FINAL

Report on Carcinogens Background Document for

Nitrobenzene

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Prepared for the:
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FOREWORD

The Report on Carcinogens (RoC) is prepared in response to Section 301 of the Public Health Service Act as amended. The RoC contains a list of all substances (i) that either are known to be human carcinogens or may reasonably be anticipated to be human carcinogens; and (ii) to which a significant number of persons residing in the United States are exposed. The Secretary, Department of Health and Human Services (DHHS) has delegated responsibility for preparation of the RoC to the National Toxicology Program (NTP) who prepares the Report with assistance from other Federal health and regulatory agencies and non-government institutions.

Nominations for listing in or delisting from the RoC are reviewed by a formal process that includes a multi-phased, scientific peer review and multiple opportunities for public comment. The review groups evaluate each nomination according to specific RoC listing criteria. This Background Document was prepared to assist in the review of the nomination of Nitrobenzene. The scientific information in this document comes from publicly available, peer reviewed sources. Any interpretive conclusions, comments or statistical calculations, etc made by the authors of this document that are not contained in the original citation are identified in brackets [ ]. If any member(s) of the scientific peer review groups feel this Background Document does not adequately capture and present the relevant information they will be asked to write a commentary for this Background Document that will be included as an addendum to the document. In addition, a meeting summary that contains a brief discussion of the respective review group's review and recommendation for the nomination will be added to the Background Document, also as an addendum.

A detailed description of the RoC nomination review process and a list of all nominations under consideration for listing in or delisting from the RoC can be obtained by accessing the NTP Home Page at http://ntp-server.niehs.nih.gov. The most recent RoC, the 9th Edition, was published in May, 2000 and may be obtained by contacting the NIEHS Environmental Health Information Service (EHIS) at http://ehis.niehs.nih.gov (800-315-3010).

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Criteria for Listing Agents, Substances or Mixtures in the Report on Carcinogens

U.S. Department of Health and Human Services
National Toxicology Program

Known to be Human Carcinogens:

There is sufficient evidence of carcinogenicity from studies in humans, which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.

Reasonably Anticipated to be Human Carcinogens:

There is limited evidence of carcinogenicity from studies in humans, which indicates that causal interpretation is credible but that alternative explanations such as chance, bias or confounding factors could not adequately be excluded; or

There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species, or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor or age at onset; or

There is less than sufficient evidence of carcinogenicity in humans or laboratory animals, however; the agent, substance or mixture belongs to a well defined, structurally-related class of substances whose members are listed in a previous Report on Carcinogens as either a *known to be human carcinogen, or reasonably anticipated to be human carcinogen* or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

Executive Summary

Introduction
Nitrobenzene is a nitro aromatic hydrocarbon used to produce aniline. It was nominated by the National Institute of Environmental Health Sciences for listing in the Report on Carcinogens based on the conclusions of an International Agency for Research on Cancer (IARC) working group that there is sufficient evidence of its carcinogenicity in experimental animals and that it is possibly carcinogenic to humans (Group 2B) (IARC 1996).

Human Exposure
Use. The primary use of nitrobenzene is in the manufacture of aniline, an intermediate in the production of dyestuffs and other products. Nitrobenzene also can be used as a solvent and in the synthesis of isocyanates, pesticides, rubber chemicals, and pharmaceuticals.

Production. Nitrobenzene is produced in a continuous process by the direct nitration of benzene. Production of nitrobenzene in the United States was over 2 billion pounds in 2000.

Environmental Exposure. Environmental exposure to nitrobenzene is expected to be primarily through inhalation of ambient air, ingestion of water, or dermal exposure to products and water containing nitrobenzene. Two environmental air surveys of more than 500 sites reported mean concentrations of nitrobenzene to be 0.117 ppb and 0.17 ppb.

Occupational Exposure. Occupational exposure to nitrobenzene generally is via inhalation of the vapor or dermal contact with the vapor or liquid. The most recent information available for occupational exposures is through the National Institute of Occupational Safety and Health's (NIOSH's) National Occupational Exposure Survey, conducted from 1981 to 1983, which estimated that 5,080 employees (475 females) potentially were exposed to nitrobenzene.

Human Cancer Studies
Only one case-control study was found describing cancer effects from exposure to nitrobenzene. Paternal exposure to nitrobenzene was associated with a 1.6 odds ratio (OR) for childhood brain cancer (95% CI = 0.4 to 6.1); this risk estimate was based on a small number of exposed cases. The paucity of data precludes evaluation of carcinogenic effects of human exposure to nitrobenzene.

Studies in Experimental Animals
When administered by inhalation to experimental animals, nitrobenzene was carcinogenic at multiple sites and in multiple species. Male B6C3F_1 mice showed significantly increased incidences of lung and thyroid follicular cell neoplasia, and female B6C3F_1 mice showed a significantly increased incidence of mammary gland adenocarcinoma. Rats showed significantly increased incidences of hepatocellular neoplasia (male F344/N and CD rats), kidney tubular cell tumors (male F344/N rats), and endometrial stromal polyps (female F344/N rats). In addition, there were marginal increases in the incidences

of hepatocellular neoplasia in female $B6C3F_1$ mice, thyroid follicular cell neoplasia in male F344/N rats, and hepatocellular neoplasia in female F344/N rats.

Genotoxicity

Nitrobenzene was not genotoxic in bacteria (with or without mammalian metabolic activation) or in several mammalian *in vitro* assays (unscheduled DNA synthesis in human or rat hepatocytes) or *in vivo* assays (sister chromatid exchange in rat spleen or blood lymphocytes or chromosomal aberrations in rat peripheral blood lymphocytes). *In vivo* exposure to nitrobenzene did induce chromosomal aberrations in human peripheral blood lymphocytes.

Other Relevant Data

Human Toxicity. Methemoglobinemia has been observed in individuals exposed to nitrobenzene and in both subchronic and chronic studies in experimental animals.

Absorption, excretion, and metabolism in animals and humans. Nitrobenzene is absorbed dermally and by inhalation in both animals and humans. Overall, *in vivo* metabolism of nitrobenzene appears to be similar in humans and animals, with the major route of excretion being in the urine.

The major metabolites of nitrobenzene in humans and animals have been isolated from urine and include ring oxidation products such as nitrophenols and aminophenols, reduction products such as aniline, modified products such as glucuronide or sulfate conjugates, and ring-cleavage product such as 4-nitrophenylmercapturic acid. Based on these products, it has been proposed that the metabolism of nitrobenzene consists of two pathways, the first being reduction of the nitro group to aniline and subsequent ring oxidation to aminophenols followed by conjugation to the glucuronide and sulfate conjugates. The second pathway is ring oxidation to nitrophenols followed by conjugation to the glucuronide and sulfate conjugates. Nitrobenzene can be reduced to aniline under anaerobic conditions (by bacteria in the intestine) or aerobic conditions (in microsomes). The former is more likely to occur when nitrobenzene is ingested, and the latter when nitrobenzene is inhaled. Reduction of nitrobenzene appears to be an important step in the production of methemoglobinemia.

Potential mechanisms. The mechanism of nitrobenzene's carcinogenicity has not been elucidated. Metabolic reduction of nitrobenzene can lead to the production of free radicals. Nitrobenzene is structurally related to other aromatic nitro and amino compounds, including several nitroarenes that are considered by the National Toxicology Program (NTP) to be *reasonably anticipated to be human carcinogens* and/or by IARC to be carcinogenic to humans (Group 2B).

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1 Introduction

Nitrobenzene is used primarily to produce aniline, an intermediate in the production of dyestuffs and other products. Nitrobenzene also is used in the manufacture of compounds including benzidine and quinoline, in the production of isocyanates, pesticides, and pharmaceuticals, and as a solvent in petroleum refining. Nitrobenzene has been detected in air and appears to volatilize from water and soil. Environmental exposure appears to be primarily through inhalation of ambient air or through dermal exposure to products containing the chemical.

Nitrobenzene was nominated by NIEHS for possible listing in the Report on Carcinogens based on the conclusions of an IARC working group that nitrobenzene is possibly carcinogenic to humans (Group 2B) (IARC 1996). This conclusion was based on inhalation studies in experimental animals, which showed that nitrobenzene induced significant increases in the incidences of tumors in female $B6C3F_1$ mice (mammary gland adenocarcinoma), male $B6C3F_1$ mice (alveolar-bronchiolar and thyroid follicular cell neoplasia), female F344/N rats (endometrial stromal polyps), male F344/N rats (hepatocellular neoplasia and kidney tubular cell tumors), and male CD rats (hepatocellular neoplasms).

1.1 Chemical identification

Nitrobenzene ($C_6H_5NO_2$, mol wt 123.11, CASRN 98-95-3) is a colorless to pale yellow, oily liquid with an odor that resembles bitter almonds. It also is known as nitrobenzol, mirbane oil, essence of mirbane, oil of mirbane, essence of myrbane, and oil of myrbane. Its RTECS number is DA6475000, its U.S. Environmental Protection Agency (EPA) hazardous waste numbers are D036 and U169 (as a solid), and its U.S. Department of Transportation shipping number and hazard class are UN 1662, Poison B. The structure of nitrobenzene is illustrated in Figure 1-1.

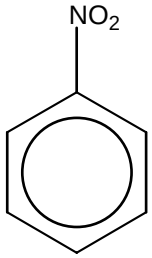


Figure 1-1. Structure of nitrobenzene

1.2 Physical-chemical properties

Nitrobenzene is a combustible liquid. It reacts with reducing agents and at high temperature with caustic soda and alkalies. It also may react with nitric acid, sulfuric acid, potassium hydroxide, silver perchlorate, and sodium chlorate (NTP 2001). It is

stable under normal laboratory conditions. The physical and chemical properties of nitrobenzene are summarized in Table 1-1.

Table 1-1. Physical and chemical properties of nitrobenzene

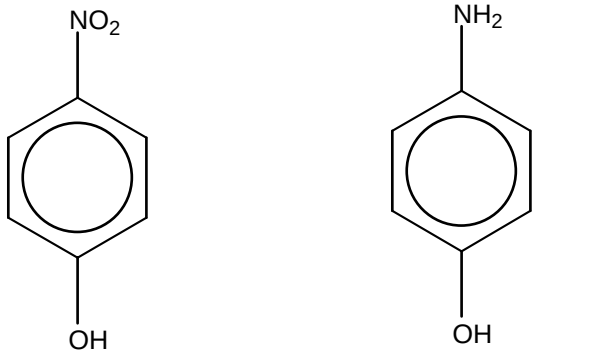
Property	Information	Reference
Molecular weight	123.11	ChemFinder 2001, Budavari *et al.* 1996
Color	colorless to pale yellow	ChemFinder 2001, Budavari *et al.* 1996
Odor	bitter almonds or black paste shoe polish	Adkins 2000, ChemFinder 2001
Physical state	oily liquid	ChemFinder 2001, Budavari *et al.* 1996
Melting point (^{o}C)	5.85	Adkins 2000
Boiling point (^{o}C)	210.9	Adkins 2000, Budavari *et al.* 1996
Flash point (^{o}C) closed cup	88	Adkins 2000, Budavari *et al.* 1996
Density (g/cm^{3}) at 25°	1.199	Adkins 2000
Vapor density (air = 1)	4.1	Adkins 2000
Vapor pressure	1 mm Hg at 44 ^{o}C	NTP 2001
Refractive index at 20°C	1.55296	Adkins 2000
Solubility (at 23^{o}C):		
water	slightly (soluble in ~500 parts water)	Budavari *et al.* 1996
alcohol	soluble	Budavari *et al.* 1996
benzene	soluble	Budavari *et al.* 1996
dimethylsulfoxide	soluble $\geq$ 10 mg/mL	NTP 2001
95% ethanol	soluble $\geq$ 10 mg/mL	NTP 2001
ether	soluble	Budavari *et al.* 1996
oils	soluble	Budavari *et al.* 1996

1.3 Metabolites

The two main human metabolites of nitrobenzene are *p*-nitrophenol (4-nitrophenol) and *p*-aminophenol (4-aminophenol). Figure 1-2 shows the chemical structures for these metabolites. Section 6 provides a detailed discussion of the metabolic pathway of nitrobenzene and its metabolites. Metabolites identified in the urine of animals exposed to nitrobenzene include the following:

- 2-Aminophenol
- 3-Aminophenol
- 4-Aminophenol
- 4-Aminophenol glucuronide
- 4-Aminophenol sulfate
- Aniline
- 4-Hydroxyacetanilidine

- 4-Hydroxyacetanilidine glucuronide
- 4-Hydroxyacetanilidine sulfate
- 4-Nitrocatechol
- 2-Nitrophenol
- 3-Nitrophenol
- 3-Nitrophenol glucuronide
- 3-Nitrophenol sulfate
- 4-Nitrophenol
- 4-Nitrophenol glucuronide
- 4-Nitrophenol sulfate
- 4-Nitrophenylmercapturic acid
- 4-Nitroquinol



p-Nitrophenol *p*-Aminophenol

Figure 1-2. Structures of *p*-nitrophenol (4-nitrophenol) and *p*-aminophenol (4-aminophenol)

2 Human Exposure

2.1 Use

The primary use of nitrobenzene, accounting for 97% or more of its use, is in the manufacture of aniline, an intermediate in the production of dyestuffs and other products (IARC 1996, ChemExpo 1999). Nitrobenzene also is used in the manufacture of benzidine, quinoline, azobenzene, and pyroxylin compounds. It is found in soaps and shoe and metal polishes, and is used as a solvent for cellulose ester, in modifying esterification of cellulose acetate, and in refining lubricating oils (NTP 2001). Nitrobenzene is used in the production of isocyanates, pesticides, rubber chemicals, pharmaceuticals, and dyes such as nigrosines and magenta (HSDB 2002). Nitrobenzene also is used as a solvent in petroleum refining and the synthesis of other organic compounds such as acetaminophen, which is in the over-the-counter analgesic Tylenol (ATSDR 1990).

2.2 Production

Nitrobenzene is produced in a continuous process through the use of "mixed acid" or "nitrating acid" (27% to 32% HNO_3, 56% to 60% H_2SO_4, 8% to 17% H_2O) in the direct nitration of benzene (IARC 1996). Four companies, BASF, ChemFirst, DuPont, and Rubicon, are known to produce nitrobenzene in the United States, with a capacity of approximately 3 billion pounds per year. Table 2-1 summarizes the annual capacity of these four companies for the year 2001.

Table 2-1. Capacities for nitrobenzene production

Company (location)	Capacity (million lb/year)
BASF (Louisiana)	600
ChemFirst (Missouri)	500
ChemFirst (Texas)	340
DuPont (Texas)	380
Rubicon (Louisiana)	1,140
Total	**2,960**

Source: CEH 2001.

Miles Corporation, also known as Bayer Corporation, previously produced nitrobenzene but ceased production of it in February 1994 (CEH 2001).

Demand for nitrobenzene in the United States for 1997, 1998, and 2000 was 1.6 billion, 1.8 billion, and 2.4 billion pounds, respectively. Nitrobenzene production growth was approximately 5% annually in recent years and is expected to continue at this rate through 2002 in response to the fast-growing aniline and methyl diphenyl diisocyanate

markets. Solvent and dye and pigment uses account for 50 to 60 million pounds of annual demand (ChemExpo 1999).

The International Trade Administration (ITA) of the U.S. Department of Commerce reported production of nitrobenzene from 1955 to 1986. The ITA production values are voluntarily provided by industry and may understate the total production of the compound. Therefore, estimated production data from the *Chemical Economics Handbook*, based on the requirement for nitrobenzene-based aniline and consumption of nitrobenzene for other uses, also are provided (CEH 2001). Table 2-2 summarizes these production values.

