2. HEALTH EFFECTS

2.1 INTRODUCTION

This chapter contains descriptions and evaluations of studies and interpretation of data on the health effects associated with exposure to nitrobenzene. Its purpose is to present levels of significant exposure for nitrobenzene based on toxicological studies, epidemiological investigations, and environmental exposure data. This information is presented to provide public health officials, physicians, toxicologists, and other interested individuals and groups with (1) an overall perspective of the toxicology of nitrobenzene and (2) a depiction of significant exposure levels associated with various adverse health effects.

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals address the needs of persons living or working near hazardous waste sites, the data in this section are organized first by route of exposure -- inhalation, oral, and dermal -- and then by health effect -- death, systemic, immunological, neurological, developmental, reproductive, genotoxic, and carcinogenic effects. These data are discussed in terms of three exposure periods -- acute, intermediate, and chronic.

Levels of significant exposure for each exposure route and duration (for which data exist) are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. These distinctions are intended to help the users of the document identify the levels of exposure at which adverse health effects start to appear, determine whether or not the intensity of the effects varies with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown on the tables and figures may differ depending on the user's perspective. For example, physicians concerned with the interpretation of clinical findings in exposed persons or with the identification of persons with the potential to develop such disease may be interested in levels of exposure associated with "serious" effects. Public health officials and project managers concerned with response actions at Superfund sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAEL) or exposure levels below which no adverse effects (NOAEL) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels, MRLs) are of interest to health professionals and citizens alike.

2.2.1 Inhalation Exposure

Health effects in humans following inhalation exposure to nitrobenzene have been described. However, as described in this section, these studies are limited in detail and technical content. There are several reliable animal studies using this route of exposure.

Table 2-l and Figure 2-l describe the health effects observed in laboratory animals associated with inhalation of nitrobenzene at varying exposure levels and durations; the results are discussed below.

2.2.1.1 Death

No studies were located regarding lethal effects of nitrobenzene in humans after inhalation exposure.

Strain and species differences in response to nitrobenzene exposure were demonstrated by Medinsky and Irons (1985). At an exposure level of 125 ppm nitrobenzene, there was a 40% rate of lethality in Sprague-Dawley (CD) rats and morbidity necessitating early sacrifice of all B6C3Fl mice. Fischer-344 rats, however, tolerated this level for 2 weeks without any adverse clinical signs. The relevance of these findings to human exposure is not known.

2.2.1.2 Systemic Effects

Hematological Effects. The outstanding toxic effect of inhalation exposure to nitrobenzene is methemoglobinemia. When the iron component of hemoglobin is converted from the ferrous state to the ferric state (oxidized), the resultant methemoglobin is no longer capable of releasing oxygen to the tissues of the body. This lowered oxygen capacity, or hypoxia, is generally associated with fatigue, weakness dyspnea, headache, and dizziness as oxygen-poor blood reaches the brain. Even under normal conditions, some (1 to 4%) methemoglobin is formed in the lungs as blood is oxygenated. Toxic or "secondary", methemoglobinemia can occur following exposure to nitrobenzene and other chemicals.

Methemoglobinemia has been reported in three-week-old twins (a male and a female) (Stevens 1928) and in a 12-month-old girl (Stevenson and Forbes 1942) exposed to nitrobenzene in insect exterminator sprays. In each case, the exposure lasted several hours and the exposure level was neither known nor estimated. Severe methemoglobinemia was reported in a 47-year-old woman who was occupationally exposed to nitrobenzene at unmeasured levels for 17 months (Ikeda and Kita 1964).

TABLE 2-1. Levels of Significant Exposure to Nitrobenzene - Inhalation

Figure Key	Species	Exposure Frequency/ Duration	Effect	NOAEL (ppm)	LOAEL (Effect) Less Serious (ppm)	LOAEL (Effect) Serious (ppm)	Reference
ACUTE EXPOSURE							
Death							
1	Rat	14 d 5d/wk 6hr/d				125[a] (LD50 males)	Medinsky and Irons 1985
Systemic							
2	Rat	14 d 5d/wk 6hr/d	Renal		10[a] (incr. kidney wt.)		Medinsky and Irons 1985
3	Rat	10 d Gd6-15 6hr/d	Other		10[a] (incr. spleen wt.)		Tyl et al. 1987
4	Rat	14 d 5d/wk 6hr/d	Hepatic		10[a] (incr. liver wt.)		Medinsky and Irons 1985
5	Rat	14 d 5d/wk 6hr/d	Hemato		10[a] (methemoglobinemia)	35 (incr. WBC's)	Medinsky and Irons 1985
6	Mouse	14 d 5d/wk 6hr/d	Other		10 (splenic hemosiderosis)	35 (lymphoid aplasia)	Medinsky and Irons 1985
Neurological							
7	Mouse	14 d 5d/wk 6hr/d				125[a] (cerebellar lesions)	Medinsky and Irons 1985
Developmental							
8	Rat	10 d Gd6-15 6hr/d		40			Tyl et al. 1987

TABLE 2-1 (Continued)

