984) Structural features of nitroaromatics that determine mutagenic activity in *Salmonella typhimurium. Environ. Mutag.*, **6**, 797–811

Verschueren, K. (1983) *Handbook of Environmental Data on Organic Chemicals*, 2nd Ed., New York, Van Nostrand Reinhold Co., pp. 910–912

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

van Zoest, R. & van Eck, G.T.M. (1991) Occurrence and behaviour of several groups of organic micropollutants in the Scheldt estuary. *Sci. total Environ.*, **103**, 57–71

Young, D.R., Gossett, R.W., Baird, R.B., Brown, D.A., Taylor, P.A. & Mille, M.J. (1983) Wastewater inputs and marine bioaccumulation of priority pollutant organics off southern California. In: Jolley, R.L., Brungs, W.A., Cotruvo, J.A., Cumming, R.B., Mattice, J.S. & Jacobs, V.A., eds, *Water Chlorination, Environmental Impact and Health Effects*, Vol. 4, Book 2, *Environment, Health, and Risk*, Ann Arbor, Ann Arbor Sciences, pp. 871–884