Table 2-2. United States production of nitrobenzene

Year	Nitrobenzene production (millions of pounds)[a]	
	Reported production	Estimated production
1955	175.9	–
1960	162.3	–
1965	280.3	–
1970	547.7	–
1975	414.3	565
1976	409.0	755
1977	552.3	805
1978	575.5	835
1979	952.4	955
1980	611.6	910
1971	901.6	–
1982	774.8	–
1983	840.7	880
1984	982.7	1,025
1985	913.5	965
1986	957.9	–
1987	–	1,100
1988	–	1,230
1989	–	1,210
1990	–	1,175
1991	–	1,185
1992	–	1,250
1993	–	1,375

Year	Nitrobenzene production (millions of pounds)[a]	
	Reported production	Estimated production
1994	–	1,550
1995	–	1,700
1996	–	1,690
1997	–	1,765
1998	–	1,900
1999	–	2,000
2000	–	2,375

Source: CEH 2001.

[a]– = not provided.

2.3 Analysis

Environmental samples containing nitrobenzene usually are extracted with an organic solvent and analyzed by gas chromatography (GC). Nitrobenzene also can be detected by flame ionization (FID) or mass spectrometry (MS) (HSDB 2002). Because of its volatility, nitrobenzene levels are difficult to determine in biological materials. The only study located for analysis of nitrobenzene in urine used a spectrophotometric method. Table 2-3 summarizes analytical methods to determine nitrobenzene in environmental and biological materials.

2.4 Environmental occurrence

2.4.1 Air

Direct release of nitrobenzene to air during the manufacturing process is minimized by passage of contaminated air through activated charcoal. Most (97% to 98%) of the nitrobenzene produced is retained in a closed system that is used to synthesize aniline and other substituted nitrobenzenes and anilines, thus limiting releases into air (ATSDR 1990).

Production of nitrobenzene and its use in the manufacture of consumer products like shoe polishes may result in its release into the environment via fugitive emissions. The amount of releases from production of consumer products and their contribution to human exposure have not been quantified (ATSDR 1990).

Table 2-3. Analytical methods to determine nitrobenzene in environmental materials and biological materials

Sample matrix	Sample preparation	Analytical method	Sample detection limit	Relative recovery (%) (mean ± s.d. or range)	Reference
Air at landfill sites	Adsorption on Tenax-GC cartridges, thermal desorption	High-resolution (HR) GC/FID	0.05 ppb	no data	Harkov *et al.* 1985
Air	Adsorption on silica gel, extraction with methanol	GC/FID	0.02 mg/sample	no data	ATSDR 1990
Air	Adsorption on silica gel, extraction with methanol	GC/FID	0.5 mg/m^3	no data	ATSDR 1990
Wastewater	Direct injection of aqueous sample	GC/FID	no data	no data	Patil and Shinde 1988
Wastewater	Extract with dichloromethane, exchange to hexane, concentrate	GC/FID	3.6 μg/L	71 ± 5.9	ATSDR 1990
Water	Extract with dichloromethane at pH 11 and 2, concentrate	GC/MS	1.9 μg/L	71 ± 31	ATSDR 1990
Soil and solid waste	Extract from sample, cleanup	GC/FID	137 mg/kg[a]	25.7–100	ATSDR 1990
Soil and solid waste	Extract from sample, cleanup	GC/MS	19 mg/kg[a]	no data	ATSDR 1990
Soil and solid waste	Extract from sample, cleanup	GC/FID	660 μg/kg[b]	54–158	ATSDR 1990
Soil and solid waste	Extract from sample, cleanup	HRGC/FTIR	12.5 μg/L[c]	no data	ATSDR 1990
Urine (spiked with nitrobenzene)	Reduce nitrobenzene, form coupled dye, extract in carbon tetrachloride	Colorimetric at 450 nm	0.8 mg/L	no data	Dangwal and Jethani 1980

Source: ATSDR 1990.

[a]Approximate detection limit in high-level soil and sludges.

[b]Approximate detection limit in low-level soil and sediments.

[c]Detection limit in water; detection limit in solids and wastes is several orders of magnitude higher.

The other principal source of nitrobenzene in the atmosphere is the photochemical reaction of nitrogen oxides with ambient benzene. Benzene in the atmosphere presumably is derived from automobile fuels and use of benzene solvents. The contribution of atmospherically produced nitrobenzene is difficult to estimate, as many of the measurements of ambient atmospheric nitrobenzene are near sites of nitrobenzene production, manufacture, use, or disposal. Levels are likely to decrease, as environmental levels of benzene are expected to drop. Although this source of nitrobenzene seems limited, it may form a significant proportion of environmental exposure (ATSDR 1990). Figure 2-1 shows the atmospheric reactions that generate and remove nitrobenzene.

EPA's Toxic Release Inventory (TRI) reported that total air emissions for nitrobenzene were 77,274 pounds in 1999 (TRI 2001). Because only certain types of facilities are required to report releases, the TRI data are not exhaustive and should be used with caution. It was estimated that from 1987 to 1994, approximately 100,000 pounds of nitrobenzene per year were released to the air (Holder 1999a).

2.4.2 Water

Occurrence of nitrobenzene in water is most likely due to effluent discharge during the manufacturing process. Nitrobenzene rarely is carried through to finished water; it is likely to be lost to air or degraded by sewage organisms (see Section 2.5.2). The U.S. EPA has surveyed nitrobenzene levels in effluents from 4,000 publicly owned treatment works and industrial sites. The highest concentrations of nitrobenzene in effluent are associated with wastewaters from the organics and plastics industries, with some reported levels exceeding 100 ppm (ATSDR 1990).

Studies seem to suggest that commercial and industrial wastes with nitrobenzene are dispersed throughout the country, with detectable levels found outside the three states in which nitrobenzene is manufactured. Of 33 industry effluents tested, nitrobenzene was detected at a concentration greater than 100 μg/L in one effluent. Reported nitrobenzene concentrations in raw and treated industrial wastewaters from several industries ranged from 1.4 to 91,000 μg/L (ATSDR 1990).

EPA's TRI reported that in 1999, total surface water releases of nitrobenzene were 372 pounds (TRI 2001). Because only certain types of facilities are required to report releases, the TRI data are not exhaustive and should be used with caution. In the years 1987 to 1994, releases of nitrobenzene to surface water were estimated to range from 500 pounds in 1993 to 10,000 pounds in 1987 (Holder 1999a).

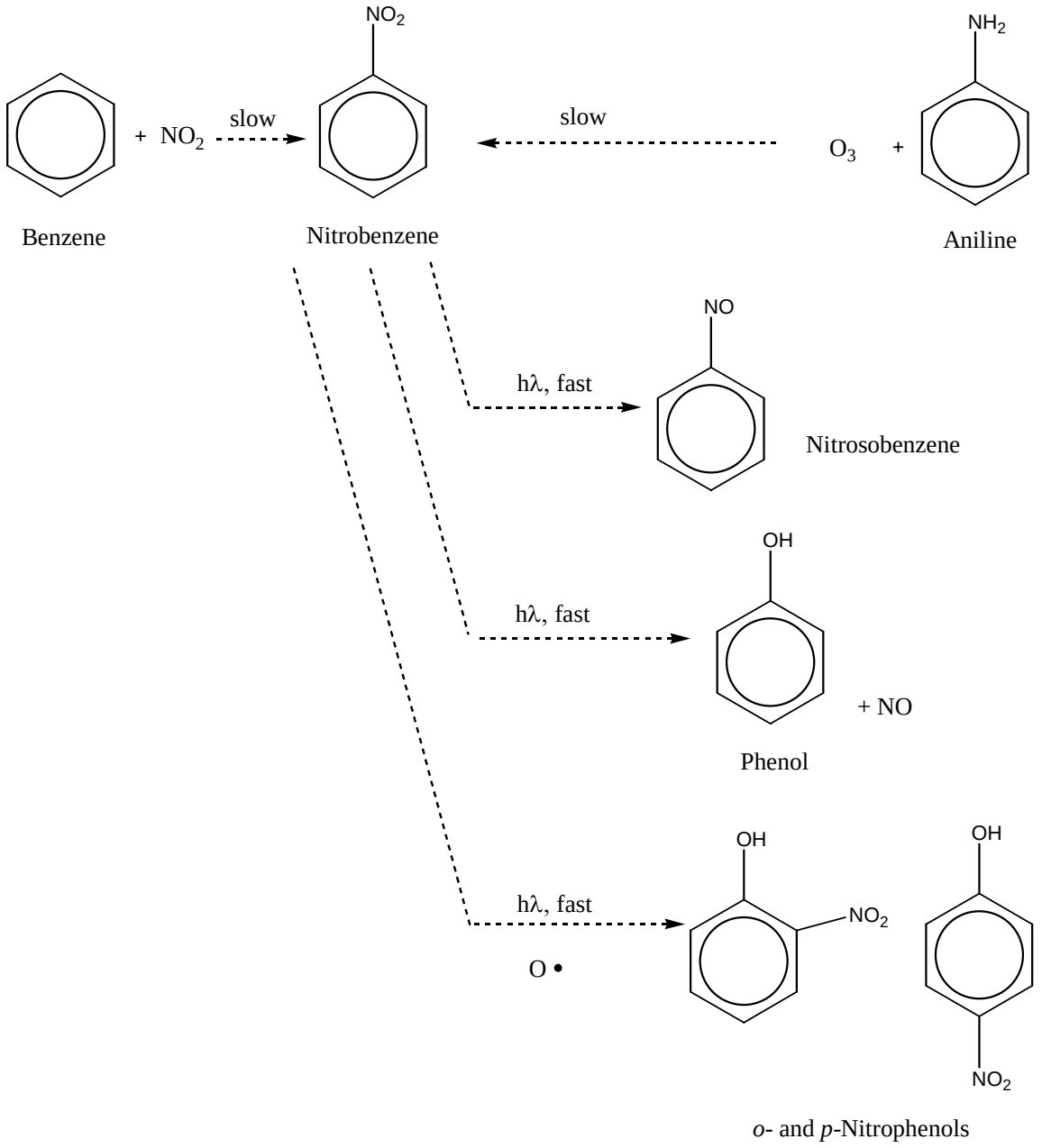


Figure 2-1. Atmospheric reactions that generate and remove nitrobenzene

Source: ATSDR 1990.

2.4.3 Soil

Few data regarding nitrobenzene concentrations in soil exist. One study reported 8 ppm nitrobenzene in the soil of a former dye-manufacturing site, but none was found in river sediments. Nitrobenzene was detected in 4 of 862 soil or sediment samples at hazardous waste sites, with a geometric mean concentration of 1,000 pg/kg. The presence of nitrobenzene in soils of abandoned hazardous wastes sites may be inferred from its presence in the atmosphere above the sites (ATSDR 1990).

EPA's TRI reported that in 1999, total underground injection releases for nitrobenzene were 211,347 pounds. Because only certain types of facilities are required to report releases, the TRI data are not exhaustive and should be used with caution. Total land releases were 65 pounds (TRI 2001).

2.5 Environmental fate

2.5.1 Air

Based on estimated vapor pressure in the atmosphere, nitrobenzene should exist mainly in the vapor (gas) phase. In the atmosphere, nitrobenzene appears to degrade primarily by photolysis (38% degradation in 5 hours), mainly to *p*-nitrophenol and nitrosobenzene (HSDB 2002). In one study, both *o*- and *p*-nitrophenols were detected when O_2 was present, whereas phenol was found when O_2 was absent (ATSDR 1990). Figure2-1 shows the degradation of nitrobenzene in air.

2.5.2 Water

Photolysis and biodegradation are expected to be the most significant environmental fate processes for nitrobenzene in water. The half-life is 133 days for photolysis, 22 days for reaction with hydrated electrons in eutrophic lakes, and 11 hours for reaction with sunlight and nitrate. Nitrobenzene is expected to volatilize from water surfaces. Estimated volatilization half-lives are 2 days for a model river and 17 days for a model lake (HSDB 2002).

Nitrobenzene may be almost completely removed from water by activated sludge treatment, depending on the conditions. One researcher obtained 98% removal of chemical oxygen demand (COD) at a rate of 14 mg of COD/h per gram dry weight of activated sludge, with nitrobenzene as the sole carbon source. A number of researchers obtained 100% degradation of nitrobenzene in water in 7 to 10 days, using a variety of model sewage treatment reactors and wastewater sources (ATSDR 1990). However, some studies have reported almost no degradation of nitrobenzene. Other studies have reported the following: no degradation in 10 days with activated sludge inoculum; 3.3% of the theoretical biological oxygen demand reached in 2 weeks using an activated sludge inoculum; and nitrobenzene degradation in more than 64 days with a soil inoculum. No simple explanation for these conflicting results is apparent (HSDB 2002).

The potential for bioaccumulation in aquatic organisms is expected to be low, based on measured bioconcentration factors (the ratio of the chemical concentration in the organism to that in the surrounding water) ranging from 1.6 to 15 (HSDB 2002).

2.5.3 Soil

Nitrobenzene is expected to have moderate to very high mobility in soil, and volatilization may be important from moist soil surfaces. In a soil column experiment, 20% to 40% of the added nitrobenzene was degraded in 45 days. Nitrobenzene had a half-life of 56 days in an aerobic soil column. The proposed catabolic pathway for nitrobenzene involves its reduction to nitrosobenzene, hydroxylaminobenzene, and 2-aminophenol. These compounds then undergo meta ring cleavage to 2-aminomuconic semialdehyde (HSDB 2002).

2.6 Environmental exposure

Environmental exposure to nitrobenzene is expected to be primarily through inhalation of ambient air, ingestion of water, or dermal exposure to products and water containing nitrobenzene (HSDB 2002).

2.6.1 Air

Information on nitrobenzene levels in air is derived mostly from a series of reports from New Jersey, where urban, rural, and waste disposal areas were extensively monitored. In the most recent studies (1982), mean concentrations in cities were 0.07 to 0.10 ppb. Studies of air concentrations of nitrobenzene seem to be confounded by weather; nitrobenzene may be detected during the warmer months of summer, but is not detected during winter or while it is snowing and raining (ATSDR 1990, HSDB 2002).

A U.S. survey of volatile organic chemicals in the atmosphere at 595 sites revealed a mean nitrobenzene concentration of 0.17 ppb and a maximum of 2.8 ppb. Of these samples, 75% contained less than 0.092 ppb nitrobenzene, with a median value of 0 ppb. An update of EPA's National Volatile Organic Compounds Database (1970 to 1987) revealed that nitrobenzene was found in 734 ambient air samples, at an average concentration of 0.117 ppb. The samples came from 2 remote, 73 rural, 111 suburban, 544 urban, and 4 source-dominated sites (ATSDR 1990, HSDB 2002).

2.6.2 Water

Nitrobenzene is only slightly soluble in water. Because of its characteristic odor (bitter almonds at levels as low as 30 ppb), nitrobenzene would be detectable if there were a large release or accumulation in groundwater. Nitrobenzene was detected in groundwater at 3 of 862 hazardous waste sites, with a geometric mean concentration of 1,400 pg/L. Nitrobenzene was not detected, however, in any of the 862 surface-water samples taken from the same sites (ATSDR 1990).

Nitrobenzene was detected in effluent discharges from facilities in 10 industrial categories. The maximum effluent concentration reported was 100,245 ppb for a site in the organics and plastics industry. In EPA's Storage and Retrieval System for Water and Biological Monitoring Data (STORET database), nitrobenzene was detected in effluents at 1.8% of the 1,245 sampling stations. In the National Urban Runoff Program, no nitrobenzene was detected in runoff samples collected from 19 cities (HSDB 2002).

In the 1970s, nitrobenzene was detected, though not quantified, in finished drinking water from two U.S. drinking-water plants (HSDB 2002).

2.6.3 Soil

The only location where nitrobenzene was detected in soil was at one of two sampling sites along the banks of the Buffalo River, in New York State. The detected concentration was 8 ppm. However, nitrobenzene was not detected in three samples of bottom sediment from the river. Nitrobenzene also was not detected at any of the 349 stations represented in the STORET database. Thus, nitrobenzene in contaminated soil does not seem to be a major source of environmental exposure (ATSDR 1990, HSDB 2002).

2.7 Occupational exposure

Occupational exposure to nitrobenzene generally is via inhalation of the vapor or dermal contact with the vapor or liquid (Howard 1989). Through the National Occupational Exposure Survey, conducted from 1981 to 1983, NIOSH estimated that 5,080 employees (475 females) potentially were exposed to nitrobenzene (IARC 1996, HSDB 2002).

2.8 Biological indices of exposure

p-Nitrophenol, one of the major metabolites of nitrobenzene, can be measured in the urine to identify exposure to nitrobenzene. However, finding this metabolite in the urine does not provide information on the concentration of nitrobenzene to which the person was exposed. Another method is to examine the blood for the presence of nitrosobenzene and phenylhydroxylamine, nitrobenzene metabolites that bind to hemoglobin (see Section 6.3). However, this method is not specific for nitrobenzene, because exposure to other chemicals also may result in production of these metabolites and their binding to hemoglobin (ATSDR 1990).

2.9 Regulations

The U.S. EPA regulates nitrobenzene under the Clean Air Act (CAA). It also regulates nitrobenzene under the Clean Water Act (CWA) and the Federal Insecticide, Fungicide, and Rodenticide Act. Nitrobenzene also is regulated under the Resource Conservation and Recovery Act (RCRA), with a hazardous waste number of U169. Under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), nitrobenzene has a reportable quantity of 1,000 pounds (454 kg). EPA also regulates nitrobenzene under the Emergency Planning and Community Right-to-Know Act. The American Conference of Governmental Industrial Hygienists (ACGIH) has established a threshold limit value (TLV) for nitrobenzene of 1 ppm (5 mg/m^3), with a skin notation, indicating potential absorption through skin. The Occupational Safety and Health Administration (OSHA) has established a permissible exposure level (PEL) of 1 ppm (5 mg/m^3) as an 8-hour time-weighted average (TWA). NIOSH has established a recommended exposure limit of 1 ppm (5 mg/m^3) as an 8-hour TWA, with a skin notation. The NIOSH “immediately dangerous to life or health” concentration is 200 ppm. Table 2-4 summarizes EPA regulations, and Table 2-5 summarizes OSHA regulations. No U.S. Food and Drug Administration regulations were located.

Table 2-4. EPA regulations

Regulatory action	Effect of regulation or other comments
40 CFR – PART 60 – STANDARDS OF PERFORMANCE FOR NEW STATIONARY SOURCES. Promulgated: 36 FR 24877, 12/31/71.	The provisions of this part apply to the owner/operator of any stationary source that contains an affected facility (a stationary source with an apparatus to which a standard is applicable) that produces nitrobenzene.
40 CFR 63 – PART 63 – NATIONAL EMISSIONS STANDARDS FOR HAZARDOUS AIR POLLUTANTS. Promulgated: 57 FR 61992, 12/29/92. U.S. Code: 7401 *et seq.*	Standards that regulate specific categories of stationary sources that emit (or have the potential to emit) a hazardous air pollutant like nitrobenzene are listed in this part pursuant to section 112(b) of the CAA. Sources include Off-Site Waste and Recovery Operations; Wood Furniture Manufacturing Operations; Synthetic Organic Chemical Manufacturing Industry for Process Vents, Storage Vessels, Transfer Operations, and Wastewater.
40 CFR 116 – PART 116 – DESIGNATION OF HAZARDOUS SUBSTANCES. Promulgated: 43 FR 10474, 03/13/78. U.S. Code: 33 U.S.C. 1251 *et seq.*	This regulation designates nitrobenzene as a hazardous substance under section 311(b)(2)(a) of the Federal Water Pollution Control Act (FWPCA). The regulation applies to the discharge of nitrobenzene to surface waters.
40 CFR 117 – PART 117 – DETERMINATION OF REPORTABLE QUANTITIES FOR HAZARDOUS SUBSTANCES. U.S. Code: FWPCA 311(b)(2)(A) and 501(a) as amended by the CWA of 1977.	Discharges to water of amounts equal to or greater than the reportable quantity (RQ) must be reported to EPA. The RQ for environmental releases of nitrobenzene to water is 1,000 lb (454 kg).
40 CFR 148.10 – Sec. 148.10 – Waste specific prohibitions — solvent wastes. Promulgated: 53 FR 28154, 07/26/88. U.S. Codes: Section 3004, RCRA, 42 U.S.C. 6901 *et seq.* Effective 08/08/88.	The spent solvent waste nitrobenzene is prohibited from underground injection unless the solvent waste is a solvent–water mixture or solvent-containing sludge containing less than 1% total solvent constituent.
40 CFR 172 – PART 172 – Subpart B – Table of Hazardous Materials and Special Provisions. Promulgated: 55 FR 52582, 12/21/90.	The Hazardous Materials Table in this section designates nitrobenzene as a hazardous material for the purpose of transportation. The identification number is UN 1662.
40 CFR 258 – PART 258 – CRITERIA FOR MUNICIPAL SOLID WASTE LANDFILLS. Promulgated: 56 FR 51016, 10/09/91. U.S. Codes: 33 U.S.C. 1345 (d) and (e); 42 U.S.C. 6907(a)(3), 6912(a), 6944(a), and 6949a(c).	The purpose of this part is to establish minimum national criteria under the RCRA, as amended, for all municipal solid waste landfill (MSWLF) units and under the CWA, as amended, for MSWLFs that are used to dispose of sewage sludge. These minimum national criteria ensure the protection of human health and the environment.
40 CFR 261 – Subpart D – Lists of Hazardous Wastes. Promulgated: 45 FR 33119, 05/19/80. U.S. Code: 42 U.S.C. 6905, 6912(a), 6921, 6922, and 6938.	Nitrobenzene, as a solid waste, is listed as a hazardous waste from non-specific sources, with a hazardous waste number of U169. It has EPA hazardous waste numbers of F004, K104, and K105.
40 CFR 268 – Subpart D – Treatment Standards. Promulgated: 59 FR 48103, 09/19/94.	Table UTS identifies nitrobenzene as a hazardous constituent in hazardous waste. The nonwastewater and wastewater treatment standard levels are 0.068 mg/L and 14 mg/L, respectively.

Regulatory action	Effect of regulation or other comments
40 CFR 302 – PART 302 – DESIGNATION, REPORTABLE QUANTITIES, AND NOTIFICATION. Promulgated: 50 FR 13474, 04/04/85. U.S. Codes: 42 U.S.C. 9602, 9603, and 9604; 33 U.S.C. 1321 and 1361.	This part designates under section 102(a) of CERCLA 1980 those substances in the statutes referred to in section 101(14) of CERCLA, identifies reportable quantities for these substances, and sets forth the notification requirements for releases of these substances. This part also sets forth reportable quantities for hazardous substances designated under section 311(b)(2)(A) of the CWA. The reportable quantity for nitrobenzene is 1,000 lb (454 kg).
40 CFR 355 – PART 355 – EMERGENCY PLANNING AND NOTIFICATION. Promulgated: 52 FR 13395, 04/22/87. U.S. Code: 42 U.S.C. 11002, 11004, and 11048.	This regulation establishes nitrobenzene as an extremely hazardous substance, with a reportable quantity of 1,000 lb and a threshold planning quantity of 10,000 lb. Facility notification responsibilities necessary for the development and implementation of state and local emergency response plans are included.
40 CFR 372 – PART 372 – TOXIC CHEMICAL RELEASE REPORTING: COMMUNITY RIGHT-TO-KNOW. Promulgated: 55 FR 4525, 02/16/88. U.S. Code: 42 U.S.C. 11013 and 11028. The effective date for reporting nitrobenzene is 01/01/87.	This part sets forth requirements for the submission of information relating to the release of toxic chemicals under section 313 of Title III of the Superfund Amendments and Reauthorization Act (1986). Information collected under this part is intended to inform the general public and the communities surrounding covered facilities about releases of toxic chemicals, to assist research, to aid in the development of regulations, guidelines, and standards.
40 CFR 401 – PART 401 – GENERAL PROVISIONS. Promulgated: 39 FR 4532, 02/01/74, as amended at 47 FR 24537, 06/04/82. U.S. Code: 33 U.S.C. 1251 *et seq*.	Nitrobenzene is included in the list of toxic pollutants, designated pursuant to section 307(a)(1) of the Act, in publicly owned treatment works treatment processes or contaminated sewage sludge.

The regulations in this table been updated through the 2001 Code of Federal Regulations, 29 CFR December 1, 2001.

Table 2-5. OSHA regulations

Regulatory action	Effect of regulation or other comments
29 CFR 1910.1000 – Air Contaminants. Table Z-1.	Established PEL for nitrobenzene of 1 ppm (5 mg/m^3) as an 8-h TWA.
29 CFR 1915.1000 – Air Contaminants.	Established PEL for nitrobenzene of 1 ppm (5 mg/m^3) as an 8-h TWA in shipyards.
29 CFR 1926.55 – Gases, vapors, fumes, dusts, and mists.	Established PEL for nitrobenzene of 1 ppm (5 mg/m^3) as an 8-h TWA in construction.

The regulations in this table been updated through the 2001 Code of Federal Regulations, 29 CFR December 1, 2001.

3 Human Cancer Studies

Only one study was found that discusses any possible relationship between human cancer and exposure specifically to nitrobenzene. Wilkins and Sinks (1990) conducted a case-control study evaluating parental occupation and intracranial neoplasms of childhood. Cases (N = 110) of primary malignant neoplasm of the brain that were diagnosed from 1975 to 1982 were identified from a pediatric hospital tumor registry. Controls (N = 193), matched to each case by age, race, and gender, were selected by random-digit dialing. Biological parents of subjects were interviewed and queried for parental occupational histories, general demographics, use of selected household products (including pesticides), hobbies, pets, index pregnancy and birth characteristics, information on pregnancies, other lifestyle factors (e.g., smoking), and medical histories related to pregnancy. Data analyses focussed on three major time periods: postnatal (birth to diagnosis), prenatal (stratified by trimester), and preconception (the 12 months before the estimated month of conception).

Exposure was assessed with a job-exposure matrix and job-clustering scheme previously developed (Hoar *et al.* 1980). Parents were classified by their industry of employment, which was derived from the *Standard Industrial Classification Manual,* and by their industry-specific task, which was based on the *Dictionary of Occupational Titles.* Parents were included *a priori* in the analysis if exposure was to any nitrosamine or nitrosamide, any nitrosatable amino compound, or any compound having a chemical structure similar to that of an *N*-nitroso group from all possible organic chemicals in the Hoar *et al.* (1980) system.

Paternal employment in the agriculture, construction, metals, or food and tobacco industries was associated with an excess risk of childhood brain cancer (ORs ranged from 1.8 to 3.3), with most of the excess risk occurring in the preconception period. However, the number of exposed cases in each category was small, ranging from 8 to 13, and most of the associations were not significant. Maternal employment in the food and tobacco industry was weakly associated with childhood brain tumors for all exposure periods (ORs ranged from 1.2 to 1.8). Job-exposure matrix analysis showed that childhood brain cancer was associated with paternal jobs linked with several aromatic amino and aromatic nitro compounds. The greater risk usually occurred with exposure to compounds in the postnatal period (ORs 3.4 to 4.4); however, small numbers precluded calculation of ORs for all chemicals. Paternal exposure to nitrobenzene during the postnatal period was associated with a small, nonsignificant risk of childhood brain cancer (OR = 1.6, 95% confidence interval = 0.4 to 6.1); ORs were not calculated for exposure during the preconception and prenatal periods.

The paucity of data precludes evaluation of any human carcinogenic effect of nitrobenzene. The study by Wilkins and Sinks (1990) is limited in its ability to evaluate specific carcinogenic effects of nitrobenzene because of its small number of exposed cases and concerns about the validity of exposure assessment.

4 Studies of Cancer in Experimental Animals

No oral or dermal carcinogenicity studies of nitrobenzene in animals were located. The only relevant carcinogenesis study is a Chemical Industry Institute of Toxicology (CIIT) two-year bioassay of the toxicity and carcinogenicity of nitrobenzene in B6C3F$_1$ mice, F344/N rats, and CD (Sprague-Dawley–derived) rats (CIIT 1993, Cattley *et al.* 1994). In addition, studies by CIIT of the acute and subchronic toxicity of nitrobenzene in B6C3F$_1$ mice, F344/N rats, and CD rats (Medinsky and Irons 1985, CIIT 1993) are briefly reviewed in this section. The inhalation bioassay data and a mechanistic analysis, including a potential mode of carcinogenic action, also were discussed in a paper by Holder (1999b).

4.1 Acute and 90-day toxicity studies

In the acute toxicity study, 10 animals of each species, strain, and sex were exposed to nitrobenzene gas at a concentration of 0, 10, 35, or 125 ppm in inhalation chambers for 6 hours/day, 5 days/week, for two weeks (Medinsky and Irons 1985). At the end of the two weeks, surviving animals were maintained in the inhalation chambers and were sacrificed 3 or 14 days later. Early morbidity and mortality were observed in mice and CD rats exposed to nitrobenzene at 125 ppm. The primary cause of death was cerebellar perivascular hemorrhage. No adverse clinical signs were observed in F344/N rats; however, dose-related increases in relative liver, spleen, and kidney weights were reported in this strain. There were marked sex, species, and strain differences in toxicity, with B6C3F$_1$ mice and CD rats being more susceptible than F344/N rats. The spleen was the most sensitive target organ, with high incidences of histologic lesions in all groups exposed to nitrobenzene at 35 or 125 ppm. At the high dose, histologic lesions also were observed in the brain, liver, lung, and testes of B6C3F$_1$ mice; brain, liver, lung, kidney, and testes of CD rats; and kidney and testes of F344/N rats. In addition, concentration-dependent increases in blood methemoglobin were observed in animals sacrificed three days after the last exposure but not in the groups sacrificed 14 days after the last exposure.

In the 90-day rangefinding study, 10 animals of each species, strain, and sex were exposed to nitrobenzene gas at a concentration of 0, 5, 16, or 50 ppm (CIIT 1993). [The complete text of the report is available from CIIT; see the reference in Section 7 for ordering information.] Based on previous studies, end points of concern were anemia and methemoglobinemia (Hamm 1984, Hamm *et al.* 1984). Mean methemoglobin concentrations showed dose-related increases from 1.4 ± 0.6% to 10.3 ± 1.4% in F344 rats, 1.4 ± 0.7% to 9.9 ± 2.3% in CD rats, and 1.0 ± 0.8% to 5.5 ± 1.3% in B6C3F$_1$ mice. Other lesions were found in the spleen, liver, adrenal glands, and kidneys of all exposed groups (Hamm 1984). These observations of methemoglobinemia agreed with the acute toxicity results and were consistent with clinical methemoglobinemia observed in humans exposed to nitrobenzene (Reddy *et al.* 1976, Kiese 1966, Beauchamp *et al.* 1982, Goldstein and Rickert 1985, Nabarro 1948, Parkes and Neill 1953, Magos and Sziza 1959, Harrison 1977). Methemoglobinemia was seen at concentrations of 5 ppm or higher in F344/N rats, 16 ppm or higher in CD rats, and 50 ppm in B6C3F$_1$ mice. These

results provided the basis for selection of the highest exposure level for the two-year inhalation exposure study.

4.2 Two-year inhalation exposure study

Groups of male and female B6C3F$_1$ mice (70 per sex per group) were exposed by inhalation to nitrobenzene at a concentration of 0, 5, 25, or 50 ppm, and groups of F344/N rats (70 per sex per group) and male CD rats (70 per group) were exposed at 0, 1, 5, or 25 ppm. Exposures were for 6 hours/day, 5 days/week, for two years (CIIT 1993). The highest exposures reflected the anticipated maximum tolerable long-term levels, and the lowest exposure (1 ppm) matched the ACIGH occupational TLV. More than 40 tissues were examined microscopically from animals in the control and high-dose groups to select the target organs for histological examination in all exposure groups (Table 4-1). Results for mice and rats are presented in Sections 4.2.1 and 4.2.2, respectively, and include both neoplastic and nonneoplastic lesions.

Table 4-1. Target organs examined following inhalation exposure of mice and rats to nitrobenzene for two years

	B6C3F$_1$ mice		F344 rats		CD rats
Target organ	Male	Female	Male	Female	Male
Nasal cavity	X	X	X	X	X
Spleen	X	X	X	X	X
Liver	X	X	X	X	X
Kidney			X	X	X
Thyroid	X	X	X		X
Parathyroid	X	X	X		X
Lung	X	X			
Bone marrow	X				
Adrenal		X			
Testes					X
Epididymis					X
Uterus				X	

4.2.1 Mice

Survival rates were unaffected by chronic inhalation exposure to nitrobenzene, except that survival of female mice exposed at 25 ppm was significantly increased ($P = 0.01$, Cox's method for pairwise comparison). Mild body weight depression was observed in high-dose male mice from week 16 until the end of the study; however, mean body weights for all groups remained within 10% of the control means (Cattley *et al.* 1994).

4.2.1.1 Neoplastic lesions

Inhalation exposure to nitrobenzene caused tumors in mice of both sexes; however, no metastatic sites were reported (Cattley *et al.* 1994; Holder 1999a, 1999b; Holder and Jinot 1998; CIIT 1993). Tumors were observed at several organ sites (Table 4-2). $B6C3F_1$ male mice had both benign and malignant alveolar and bronchiolar lung tumors. The incidence of adenoma, but not carcinoma, increased in a dose-related manner. However, the exposed male mice had higher incidences of alveolar/bronchiolar carcinoma (12% to 15%) than did the concurrent control mice (6%). In addition, thyroid follicular cell adenoma was significantly increased in male mice. In female mice, the incidence of malignant mammary gland tumors in the high-dose group was about 8% (5/60), significantly higher than in the controls. [The spontaneous incidence of mammary gland carcinoma in the NTP carcinogenicity studies is about 1.6% (31/1,902) (Haseman *et al.* 1998).] Not all animals in the 5- and 25-ppm groups were analyzed histologically for mammary gland tumors, because the mammary gland was not selected as a target organ. Neither benign nor malignant liver tumors were significantly increased in mice of either sex (Fisher's exact test); however, female mice showed a significant dose-related increase in hepatocellular adenoma (Cattley *et al.* 1994, CIIT 1993).

Table 4-2. Tumor incidence in B6C3F$_1$ mice following inhalation exposure to nitrobenzene for two years

Sex	Conc. (ppm)	No. with tumor/no. examined (%)							
		Lung alveolar/bronchiolar			Liver hepatocellular				
		Adenoma	Carcinoma	Combined	Adenoma	Carcinoma	Combined	Thyroid follicular cell adenoma	Mammary gland adenocarcinoma[a]
Male	0	7/68 (10)	4/68 (6)	9/68 (13)	14/68 (21)	12/68 (18)	25/68 (37)	0/65 (0)	0/4[c] (0)
	5	12/67 (18)	10/67 (15)	21/67 (31)**	18/65 (28)	13/65 (20)	30/65 (46)	4/65 (6)	NE
	25	15/65 (23)*	8/65 (12)	21/65 (32)**	15/65 (23)	12/65 (18)	22/65 (34)	1/65 (2)	NE
	50	18/66 (27)**	8/66 (12)	23/66 (35)**	14/64 (22)	8/64 (13)	21/64 (33)	7/64 (11)**	0/1[c] (0)
	Trend[b]	*P* = 0.01	NS	*P* = 0.017	NS	NS	NS	*P* = 0.015	NAP
Female	0	4/53 (8)	2/53 (4)	6/53 (11)	6/51 (12)	1/51 (2)	7/51 (14)	2/49 (4)	0/48 (0)
	5	11/60 (18)	0/60 (0)	11/60 (18)	5/61 (8)	2/61 (3)	7/61 (11)	0/59 (0)	0/2[c] (0)
	25	3/64 (5)	4/64 (6)	6/64 (9)	5/64 (8)	3/64 (5)	7/64 (11)	3/61 (5)	NE
	50	2/62 (3)	4/62 (6)	6/62 (10)	13/62 (21)	1/62 (2)	14/62 (23)	2/61 (3)	5/60 (8)*
	Trend[b]	*P* = 0.018N	NS	NS	*P* = 0.036	NS	NS	NS	NAP

Sources: CIIT 1993, Cattley *et al.* 1994, Holder 1999b.

*$P \leq 0.05$, **$P \leq 0.01$ (Fisher's exact test).

[a]NE = not examined; NAP = not applicable (no statistical evaluation, because the full groups were not examined or the data did not meet the criteria for the statistical trend test).

[b]Cochran-Armitage trend test; N = negative trend; NS = not significant.

[c][The lack of pathology may be considered a data gap, as the small number of animals reported does not represent a statistically valid sample.]

4.2.1.2 Nonneoplastic lesions

Exposed mice exhibited clinical blood dyscrasias, anemia, and methemoglobinemia. Female B6C3F_1 mice showed decreased red blood cell count, hematocrit, and hemoglobin at 5 and 25 ppm but, oddly, not at 50 ppm. Male B6C3F_1 mice showed decreased red blood cell count, hematocrit, and hemoglobin at 50 ppm. Both exposed and control mice exhibited Howell-Jolley bodies and polychromasia. Methemoglobin was observed at the highest exposure level in all mice and appeared to be the most persistent and characteristic toxicological end point of nitrobenzene exposure. Methemoglobin production likely is involved in the hemotoxic effects initiated by the reduction and oxidation (redox) properties of nitrobenzene and its metabolites in red blood cells (Kiese 1966, Kiese *et al.* 1972, Holder 1999b) (see Sections 6.2–6.4).

Organ effects occurred mostly at the highest exposure level. Toxic effects or degenerative changes occurred in the nose and lung (the route of entry), liver, and thyroid (Table 4-3) (Cattley *et al.* 1994, CIIT 1993). Several nonneoplastic lesions were increased in the bone marrow, testes, thymus, kidney, and pancreas in the high-dose group. In mice, increased incidences of inflammatory cells were seen in the submucosa of the sinus cavity, as well as degeneration and loss of olfactory epithelium and pigment deposition. Bronchialization of the alveolar walls was significantly increased at all exposure concentrations in both sexes. Incidences of alveolar/bronchiolar hyperplasia (a presumed preneoplastic lesion) were significantly increased in male mice at the mid and high doses and in female mice at the mid dose. In male mice, sperm counts were decreased at the high dose, and the incidences of multinucleated hepatocytes and centrilobular hepatocellular enlargement were increased at all dose levels. High-dose female mice showed centrilobular hepatocellular enlargement.

Table 4-3. Incidences of selected nonneoplastic lesions in B6C3F$_1$ mice following inhalation exposure to nitrobenzene for two years

Sex	Conc. (ppm)	No. with lesion/no. examined (%)							
		Lung		Liver		Nose[a]			
		Alveolar/ bronchiolar hyperplasia	Bronchial-ization	Centrilobular hepato-cytomegaly	Multi-nucleated hepatocytes	Increased secretory product respiratory epithelium	Degeneration olfactory epithelium	Pigment deposition olfactory epithelium	Thyroid follicular cell hyperplasia
M	0	1/68 (1)	0/68 (0)	1/68 (1)	2/68 (3)	0/67 (0)	1/67 (1)	0/67 (0)	1/65 (2)
	5	2/67 (3)	58/67 (87)***	15/65 (23)***	14/65 (22)***	0/66 (0)	1/66 (2)	7/66 (11)**	4/65 (6)
	25	8/65 (12)*	58/65 (89)***	44/65 (68)***	45/65 (69)***	3/65 (5)	32/65 (49)***	46/65 (71)***	7/65 (11)*
	50	13/66 (20)***	62/66 (94)***	57/64 (89)***	56/64 (88)***	6/66 (9)*	41/66 (62)***	49/66 (74)***	12/64 (19)***
	Trend[b]	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
F	0	0/53 (0)	0/53 (0)	0/51 (0)	0/51 (0)	2/52 (4)	0/52 (0)	0/52 (0)	2/49 (4)
	5	2/60 (3)	55/60 (92)***	0/61 (0)	0/61 (0)	7/60 (12)	19/60 (32)***	6/60 (10)*	1/59 (2)
	25	5/64 (8)*	63/64 (98)***	0/64 (0)	0/64 (0)	19/63 (30)***	47/63 (75)***	37/63 (59)***	1/61 (2)
	50	1/62 (2)	62/62 (100)***	7/62 (11)*	2/62 (3)	32/61 (52)***	42/61 (69)***	29/61 (48)***	8/61 (13)
	Trend[b]	NS	$P < 0.001$	$P < 0.001$	NS	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.007$

Sources: CIIT 1993, Cattley *et al.* 1994.

*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$ (Fisher's exact test).

[a]Four transverse sections of the nose were examined. The data are for level 3.

[b]Cochran-Armitage trend test; NS = not significant..

4.2.2 Rats

As in mice, survival rates were unaffected by chronic inhalation exposure to nitrobenzene. Mild body weight depression was observed in low-dose female F344/N rats and low- and high-dose male F344/N rats from week 16 until the end of the study; however, mean body weights for all groups remained within 10% of the control means (Cattley *et al.* 1994).

4.2.2.1 Neoplastic lesions

Inhalation exposure to nitrobenzene caused benign and malignant tumors at several organ sites and in both strains of rats (Cattley *et al.* 1994, Holder 1999a, 1999b, Holder and Jinot 1998, CIIT 1993). No metastatic sites were reported. Table 4-4 summarizes the results for each organ site at which a biologically and statistically significant tumorigenic response was seen in at least one sex or strain.

Male F344/N rats exposed to nitrobenzene at 25 ppm had significantly increased incidences of hepatocellular adenoma, hepatocellular adenoma or carcinoma combined, kidney tubular cell adenoma, and kidney tubular cell adenoma or adenocarcinoma combined. These tumor incidences exhibited significant dose-related trends. Incidences of kidney tubular cell adenocarcinoma were not significantly increased; however, these tumors are rare. [The reported rate for these tumors in male F344 inhalation chamber control rats in NTP carcinogenicity studies is 1/902 (Haseman *et al.* 1998).] Male F344/N rats also showed significant dose-related trends in the incidences of hepatocellular carcinoma, thyroid follicular cell adenocarcinoma, and thyroid follicular cell adenoma or adenocarcinoma combined, although the incidences were not significantly higher than in controls when tested by pairwise comparison. In female F344/N rats, the incidence of benign uterine tumors (endometrial polyps) was significantly higher in the high-dose group than in the control group. The incidences of endometrial polyps, hepatocellular carcinoma, and hepatocellular adenoma or carcinoma combined also showed significant dose-related trends in female rats.

Cattley *et al.* (1994) reported that male CD rats were selected as a second rat strain because male F344/N rats exhibit high spontaneous rates of testicular neoplasia. No testicular neoplasia was observed in male CD rats. However, male CD rats did show increased incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma combined that were significant only at 25 ppm (Table 4-4). These obervations of liver cancer in CD rats are concordant with the results for F344/N rats.

Table 4-4. Tumor incidence in F344/N or CD rats following inhalation exposure to nitrobenzene

Strain & sex	Conc. (ppm)	No. with tumors/no. examined (%)									
		Liver hepatocellular			Kidney tubular cell			Thyroid follicular cell			Uterus endometrial polyp[a]
		Adenoma	Carcinoma	Comb.	Adenoma	Adeno-carcinoma	Comb.	Adenoma	Adeno-carcinoma	Comb.	
F344/N M	0	1/69 (1)	0/69 (0)	1/69 (1)	0/69 (0)	0/69 (0)	0/69 (0)	0/69 (0)	2/69 (3)	2/69 (3)	NAP
	1	3/69 (4)	1/69 (1)	4/69 (6)	0/68 (0)	0/68 (0)	0/68 (0)	0/69 (0)	1/69 (1)	1/69 (1)	
	5	3/70 (4)	2/70 (3)	5/70 (7)	0/70 (0)	0/70 (0)	0/70 (0)	2/70 (3)	3/70 (4)	5/70 (7)	
	25	15/70 (21)***	4/70 (6)	16/70 (23)***	5/70 (7)*	1/70 (1)	6/70 (9)*	2/70 (3)	6/70 (9)	8/70 (11)	
	Trend[b]	$P < 0.001$	$P = 0.04$	$P < 0.001$	$P < 0.001$	NS	$P < 0.001$	NS	$P = 0.035$	$P = 0.01$	
F344/N F	0	0/70 (0)	0/70 (0)	0/70 (0)	0/70 (0)	0/70 (0)	0/70 (0)	0/69 (0)	0/69 (0)	0/69 (0)	11/69 (16)
	1	2/66 (3)	0/66 (0)	2/66 (3)	0/66 (0)	0/66 (0)	0/66 (0)	0/7[c] (0)	0/7[c] (0)	0/7[c] (0)	17/65 (26)
	5	0/66 (0)	0/66 (0)	0/66 (0)	0/66 (0)	0/66 (0)	0/66 (0)	0/4[c] (0)	0/4[c] (0)	0/4[c] (0)	15/65 (23)
	25	3/70 (4)	2/70 (3)	4/70 (6)	0/70 (0)	0/70 (0)	0/70 (0)	2/68 (3)	1/68 (1)	3/68 (4)	25/69 (36)**
	Trend[b]	NS	$P = 0.047$	$P = 0.036$	NAP	NAP	NAP	NAP	NAP	NAP	$P = 0.01$
CD M	0	1/63 (2)	2/63 (3)	2/63 (3)	2/63 (3)	0/63 (0)	2/63 (3)	2/63 (3)	4/63 (6)	5/63 (8)	NAP
	1	1/67 (1)	0/67 (0)	1/67 (1)	0/67 (0)	1/67 (1)	1/67 (1)	4/64 (6)	1/64 (2)	5/64 (8)	
	5	2/70 (3)	2/70 (3)	4/70 (6)	2/70 (3)	0/70 (0)	2/70 (3)	2/68 (3)	1/68 (1)	3/68 (4)	
	25	7/65 (11)*	2/65 (3)	9/65 (14)*	0/65 (0)	0/65 (0)	0/65 (0)	3/64 (5)	2/64 (3)	5/64 (8)	
	Trend[b]	$P = 0.003$	NS	$P = 0.002$	NS	NS	NS	NS	NS	NS	

Sources: CIIT 1993, Cattley *et al.* 1994, Holder 1999b.

*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$ (Fisher's exact test).

[a]NAP = not applicable.; no statistical evaluation because the full groups were not examined or the data did not meet the criteria for the statistical test.

[b]Cochran-Armitage trend test; NS = not significant ($P > 0.05$).

[c][The number of animals was too small for statistical comparison.]

4.2.2.2 Nonneoplastic effects

Rats exhibited clinical blood dyscrasias, anemia, and methemoglobinemia. Decreases in red blood cells count, hematocrit, and hemoglobin protein occurred in high-dose F344/N rats but not in high-dose CD rats. Incidences of Howell-Jolley bodies and polychromasia were increased in both rat strains. The spleens of F344/N rat showed congestion, extramedullary hematopoiesis, follicular atrophy, and pigment deposition, and those of CD rats showed pigmentation. As with mice, methemoglobin was observed in all rats at the highest exposure level and appeared to be the most persistent and characteristic toxicological end point of nitrobenzene exposure.

Effects on organs in F344/N and CD rats occurred mostly at the highest exposure level (25 ppm) (Table 4-5). Toxic effects or degenerative changes occurred in the nose, liver, thyroid, testes, and kidney (Cattley *et al.* 1994, CIIT 1993). Nasal tissue pigmentation and epithelialization (i.e., orderly regrowth of denuded epithelium) were observed. The livers of F344/N rats exhibited eosinophilic foci, and centrilobular hepatocytomegaly was seen in F344/N rats and mid- and high-dose male CD rats. In F344/N rats, significant dose-related increases were observed in the incidences of chronic nephropathy in females and kidney tubular hyperplasia in males. These kidney lesions were not increased in CD rats. In male F344/N rats, dose-related increases were seen in thyroid follicular cell hyperplasia and kidney tubular hyperplasia. CD rats showed bilateral atrophy of the testes and epididymal hypospermia at the highest exposure level.

Table 4-5. Incidences of selected nonneoplastic lesions in F344 and CD rats following inhalation exposure to nitrobenzene for two years

Strain & sex	Conc. (ppm)	No. with lesion/no. examined (%)							
		Liver			Kidney		Thyroid[a]	Nose[b]	Testes[c]
		Eosinophilic foci	Centrilobular hepato-cytomegaly	Spongiosis hepatis	Chronic nephropathy	Tubular hyperplasia	Follicular cell hyperplasia	Pigment deposition olfactory epithelium	Bilateral atrophy
F344 M	0	29/69 (42)	0/69 (0)	25/69 (36)	69/69 (100)	2/69 (3)	0/69 (0)	40/67 (60)	61/69 (88)
	1	25/69 (36)	0/69 (0)	24/69 (35)	64/68 (94)	2/68 (3)	1/69 (1)	53/67 (79)*	50/56 (89)
	5	44/70 (63)*	8/70 (11)**	33/70 (47)	70/70 (100)	2/70 (3)	2/70 (3)	67/70 (96)***	59/61 (97)
	25	57/70 (81)***	57/70 (81)***	58/70 (83)***	70/70 (100)	13/70 (19)**	4/70 (6)	68/69 (99)***	61/70 (87)
	Trend[d]	$P < 0.001$	$P < 0.001$	$P < 0.001$	NS	$P < 0.001$	$P = 0.04$	$P < 0.001$	NS
F344 F	0	6/70 (9)	0/70 (0)	0/70 (0)	58/70 (83)	0/70 (0)	1/69 (1)	37/67 (55)	NAP
	1	9/66 (14)	0/66 (0)	0/66 (0)	51/66 (77)	0/66 (0)	NE	54/65 (83)***	
	5	13/66 (20)	0/66 (0)	0/66 (0)	60/66 (91)	2/66 (3)	NE	60/65 (92)***	
	25	16/70 (23)*	0/70 (0)	6/70 (9)*	67/70 (96)*	2/70 (3)	0/68 (0)	66/66 (100)***	
	Trend[d]	$P = 0.03$	NS	$P < 0.001$	$P = 0.004$	NS	NS	$P < 0.001$	
CD M	0	11/63 (17)	3/63 (5)	25/63 (40)	54/63 (86)	3/63 (5)	2/63 (3)	42/63 (67)	11/62 (18)
	1	3/67 (4)	1/67 (1)	25/67 (37)	60/67 (90)	1/67 (1)	2/64 (3)	49/64 (77)	17/66 (26)
	5	8/70 (11)	14/70 (20)**	25/70 (36)	63/70 (90)	5/70 (7)	1/68 (1)	60/66 (91)***	22/70 (31)
	25	19/65 (29)	39/65 (60)***	37/65 (57)*	59/65 (91)	6/65 (9)	4/64 (6)	58/61 (95)***	35/61 (57)***
	Trend[d]	$P < 0.001$	$P < 0.001$	$P = 0.006$	NS	NS	NS	$P < 0.001$	$P < 0.001$

Sources: CIIT 1993, Cattley *et al.* 1994.
*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$ (Fisher's exact test).
[a]NE = not examined.
[b]Four transverse sections of the nose were examined. The data are for level 3.
[c]NAP = not applicable.
[d]Cochran-Armitage trend test; NS = not significant.

4.3 Summary

Following inhalation exposure to nitrobenzene, male B6C3F_1 mice showed significant increases in lung and thyroid follicular cell neoplasms, and female B6C3F_1 mice showed increased incidences of mammary gland adenocarcinoma. Neoplasms showing exposure-related increases in rats included hepatocellular neoplasia (male F344/N and male CD rats), kidney tubular cell tumors (male F344/N rats), and endometrial stromal polyps (female F344/N rats). In addition, there were marginal increases in the incidences of hepatocellular neoplasia in female B6C3F_1 mice and F344/N rats, and thyroid follicular cell neoplasia in male F344/N rats.

5 Genotoxicity

In 1996, IARC reviewed the genetic effects of nitrobenzene (IARC 1996). Nitrobenzene did not induce reverse mutation in *Salmonella typhimurium*, with or without mammalian metabolic activation. Inhalation exposure of rats to nitrobenzene did not induce unscheduled DNA synthesis (UDS), sister chromatid exchange (SCE), or chromosomal aberrations in lymphocytes in the blood or spleen. Table 5-1 summarizes the studies examined by IARC (1996) and several more recent studies (discussed below).

Table 5-1. Genetic and related effects of nitrobenzene exposure

Test System	End Point	Results[a]		References
		without metabolic activation	with metabolic activation	
S. typhimurium TA98	reverse mutation	–	–	Haworth *et al.* 1983, Shimizu *et al.* 1983, Aßmann *et al.* 1997
S. typhimurium TA98	reverse mutation	–	–[b]	Dellarco and Prival 1989
S. typhimurium TA98	reverse mutation	–	ND	Chiu *et al.* 1978, Vance and Levin 1984
S. typhimurium TA1537	reverse mutation	–	–	Shimizu *et al.* 1983
S. typhimurium TA1538	reverse mutation	–	–	Shimizu *et al.* 1983
S. typhimurium TA100	reverse mutation	–	–	Haworth *et al.* 1983, Shimizu *et al.* 1983, Aßmann *et al.* 1997
S. typhimurium TA100	reverse mutation	–	ND	Chiu *et al.* 1978, Vance and Levin 1984
S. typhimurium TA100	reverse mutation	–	–[b]	Dellarco and Prival 1989
S. typhimurium TA1535	reverse mutation	–	–	Haworth *et al.* 1983, Shimizu *et al.* 1983
S. typhimurium TA1535	reverse mutation	–	ND	Vance and Levin 1984
S. typhimurium TA98NR	reverse mutation	ND	–	Suzuki *et al.* 1987, Vance and Levin 1984
S. typhimurium TA98/1,8-DNP_6	reverse mutation	ND	–	Suzuki *et al.* 1987
S. typhimurium TA100NR, TA1537NR, TA97a	reverse mutation	–	ND	Vance and Levin 1984
Rat hepatocytes	UDS	–	NAP	Butterworth *et al.* 1989

Test System	End Point	Results[a]		References
		without metabolic activation	with metabolic activation	
Human hepatocytes	UDS	–	NAP	Butterworth *et al.* 1989
Human peripheral blood lymphocytes	chromosomal aberrations	+	NAP	Huang *et al.* 1995, 1996
Male F344 rat peripheral blood lymphocytes *in vivo*	SCE, chromosomal aberrations	–	NAP	Kligerman *et al.* 1983
Male F344 rat splenic lymphocytes *in vivo*	SCE	–	NA	Kligerman *et al.* 1983

[a]ND = not determined; NAP = not applicable.

[b]Preincubation with flavin mononucleotide to facilitate nitro reduction.

Few studies on genetic effects of nitrobenzene have been published since 1995. The more recent studies are summarized below.

5.1 Prokaryotic systems: induction of mutation in *Salmonella typhimurium*

Aßmann *et al*. (1997) assessed the genotoxicity of nitrobenzene in *S. typhimurium* strains TA98 and TA100, both in the presence and in the absence of induced-rat liver S9 metabolic activation. At concentrations ranging from 316 to 5,050 μg/plate, after incubation for 48 hours, nitrobenzene did not induce reverse mutation in either strain.

5.2 Mammalian systems: chromosomal aberrations *in vitro*

In two separate studies, Huang *et al*. (1995, 1996) examined the genotoxic effects of nitrobenzene in human peripheral blood lymphocytes. The percentage of aberrant cells (the number of cells with structural chromosomal aberrations among 100 metaphase cells) was 33.2% after exposure to nitrobenzene at a concentration of 0.8 mmol/L for 24 hours (Huang *et al.* 1995), clearly higher than in the dimethylsulfoxide control (1.8%). In a later study, exposure to nitrobenzene at 50 mmol/L for 24 hours significantly increased the percentage of aberrant cells (*t*-test, $P < 0.01$) (Huang *et al.* 1996).

5.3 Summary

IARC (1996) concluded that nitrobenzene was not genotoxic in bacteria, mammalian cells *in vitro*, or mammals *in vivo*. Few studies on nitrobenzene have been published since the 1996 IARC review of nitrobenzene. In agreement with the studies summarized by IARC (1996), the study by Aßmann *et al*. (1997) found that nitrobenzene was not genotoxic in *S. typhimurium*. However, Huang *et al*. (1995, 1996) found that exposure to nitrobenzene *in vitro* induced chromosomal aberrations in human peripheral blood lymphocytes.

6 Other Relevant Data

6.1 Absorption, excretion, and metabolism in animals and humans

6.1.1 Absorption and excretion

Skin absorption of liquid nitrobenzene in humans and monkeys has been measured *in vitro* and *in vivo*. At high concentrations on the human forearm (15 mg/cm^2), an initial absorption rate of 1.85 mg/cm^2 was observed (Salmowa and Piotrowski 1960). At lower concentrations (4 μg/cm^2), 1.5% of the applied dose was absorbed in 24 hours (Feldmann and Maibach 1970). In monkeys, *in vivo* absorption of nitrobenzene applied as an acetone solution (4 μg/cm^2) to abdominal skin was 4.2% in 24 hours. *In vitro* penetration of human and monkey abdominal skin was 7.8% and 6.2%, respectively, of the applied dose (4 μg/cm^2) in 24 hours (Bronaugh and Maibach 1985).

Human subjects retained about 80% of nitrobenzene inhaled at concentrations of 5 to 30 μg/L (Beauchamp *et al.* 1982 and references therein). Nitrobenzene in the vapor (gas) phase also is readily absorbed through the skin. Between 8 and 19 mg was absorbed by naked humans exposed to nitrobenzene for six hours at a concentration of 10 mg/m^3 (Piotrowski 1967). Inhaled nitrobenzene is extensively absorbed in humans.

A major route of excretion of nitrobenzene in humans and laboratory animals is the urine. Urinary excretion accounts for about two thirds of the nitrobenzene administered to rats (Rickert *et al.* 1983) and rabbits (Robinson *et al.* 1951, Parke 1956), about one third of the dose in mice (Rickert *et al.* 1983), and from 6% to 37% in humans (Salmowa *et al.* 1963). Feces account for the rest of the dose in experimental animals, with expired air accounting for 1% to 2% of the dose in rabbits, rats, and mice. Nearly all of the nitrobenzene excreted in the urine is in the form of metabolites (see Table 6-1).

Table 6-1. Urinary excretion of nitrobenzene metabolites by rats and mice in the 72 hours following an oral dose of nitrobenzene (225 mg/kg body weight)

	Percentage of dose excreted (N = 3; mean ± s.d.)		
Metabolites	**Fischer 344 rats**	**CD rats**	**B6C3F$_1$ mice**
4-Aminophenol	0	0	0.1 ± 0.1
4-Aminophenol glucuronide	0	0	0.2 ± 0.2
4-Aminophenol sulfate	0	0	9.4 ± 1.3
4-Nitrophenol	0	2.2 ± 0.6	0.8 ± 0.1
4-Nitrophenol glucuronide	0	0.5 ± 0.1	6.3 ± 1.1
4-Nitrophenol sulfate	19.9 ± 1.1	13.0 ± 2.4	7.2 ± 1.2
3-Nitrophenol	0	1.2 ± 0.4	0.1 ± 0.1
3-Nitrophenol glucuronide	0	0.5 ± 0.2	0
3-Nitrophenol sulfate	10.2 ± 0.6	6.2 ± 1.7	6.1 ± 1.2

	Percentage of dose excreted (N = 3; mean ± s.d.)		
Metabolites	**Fischer 344 rats**	**CD rats**	**B6C3F$_1$ mice**
4-Hydroxyacetanilide	0	1.3 ± 0.2	0.4 ± 0.0
4-Hydroxyacetanilide glucuronide	0	1.8 ± 0.6	3.1 ± 0.3
4-Hydroxyacetanilide sulfate	19.0 ± 0.9	5.8 ± 1.2	0.4 ± 0.1

Source: Rickert *et al.* 1983.

6.1.2 Metabolism

The metabolism of nitrobenzene *in vivo* has been studied in several laboratory animals and in humans after accidental and experimental exposures. Overall, the metabolism of nitrobenzene in humans and animals is quite similar (see Section 6.1.2.1). The metabolism of nitrobenzene in *in vitro* preparations from human tissues does not seem to have been studied. [However, similarities in the metabolism of other nitroaromatic compounds by human and animal tissue preparations suggest that data derived from animal preparations may be extrapolated to human preparations] (see Section 6.1.2.2).

6.1.2.1 In vivo

The metabolism of nitrobenzene has been studied after oral administration in rabbits (Robinson *et al.* 1951, Parke 1956), several strains of rat (Tomaszewski *et al.* 1975, Rickert *et al.* 1983), and B6C3F$_1$ mice (Rickert *et al.* 1983). Table 6-2 lists metabolites of nitrobenzene in various species and strains. In all cases, the most abundant excreted metabolites were *p*-nitrophenol, *m*-nitrophenol, and *p*-hydroxyacetanilide (which may have been converted to *p*-aminophenol by isolation procedures used in the earlier studies) (Robinson *et al.* 1951, Parke 1956, Kao *et al.* 1978). These metabolites were excreted mainly as their sulfate or glucuronide conjugates. In each case, nitro group reduction (yielding *p*-hydroxyacetanilide or *p*-aminophenol) was an important pathway. The percentage of dose proceeding through nitro group reduction varied among strains and species, from about 13% in B6C3F$_1$ mice to about 31% in rabbits.

Parke (1956) also examined the urine collected from rabbits and guinea pigs orally exposed to [^{14}C]nitrobenzene for the presence of azoxybenzene, azobenzene, and benzidine. Although Robinson *et al.* (1951) had reported that phenolhydroxylamine derived from metabolism of nitrobenzene gave rise to azoxy-, azo-, and hydrazo-benzene (recovered as benzidine) *in vitro*, no radioactivity associated with these putative products was recovered by Parke (1956).

In an attempt to elucidate the marked differences in the toxicity of inhaled nitrobenzene in a number of species and strains, Rickert *et al.* (1983) gave F344 rats, CD rats, and B6C3F$_1$ mice a single oral dose of [^{14}C]nitrobenzene. Urinary metabolites were separated by high-pressure liquid chromatography, with quantitation of radioactivity by liquid scintillation spectrometry. Excretion of nitrobenzene metabolites as a percentage of the total dose is summarized in Table 6-1 (above). Based on the urinary metabolites identified in these strains of rats and mice, Rickert (1987) proposed the overall

mammalian metabolic pathway illustrated in Figure 6-1. Table 6-2 lists the potential human metabolites of nitrobenzene identified in animal urine.

Humans have been exposed to nitrobenzene accidentally (Ikeda and Kita 1964) and experimentally by inhalation (Piotrowski 1967, Salmowa *et al.* 1963). As in animals, the major metabolites isolated from urine are *p*-nitrophenol (4-nitrophenol) and *p*-aminophenol (4-aminophenol) (or perhaps *p*-hydroxyacetanilide that was converted to *p*-aminophenol during sample preparation). *p*-Aminophenol was found after acute poisoning, but lower nitrobenzene exposures yielded mostly *p*-nitrophenol. [It is possible that *p*-aminophenol still was present but at a concentration below the relatively poor sensitivity of the assay.]

Table 6-2. Potential human nitrobenzene metabolites identified in animal urine, grouped by general chemical structure

Metabolite	Animal[a]	Reference[b]
Ring oxidation products		
2-Nitrophenol	rabbit	1, 2
3-Nitrophenol	rabbit, CD rat, mouse, guinea pig	1, 2, 3, 4
3-Nitrophenol glucuronide	CD rat, mouse	3, 4
3-Nitrophenol sulfate	F344 rat, CD rat, mouse, guinea pig	3, 4
4-Nitrophenol	rabbit, CD rat, mouse, guinea pig	1, 2, 3, 4
4-Nitrophenol glucuronide	CD rat, mouse	3, 4
4-Nitrophenol sulfate	F344 rat, CD rat, mouse	3, 4
4-Nitrocatechol	rabbit, guinea pig	1, 2
2-Aminophenol	rabbit, guinea pig	1, 2
3-Aminophenol	rabbit, guinea pig	1, 2
4-Aminophenol	rabbit, mouse, guinea pig	1, 2, 3, 4
4-Aminophenol glucuronide	rabbit, mouse	1, 3
4-Aminophenol sulfate	rabbit, mouse	1, 3
Nitroquinol	rabbit, guinea pig	2
Reduction product		
Aniline	rabbit	1, 2
Modified products		
4-Hydroxyacetanilidine	CD rat, mouse	3, 4
4-Hydroxyacetanilidine glucuronide	CD rat, mouse	3, 4
4-Hydroxyacetanilidine sulfate	F344 rat, CD rat, mouse	3, 4
Ring-cleavage product		
4-Nitrophenylmercapturic acid	rabbit, guinea pig	2

[a]F344 rat = Fischer 344 rat; mouse = $B6C3F_1$ mouse.

[b]1 = Robinson *et al.* 1951; 2 = Parke 1956; 3 = Rickert *et al.* 1983; 4 = Rickert 1987.

6.1.2.2 In vitro

The major site for reduction of the nitro group to an amine in animals, and probably humans as well, likely is in the bacteria inhabiting the intestine. On the other hand, a one-electron reduction can take place, and this may occur throughout the mammalian body.

In vitro studies using liver microsomal preparations have shown that reduction of nitrobenzene to aniline occurs rapidly under anaerobic conditions and poorly, if at all, in the presence of oxygen (Harada and Omura 1980, Levin and Dent 1982). Levin and Dent (1982) calculated that in Fischer 344 rats, reduction of nitrobenzene to aniline was 150-fold faster in cecal contents than in liver, even if the calculation was based on the rate of nitrobenzene reduction in liver microsomal preparations incubated anaerobically. Reduction rates could not be compared between cecal contents and liver microsomes incubated aerobically because of the extremely low rates in the liver preparation.

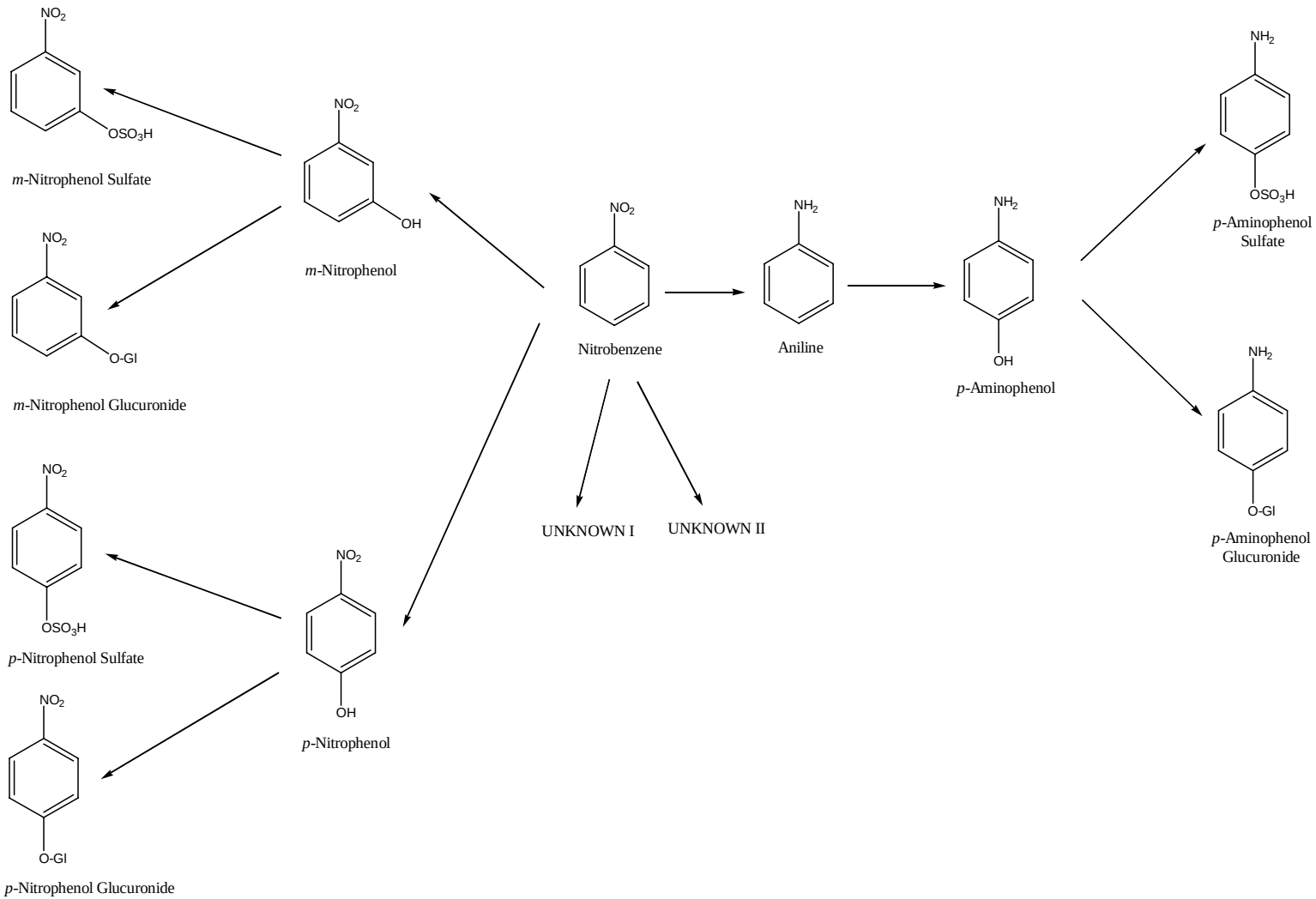


Figure 6-1. Potential nitrobenzene metabolites in mammals (Adapted from Rickert 1987)

Although reduction of nitrobenzene under aerobic conditions has been detected in incubations with isolated rat hepatocytes (Blaauboer and Van Holsteijn 1983), the two studies discussed below suggest that hepatic reductases may contribute less to the production of reduced metabolites *in vivo* than do anaerobic bacteria. Reduction of nitrobenzene is important in the production of methemoglobinemia (Holder 1999b), and Reddy *et al*. (1976) showed that rats whose intestines were not colonized by bacteria did not develop measurable methemoglobinemia after doses of nitrobenzene that produced 30% to 40% methemoglobinemia in conventional rats. Levin and Dent (1982) followed up their observations *in vitro* with an *in vivo* experiment in which control and antibiotic-treated rats were given a dose of nitrobenzene, and the urinary metabolites were quantified. The one identified metabolite arising from nitrobenzene reduction, *p*-hydroxyacetanilide, accounted for about one-sixteenth as much of the dose in antibiotic-treated animals as it did in normal rats.

Although a complete six-electron reduction from nitrobenzene to aniline does not occur in aerobic incubations, one-electron reduction does, and this likely has toxicological implications. Studies with various nitroaromatic compounds suggested that in the presence of NADPH, a flavin component of microsomes effects a one-electron reduction of a nitro group to a nitroanion radical (Mason and Holtzman 1975a, Sealy *et al.* 1978). In the presence of molecular oxygen, the nitroanion radical is quickly oxidized to the original compound, and the superoxide anion is produced in what is called a “futile reaction,” in which the parent nitrobenzene is regenerated.

While most studies of *in vitro* nitrobenzene metabolism have been performed with preparations of liver or intestinal contents, Yoshioka *et al*. (1989) incubated nitrobenzene with boar spermatozoa. They demonstrated a small amount of conversion of the parent compound to phenylhydroxylamine.

6.2 Human toxicity

IARC (1996) reviewed toxic effects following acute exposure of humans to nitrobenzene. Acute nitrobenzene poisoning is characterized by methemoglobinemia with cyanosis, headache, dyspnea, and coma or death. IARC (1996) cited several case studies of methemoglobinemia occuring in individuals exposed to nitrobenzene; however, the data were not sufficient to indicate dose-response relationships.

6.3 Considerations on whether nitrobenzene may be a human carcinogen

A central question is whether the carcinogenicity of nitrobenzene in animals (see Section 4) is applicable to humans. This question is addressed in the following sections, which discuss metabolism, structure-activity relationships (SARs), and relevance of results in animals to potential human hazard.

6.3.1 Metabolic manifestations implicating nitrobenzene carcinogenicity

Nitrobenzene organ toxicity is manifest by its metabolism and organ concentrations of parent and metabolites. As discussed in Section 6.1.2, it is expected that nitrobenzene can be chemically reduced anywhere in the body. Nitrobenzene can be reduced at the

nitrogen atom to produce various reactive free radical intermediates, while also being ring oxidized to form various phenolic intermediates (see Figures 1-2 and 6-1).

6.3.1.1 Reduction of nitrobenzene

In rats, endogenous intestinal bacteria efficiently convert orally ingested nitrobenzene to reduced nitroxide intermediates. Mechanistically, this reduction is a concerted two-electron-step process from nitrobenzene to nitrosobenzene to phenylhydroxylamine to aniline (Figure 6-2a) (Holder 1999b). Once nitrobenzene and its metabolites are absorbed into the body, a microsomal one-electron step reduction process can produce reduced nitroxides, with aniline as its final product (Figure 6-2b). Thus, oral exposure results in formation of nitroxides in the cecum by bacterial nitroreductases, but inhalation exposure produces nitroxides mostly in microsomes (and possibly the mitochondria). Reduction at these two sites, by one-electron and two-electron steps, is catalyzed by different nitroreductase enzymes (Wheeler *et al.* 1975, Peterson *et al.* 1979, Levin and Dent 1982). When exposure is by inhalation, the gastroenteral reduction process is mostly bypassed; however, a small amount of nitrobenzene would be swallowed during inhalation exposre and thus be subject to cecal bacterial reduction.

Although chemically reactive intermediates nitrosobenzene and phenylhydroxylamine are produced in both reduction processes (Figure 6-2a and b), only the one-electron-step reduction results in production of free-radical intermediates, such as the nitroanion free radical (Figure 6-2b) (Mason 1982, Mason and Holtzman 1975a). Whereas only modest steady-state levels of the nitroxides nitrosobenzene and phenylhydroxylamine occur in rat liver, significant amounts of these nitroxides persist in circulating red blood cells (Figure 6-3) (Eyer *et al.* 1980, Blaauboer and Van Holsteijn 1983). Nitrosobenzene and phenylhydroxylamine drive reactions forming methemoglobin and consuming NAD(P)H, thus maintaining a persistent redox couple (Eyer and Lierheimer 1980). Hence, frequent nitrobenzene re-exposures of rodents, as in the two-year bioassay, tend to initiate and maintain the cycling actions of the redox couple. This redox couple likely contributes to the slow kinetic elimination of nitrobenzene in addition to the “futile reaction” proposed by Rickert (1987). Because the circulation involves all tissues, the redox couple accounts in part for the pervasive and stable system toxicity set up by nitrobenzene exposure. The redox couple in RBCs constitutes an ongoing catalytic pool that resists nitrobenzene metabolic clearance and could affect many tissue types. This pervasiveness among organs may explain why each of the three rodent strains (two species) tested in the CIIT bioassay responded with tumors at many organ sites (eight). By extension, humans also may generate the redox couple via frequent exposures that could occur from frequent use of nitrobenzene.

6.3.1.2 Oxidation of nitrobenzene

Nitrobenzene can be oxidized by various microsomal oxygenases to form nitrophenols and derivative aminophenols (Rickert 1987, Robinson *et al.* 1951, Parke 1956, Medinsky and Irons 1985) (see Table 6-2). Ring oxidation produces mostly *p*-nitrophenol, *p*-aminophenol, and *p*-hydroxyacetanilide metabolites (among others), which are eliminated in the excreta (Rickert 1987, Parke 1956, Ikeda and Kita 1964). Nitrobenzene is modified toward more polarity by sulfation, acetylation, or glucuronidation.

The kinetics of urinary elimination of *p*-nitrophenol, the characteristic nitrobenzene elimination metabolite, are slow, suggesting that nitrobenzene is either recycled in the bile or retained by other means. Experimental results suggest that no significant bile recycling takes place (Salmowa *et al.* 1963, Rickert *et al.* 1983). It has been suggested that retention may be due, in part, to the oxidation "futile reaction" (Figure 6-2b), which may continually regenerate nitrobenzene, thereby slowing its net elimination from the body (Rickert 1987). The sustainable pool of persistent nitroxide intermediates and regeneration of the parent compound (Figure 6-2b) would result in resistance to the efficient elimination of nitrobenzene. By means of the "futile reaction," tissues with sufficient O_2 are able to oxidize nitroanion free radicals, thus producing superoxide anions while regenerating parent nitrobenzene (Bus and Gibson 1982, Mason and Holtzman 1975b, Sealy *et al.* 1978, Levin and Dent 1982, Holder 1999b). Considering the suspected carcinogenic properties of superoxide anion radicals, the "futile reaction" may account for a number of the toxic and/or carcinogenic effects of nitrobenzene (Trush and Kensler 1991, Flohé *et al.* 1985, Guyton and Kensler 1993, Feig *et al.* 1994, Cerutti 1994, Dreher and Junod 1996).

6.3.1.3 Conjugation of nitrosobenzene

Nitrosobenzene is known to bind glutathione (GSH) during elimination, forming a relatively stable circulating glutathione–nitrosobenzene conjugate (GS-NOB). As shown in Figure 6-3, that GS-NOB may translocate throughout the body, where it may (1) homeolytically cleave to form the reactive glutathiyl radical, (2) undergo a redox reaction to form phenylhydroxylamine, or (3) rearrange to form glutathionesulfinamide, which in turn cleaves to produce aniline (Maples *et al.* 1989, Eyer 1979, Eyer and Lierheimer 1980, Eyer and Ascherl 1987, Holder 1999b). The third sequence is a major pathway for aniline production (see Figure 6-2). Aniline itself is a suspected animal carcinogen (NTP 1978) and may act as a nitroxide reservoir upon oxidation (not shown in Figure 6-1).

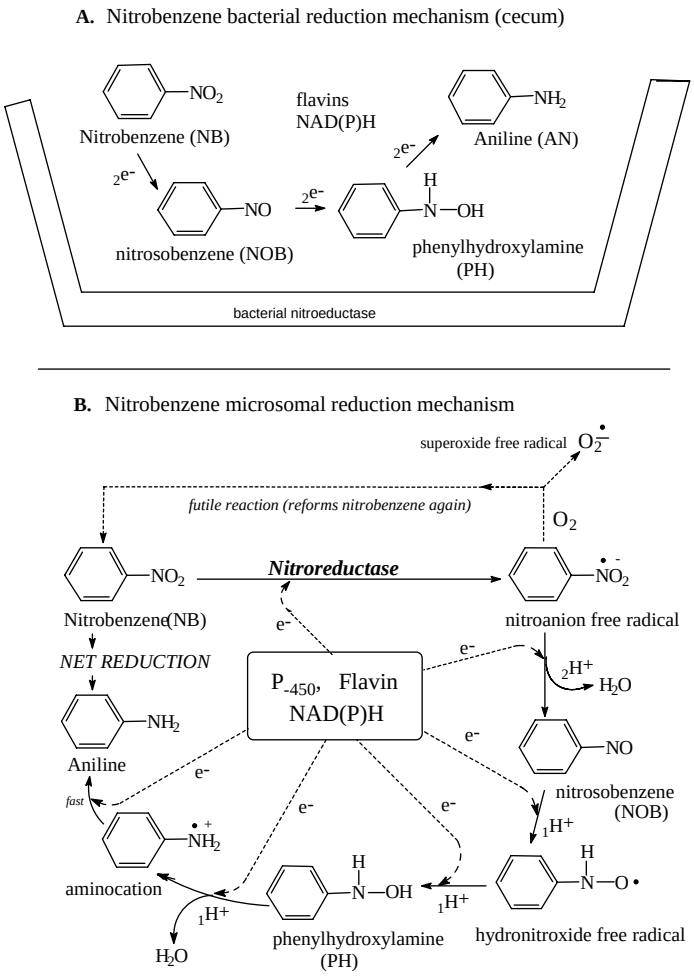


Figure 6-2. Nitrobenzene metabolic reduction processes

Source: Holder 1999b (used by permission of the publisher, Arnold Publishers, and the author, Dr. James Holder).

a. Microbial nitroreductase reaction in the cecum**.** Free radicals are concerted and not released locally from the catalytic center of the nitroreductase. This could be the most important nitroreduction following oral exposure to nitrobenzene. The reactive intermediates nitrosobenzene, phenylhydroxylamine, and aniline are released and then absorbed and distributed in the body.

b. Microsomal one-electron-step reduction mechanism**.** The reactive free radicals superoxide, nitroanion, hydronitroxide, and aminocation are produced. At fast rates of nitrobenzene catalysis, these reactive species may exceed the capacity of the local syncytia to quench them by spin traps. Not shown are enzymatic steps that can oxidize aniline to phenylhydroxylamine and nitrosobenzene. These reactions are driven by microsomal P-450s and NAD(P)H pools and perhaps mitochondrial flavins. These mechanisms are not completely understood. The aminocation free radical, although diagrammed here, has not been isolated, and its production remains theoretical.

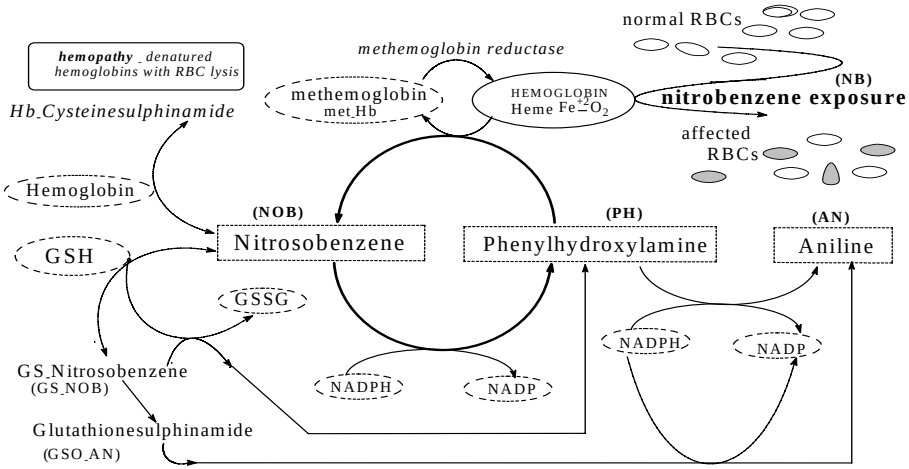


Figure 6-3. Red blood cell cycling of nitrosobenzene and phenylhydroxylamine

Source: Holder 199b (used by permission of the publisher, Arnold Publishers, and the author, Dr. James Holder).

The conversion of nitrosobenzene to phenylhydroxylamine is driven by the oxidation of NAD(P)H on the one side and the oxidation of endogenous heme-Fe^{2+} on the other. Nitrosobenzene can outcompete O_2 for binding to functional hemoglobin. Not shown is the destabilization of tetrameric hemoglobin to $\alpha\beta$ dimers. Nitrosobenzene can bind cysteine groups on functional hemoglobin, thereby denaturing the globin chain, resulting in hemopathy. Because of the redox disturbances, red blood cells are turned over more rapidly in the spleen, which becomes engorged. Glutathione can bind nitrosobenzene; the conjugate can move "masked" systemically and regenerate nitrosobenzene elsewhere, to restart the pernicious cycle. Other cell types are likely to regenerate nitrosobenzene and phenylhydroxylamine, but these mechanisms are less well understood.

6.3.1.4 Nitrobenzene structure-activity relationships

Nitrobenzene has structure-activity relationships with other aromatic nitro and amino compounds of concern. The NTP currently lists five selected nitroarenes in the Report on Carcinogens as *reasonably anticipated to be human carcinogens*. The tumor sites reported for these five nitroarenes are summarized in Table 6-3, together with the tumor sites identified for nitrobenzene in Section 4; the structures of these molecules are illustrated in Table 6-4. These five nitroarenes plus a sixth, 2-nitrofluorene, have been reviewed by IARC (1989) and evaluated as possibly carcinogenic to humans (Group 2B). Another nine nitroarenes also were reviewed by the same IARC working group, and each was designated as not classifiable as to its carcinogenicity to humans (Group 3); these included 3,7-dinitrofluoranthrene, 3,9-dinitrofluoranthrene, 1,3-dinitropyrene, 7-nitrobenz[*a*]anthracene, 6-nitrobenzo[*a*]pyrene, 1-nitronaphthalene, 2-nitronaphthalene, 3-nitroperylene, and 2-nitropyrene.

Holder (1999b) reported that of 16 nitroarenes tested by the NTP, 62.5% (10/16) were carcinogenic in mice and/or rats. Further SAR analysis of these NTP data suggest that other functional groups, such as multiple strong electron withdrawing groups, can suppress nitroarene carcinogenicity. Therefore, the mere presence of a nitro group in a compound does not necessarily connote carcinogenicity (Rosenkranz and Mermelstein 1983). A systematic study was made of certain amines that might produce nitroxide intermediates. Eleven of the chosen amines were known to be carcinogens, and eight were not carcinogenic (Stier *et al.* 1980). The characteristic nitroxide electron spin resonance (ESR) signal (stable free radicals) was found in 91% of the carcinogenic amines, but only 25% of the noncarcinogenic amines. While not completely deterministic, this study suggests that those amines which generated the nitroxide ESR signal in metabolism have a tendency to be involved with carcinogenesis. No analogous systematic study was located for nitroarenes.

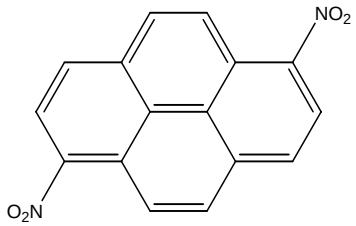
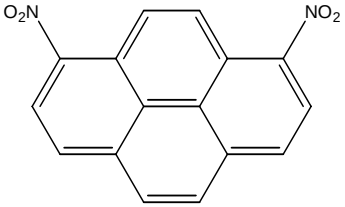
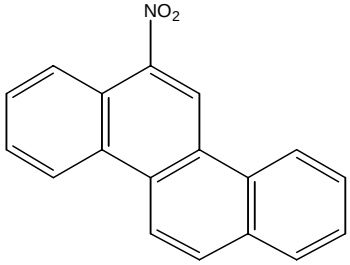
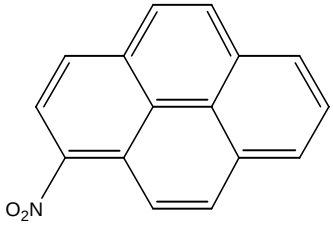
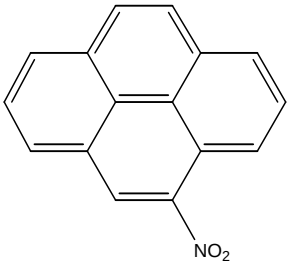
Since publication of Holder's (1999b) review, additional aromatic compounds with one or more nitro groups have been the subjects of NTP bioassays. The carcinogenicity of 27 nitroaromatic compounds identified in the NTP bioassay database is summarized in Appendix B (Table B-1). The results were as follows: 12 compounds were carcinogenic in at least one study, five showed some evidence of carcinogenicity, the evidence for five was equivocal (a marginal increase in neoplasia), and six were not carcinogenic. The percentage of compounds showing evidence of carcinogenicity is 59% (16 of 27). [If compounds with equivocal results are excluded, the percentage for which there is evidence of carcinogenicity increases to 73% (16 of 22).]

Table 6-3. Carcinogenicity of nitrobenzene and selected nitroarenes listed in the Report on Carcinogens[a]

Site	Nitrobenzene	1,6-Dinitropyrene	1,8-Dinitropyrene	6-Nitrochrysene	1-Nitropyrene	4-Nitropyrene
Injection-site sarcoma	–	mice: M rats: M & F	mice: M rats: M & F	–	rats: M & F	rats: F
Leukemia	–	rats: F hamsters: M & F	rats: F	–	–	rats: F
Mammary gland	mice: F	–	rats: F	rats: F	rats: F	rats: F
Liver	rats: M	mice: M	–	mice: M & F	mice: M	mice: M
Lung	mice: M	rats: M hamsters: M & F	–	mice: M & F	mice: M & F hamsters	mice: M & F
Peritoneal cavity	–	rats: F	rats: F	–	–	–
Colon	–	–	–	rats: M & F	–	–
Kidney	rats: M	–	–	–	–	–
Pituitary	–	rats	–	–	–	–
Thyroid	mice: M	–	–	–	–	–
Endometrium	rats: F	–	–	–	–	–
Malignant lymphoma	–	–	–	mice: M	–	–
Zymbal gland	–	–	–	–	–	rats: F

[a]All of the nitroarenes listed in the Report on Carcinogens exist as solids at room temperature (melting points ≥ 155ºC) and were tested by injection or oral administration. Nitrobenzene was administered by inhalation in the two-year carcinogenicity bioassay.

Table 6-4. Structure of nitrobenzene analogs listed in the Report on Carcinogens

Chemical	Structure
1,6-Dinitropyrene	
1,8-Dinitropyrene	
6-Nitrochrysene	
1-Nitropyrene	
4-Nitropyrene	

Aniline is the fully reduced form of nitrobenzene and thus is structurally related to nitrobenzene in that both can produce the nitroxide intermediates shown in Figure 6-2b. Any aniline produced from nitrobenzene, or already present from other sources, may serve as a pool to later be oxidized to reform the nitroxide intermediates, which would also reinforce the redox couple tending to resist nitrobenzene metabolite clearance and utltimately contributing to the slow elimination of nitrobenzene. Although aniline is the final product of both reduction processes (Figure 6-2a,b), it is likely that the nitroxide intermediates (nitrosobenzene and phenylhydroxylamine) and their associated free radicals in the one-electron-step process are the most chemically reactive intermediates and hence most likely the cause of nitrobenzene toxicity. As an indication of aniline's potential reservoir activity, oxidation of aniline has been linked to lipid peroxidation (Khan *et al.* 1997, Stier *et al.* 1980).

Aniline is carcinogenic to rats by oral exposure (NTP 1978). It induces hemangiosarcomas, fibrosarcomas, and sarcomas in the spleen, as well as sarcomas in other organs to limited degree. Moreover, aniline is thought to be associated with excess human bladder cancers (Ward *et al.* 1996). Aniline is known to cause stress in the spleen by promoting systemic redox reactions (Khan *et al.* 1997). Oxidation of aniline forms the same nitroxide intermediates as reduction of nitrobenzene (Kadlubar and Ziegler 1974, Mason and Holtzman 1975a, Mason 1982). Hence, because aniline is toxic and is considered a likely animal carcinogen, nitrobenzene also may be predicted by inference to be a likely carcinogen. Although no evidence has been reported on carcinogenicity of nitrobenzene to humans, nitrobenzene's predicted carcinogenicity is borne out experimentally in animals (see Section 4, Tables 4-1 and 4-2).

Nitrobenzene has structure-activity relationships with other aromatic nitro and amino compounds that produce common reactive nitroxide intermediates — aromatic nitroso- and hydroxyl-amine compounds and their free radicals — that relate to their mutagenicity and to metabolic imbalances that lead to cancer (Rickert 1987, Rosenkranz 1996, Blaauboer and Van Holsteijn 1983, Kiese 1966, Miller 1970, Weisberger and Weisberger 1973, Mason 1982, Verna *et al.* 1996). Nitroaromatics are of concern for chemical carcinogenesis because of their metabolic activation in various environmental media (Miller 1970, Rosenkranz and Mermelstein 1983, Rickert 1984) and occurrence in complex mixtures, such as municipal waste incineration emissions, diesel emissions, azo dyes, and food pyrolysates (DeMarini *et al.* 1996, Crebelli *et al.* 1995, King *et al.* 1988). Other examples of carcinogenic nitroxides are tobacco products 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone and *N*'-nitrosonornicotine, which are microsomally activated intermediates of tobacco combustion (redox) ingredients, nicotine and related plant alkaloids (Staretz *et al.* 1997, Hecht 1996). These nitroxides are linked to human lung, oral cavity, esophageal, and pancretic cancer from direct and/or indirect sources such as passive smoking (Hecht 1996, Pryor 1997). For a discussion of passive smoking, see U.S. EPA (1998) and Witschi *et al*. (1997). Free radicals currently are being analyzed in cigarette smoke, the toxicological activity of which depends on host factors such as vitamin concentrations, dietary lipids, superoxide dismutase, catalase, and cytochrome P-450 complements (Kodama *et al.* 1997, Maser 1997). It is reasonable to consider that nitrobenzene carcinogenicity may depend on these same host factors.

6.3.2 Considerations in human carcinogenicity of inhaled nitrobenzene

The likely mode of action for human carcinogenicity of nitrobenzene is that nitrobenzene-induced toxicity integrated over time leads to chemical carcinogenesis in various organ sites by unbalanced redox systems (Holder 1999). Because of the ubiquity of the redox conditions capable of producing amino- and nitro-phenols, nitrosobenzene, and phenylhydroxylamine in various organs, a variety of tissues can be damaged. Because of translocation and free radical chain-reactions, the tissue damage need not necessarily occur where the metabolites or their associated free radicals originally are produced. Specific toxicity profiles in different organs depend on detoxifying enzyme levels and many host- and tissue-specific factors, such as the number of endogenous free radical producers, quenching agents, spin traps (agents acting as stabilizers), and free radical carriers (Netke *et al.* 1997, Stier *et al.* 1980, Gutteridge 1995, Kehrer 1993). It is notable that target organs in rodents — nose, spleen, and testes — showed considerable toxicity in the CIIT bioassays, but not concordant tumorigenicity (Holder 1999b). This seems a contradiction unless it is assumed that these organs are specifically protected from nitrobenzene carcinogenicity. Whether this is so is not known, and the difficulties of necessarily associating toxicity with chemical carcinogenesis have been pointed out (Huff 1992).

6.3.3 Summary

Nitrobenzene is absorbed dermally and by inhalation in both animals and humans. Overall the metabolism *in vivo* of nitrobenzene appears to be similar in humans and animals with the major route of excretion being in the urine.

The major metabolites in humans and animals have been isolated from urine and include ring oxidation products such as nitrophenols, and aminophenols, reduction products such as aniline, modified products such as glucuronide or sulfate conjugates, and ring-cleavage product such as 4-nitrophenylmercapturic acid. Based on these products, the metabolism of nitrobenzene has been proposed to consist of two pathways, the first being reduction of the nitro group to aniline and subsequent ring oxidation to aminophenols followed by conjugation to the glucuronide and sulfate conjugates. The second pathway is ring oxidation to nitrophenols followed by conjugation to the glucuronide and sulfate conjugates. Reduction of nitrobenzene to aniline can occur under anaerobic (bacteria in the intestine) or aerobic conditions (cellular microsomes). The former is more likely to occur when nitrobenzene is ingested whereas the later is more likely to occur when nitrobenzene is inhaled. The reduction of nitrobenzene appears to be an important step in the production of methemoglobinemia.

Regarding the noncarcinogenic, toxic effects of nitrobenzene, methemoglobinemia has been observed in individuals exposed to nitrobenzene, and has also been observed in both subchronic and chronic studies in experimental animals.

The mechanism of carcinogenicity of nitrobenzene has not been elucidated. The metabolic reduction of nitrobenzene can lead to the production of free radicals. Nitrobenzene is structurally related to other aromatic nitro and amino compounds

including several nitroarenes that are considered by the National Toxicology Program and/or IARC to be reasonably anticipated or possible human carcinogens.

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NITROBENZENE

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

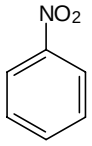
Chem. Abstr. Serv. Reg. No.: 98-95-3

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 ºC (Lide, 1993)

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

(*d*) *Density*: 1.2037 at 20 ºC/4 ºC (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 ºC); soluble in acetone, benzene, diethyl ether and ethanol (Booth, 1991; Lide, 1993)

(*g*) *Volatility*: Vapour pressure, 0.15 mm Hg [20 Pa] at 20 ºC; 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 ºC), 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-

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[1]Calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming temperature (25 ºC) 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 ºC. The mixed acid was then added slowly below the surface of the benzene and the temperature raised to 80–90 ºC. 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 ºC 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/m^3] 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/m^3] 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/m^3, 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 ºC. 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 pretreatment 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. Pretreatment 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 $\geq$ 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 $\geq$ 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 $\geq$ 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 $\geq$ 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 $\geq$ 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.

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)*.

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[1]For definition of the italicized terms, see Preamble.

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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

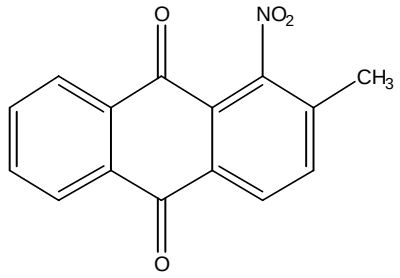
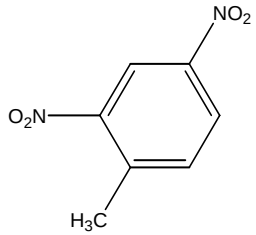
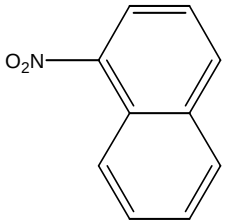
[c] Negative with or without 2mM flavin mononucleotide (FMN) in preincubation mix

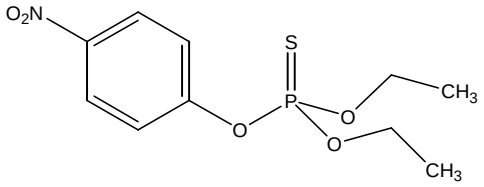
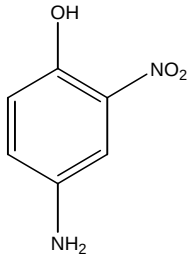
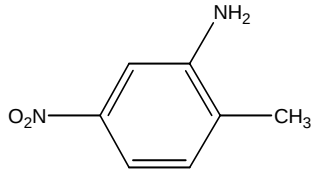
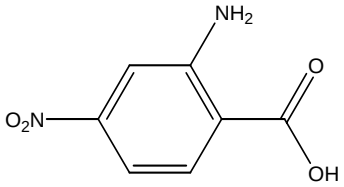
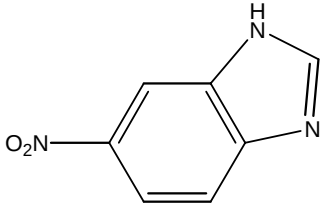
[d] Positive in the presence of 200 μg/plate norharman

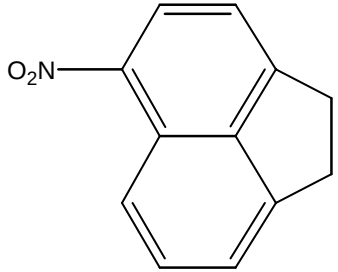
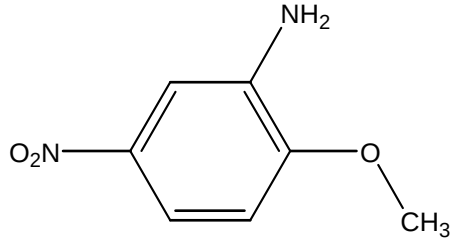
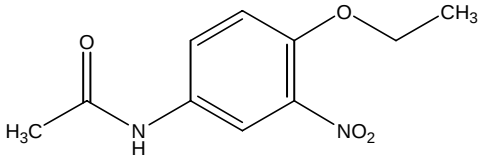
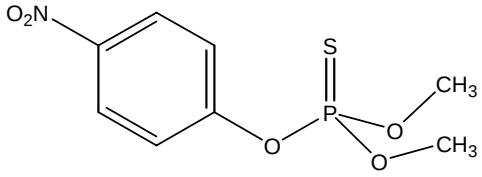
Appendix B: Selected Nitro-aromatic Compounds Bioassayed by the NTP

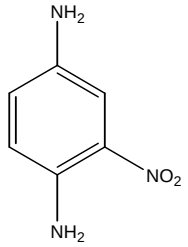
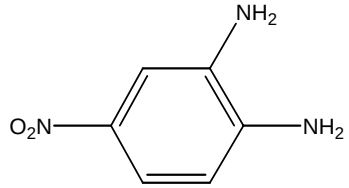
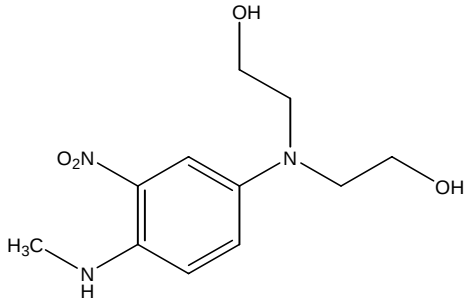
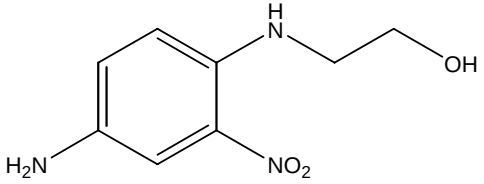
Table B-1. 27 Selected nitro-aromatic compounds bioassayed by the NTP. Rats refers to Fischer 344/N rats and mice to B6C3F$_1$ mice, except as noted below.

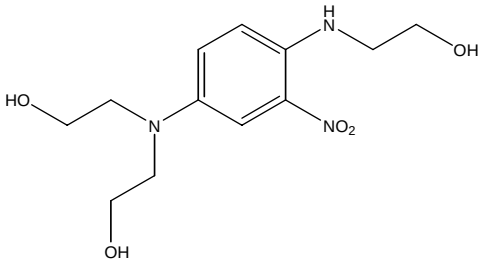
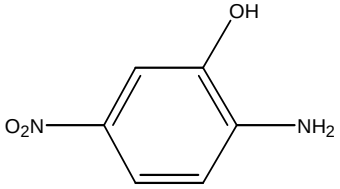
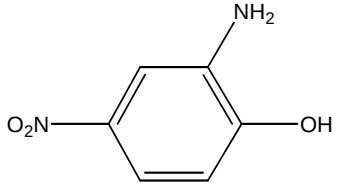
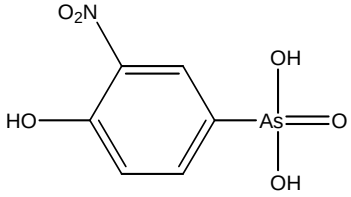
Results: 12 Positive, 4 Some Evidence, 5 Equivocal, 6 Negative. 16/22 (excluding equivocal results) = 73%; 16/27 (all compounds tested) = 59%.

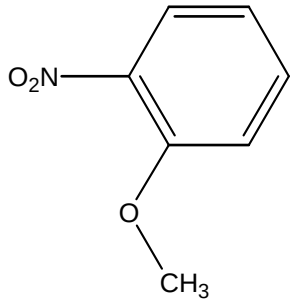
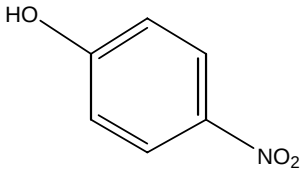
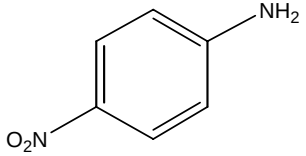
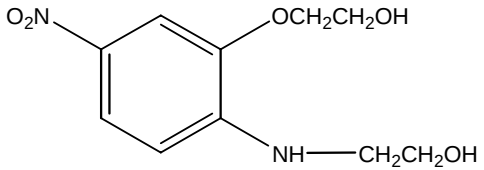
TR-#	Compound	CAS #	Structure	Carcinogenicity
TR-029	2-Methyl-1-nitroanthraquinone	129–15–7		Male rats – **Positive**; liver and forestomach tumors Female rats – **Positive**; liver, forestomach, and bladder tumors Male mice – **Positive*** Female mice – **Positive***
TR-054	2,4-Dinitrotoluene	121–14–2		Male rats – **Positive**; dermal and subcutaneous tumors Female rats – **Positive**; mammary gland tumors Male mice – Negative Female mice – Negative
TR–064	1-Nitronaphthalene	86–57–7		Male rats – Negative Female rats – Negative Male mice – Negative Female mice – Negative

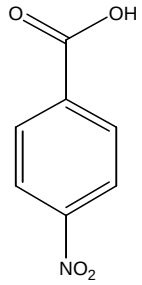
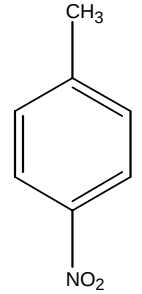
TR-#	Compound	CAS #	Structure	Carcinogenicity
TR–070	Parathion	56–38–2		Male rats[c] – *Equivocal*; adrenal tumors Female rats[c] – *Equivocal*; adrenal tumors Male mice – Negative Female mice – Negative
TR–094	4-Amino-2-nitrophenol	119–34–6		Male rats– **Positive**; bladder tumors Female rats – *Equivocal*; bladder tumors Male mice – Negative Female mice – Negative
TR–107	5-Nitro-o-toluidine	99–55–8		Male rats – Negative Female rats – Negative Male mice – **Positive**; liver tumors Female mice – **Positive**; liver tumors
TR–109	4-Nitroanthranilic acid	619–17–0		Male rats – Negative Female rats – Negative Male mice – Negative Female mice – Negative
TR–117	6-Nitrobenzimidazole	94–52–0		Male rats – Negative Female rats – Negative Male mice – **Positive**; liver, eye, and Harderian gland tumors Female mice – **Positive**; liver, eye, and Harderian gland tumors

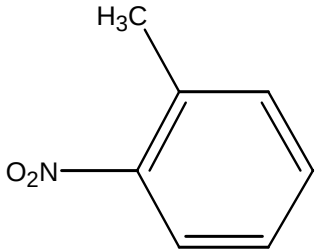
TR-#	Compound	CAS #	Structure	Carcinogenicity
TR–118	5-Nitroacenaphthene	602–87–9		Male rats – **Positive**; ear canal and alveolar/bronchiolar tumors Female rats – **Positive**; ear canal, clitoral gland, mammary gland, and alveolar/bronchiolar tumors Male mice – Negative Female mice – **Positive**; liver and ovary tumors
TR–127	5-Nitro-o-anisidine	99–59–2		Male rats – **Positive**; integumentary system (skin, Zymbal gland) tumors Female rats – **Positive**; integumentary system (skin, Zymbal gland, clitoral gland) tumors Male mice – *Equivocal* Female mice – **Positive**; liver tumors
TR–133	3-Nitro-P-acetophenetide	1777–84–0		Male rats – Negative Female rats – Negative Male mice – **Positive**; liver tumors Female mice – Negative
TR–157	Methyl parathion	298–00–0		Male rats – Negative Female rats – Negative Male mice – Negative Female mice – Negative

TR-#	Compound	CAS #	Structure	Carcinogenicity
TR–169	2-Nitro-p-phenylenediamine	5307–14–2		Male rats – Negative Female rats – Negative Male mice – Negative Female mice – **Positive**; liver tumors
TR–180	4-Nitro-o-phenylenediamine	99–56–9		Male rats – Negative Female rats – Negative Male mice – Negative Female mice – Negative
TR–271	HC Blue No. 1	2784–94–3		Male rats – *Equivocal Evidence*; liver tumors Female rats – Some Evidence; alveolar/bronchiolar tumors Male mice – **Clear Evidence**; liver and thyroid gland tumors Female mice – **Clear Evidence**; liver tumors
TR–281	HC Red No. 3b	2871–01–4		Male rats – No Evidence Female rats – No Evidence Male mice – *Equivocal Evidence*; liver tumors Female mice – Inadequate Study

TR-#	Compound	CAS #	Structure	Carcinogenicity
TR–293	HC Blue No. 2	33229–34–4		Male rats – No Evidence Female rats – No Evidence Male mice – No Evidence Female mice – No Evidence
TR–334	2-Amino-5-nitrophenol	121–88–0		Male rats – Some Evidence; pancreas tumors Female rats – No Evidence Male mice – No Evidence Female mice – No Evidence
TR–339	2-Amino-Nitrophenol	99–57–0		Male rats – Some Evidence; kidney tumors Female rats – No Evidence Male mice – No Evidence Female mice – No Evidence
TR–345	Roxarsone	121–19–7		Male rats – *Equivocal Evidence*; pancreas tumors Female rats – No Evidence Male mice – No Evidence Female mice – No Evidence

TR-#	Compound	CAS #	Structure	Carcinogenicity
TR–416	o-Nitroanisole[a]	91–23–6		Male rats – **Clear Evidence**; bladder, kidney, and large intestine tumors and leukemia Female rats – **Clear Evidence**; bladder, kidney, and large intestine tumors and leukemia Male mice – **Clear Evidence**; liver tumors Female mice – Some Evidence; liver tumors
TR–417	p-Nitrophenole	100–02–7		Male mice[d] – No Evidence Female mice[d] – No Evidence
TR–418	p-Nitroaniline[b]	100–01–6		Male mice – *Equivocal Evidence*; liver tumors Female mice – No Evidence
TR–419	HC Yellow 4	59820–43–8		Male rats – *Equivocal Evidence*; pituitary gland tumors Female rats – No Evidence Male mice – No Evidence Female mice – No Evidence

TR-#	Compound	CAS #	Structure	Carcinogenicity
TR–442	p-Nitrobenzoic acid	62–23–7		Male rats – No Evidence Female rats – Some Evidence; clitoral gland tumors Male mice– No Evidence Female mice– No Evidence
TR–498	para-Nitrotoluene (Draft Report)	99–99–0		Male rats – *Equivocal Evidence*; subcutaneous skin tumors Female rats – Some Evidence; clitoral gland tumors Male mice – *Equivocal Evidence*; alveolar/bronchiolar tumors Female mice – No Evidence

TR-#	Compound	CAS #	Structure	Carcinogenicity
TR–504	ortho-Nitrotoluene (Draft Report)	88–72–2		Male rats – **Clear Evidence**; mesotheliomas, subcutaneous skin, mammary gland, liver, and lung tumors Female rats – **Clear Evidence**; subcutaneous skin and mammary gland tumors Male mice – **Clear Evidence**; hemangiosarcomas, large intestine (cecum) and liver tumors Female mice – **Clear Evidence**; hemangiosarcomas, large intestine (cecum) and liver tumors

Note: The table contains those compounds with one ring or fused rings with no more than 2 substituents other than the nitro group.

[a]Listed in the Ninth Report on Carcinogens as *"reasonably anticipated to be a human carcinogen"*.

[b]Compound administered by corn-oil gavage; all other compounds were administered in the feed.

[c]Osborne-Mendel rats; Fischer 344/N rats were used for all other bioassays.

[d]Swiss-Webster mice; $B6C3F_1$ mice were used for all other bioassays.

[e]Dermal administration.

*NCI mouse study (A.S. Krishna Murthy, J.R. Baker, E.R. Smith and G.G. Wade. Development of Hemangiosarcomas in B6C3F1 Mice Fed 2-Methyl-1-Nitroanthraquinone. Int. J. Cancer: 19, 117-121 (1977)) not available.