Figure Key	Species	Exposure Frequency/ Duration	Effect	NOAEL (ppm)	LOAEL (Effect) Less Serious (ppm)	LOAEL (Effect) Serious (ppm)	Reference
INTERMEDIATE EXPOSURE							
Systemic							
9	Rat	21 d 5d/wk 6hr/d	Other	50			Kligerman et al. 1983
10	Rat	90 d 5d/wk 6hr/d	Renal		5[a] (nephrosis)	50 (necrosis)	Hamm 1984
11	Rat	90 d 5d/wk 6hr/d	Other		5 (splenic hyperplasia)	50 (splenic lesions)	Hamm 1984
12	Rat	90 d 5d/wk 6hr/d	Hemato		5 (methemoglob-inemia)		Hamm 1984
13	Rat	90 d 5d/wk 6hr/d	Hepatic			50[a] (necrosis)	Hamm 1984
14	Mouse	90 d 5d/wk 6hr/d	Other		5 (adrenal lesions)		Hamm 1984
15	Mouse	90 d 5d/wk 6hr/d	Hemato			50 (methemoglob-inemia)	Hamm 1984
16	Rat	10 wk (2 gen) 5d/wk 6hr/d	Other	10			Dodd et al. 1987
Reproductive							
17	Rat	90 d 5d/wk 6hr/d				50[a] (testicular degeneration)	Hamm 1984

TABLE 2-1 (Continued)

Figure Key	Species	Exposure Frequency/ Duration	Effect	NOAEL (ppm)	LOAEL (Effect) Less Serious (ppm)	LOAEL (Effect) Serious (ppm)	Reference
18	Mouse	90 d 5d/wk 6hr/d		50			Hamm 1984
19	Rat	10 wk (2 gen) 5d/wk 6hr/d		10		40 (testic. lesions, decr. fertility)	Dodd et al. 1987

[a]Presented in Table 1-2.

LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; ppm = parts per million; d = day; LD_{50} = lethal dose, 50% mortality; wk = week; hr = hour; incr. = increase; wt = weight; Gd = gestation day; hemato = hematological; WBC's = white blood cells; gen = generation; testic. = testicular; decr. = decreased.

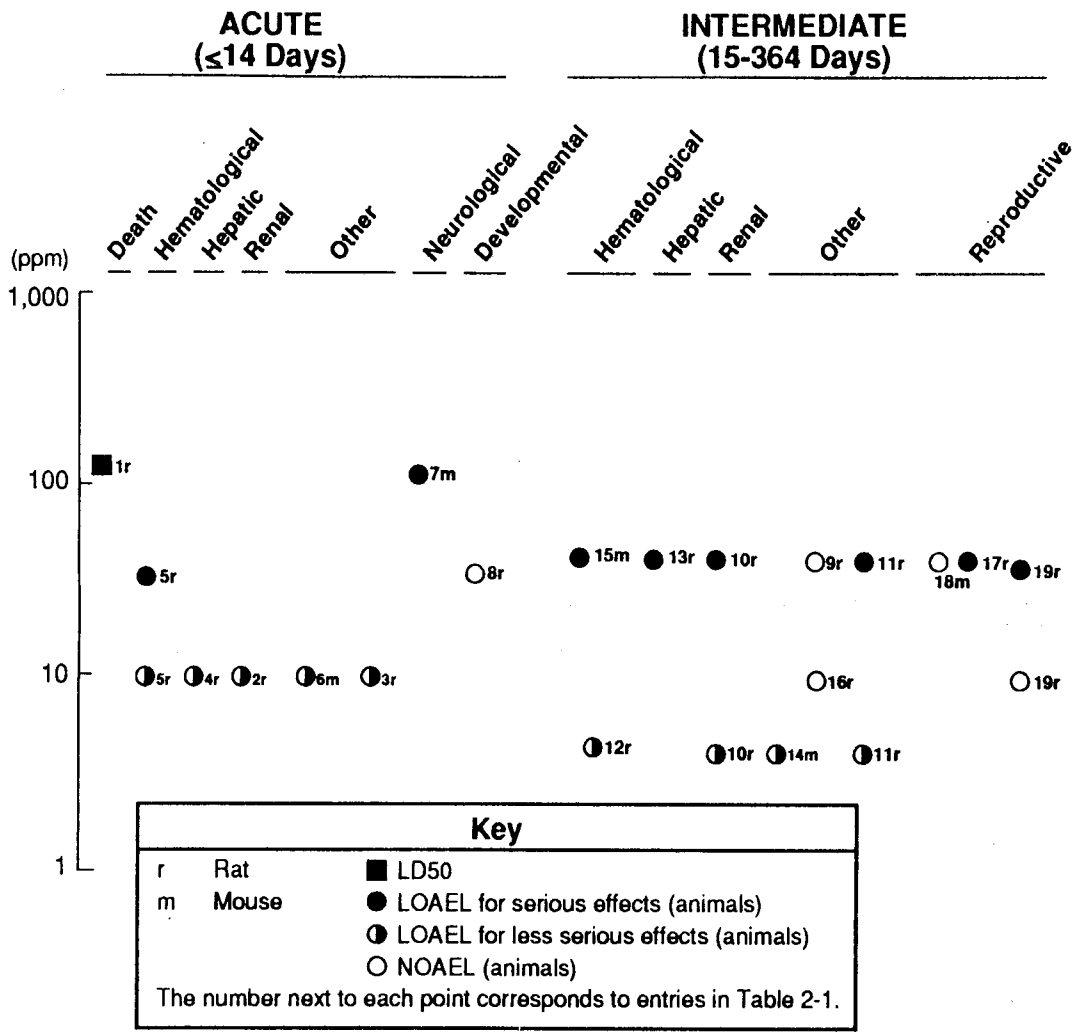

FIGURE 2-1. Levels of Significant Exposure to Nitrobenzene – Inhalation

2. HEALTH EFFECTS

Increased levels of blood methemoglobin have been reported in rate Exposed to nitrobenzene at levels as low as 10 ppm for two weeks Medinsky and Irons 1985) or 5 ppm for 90 days (Hamm 1984).

Hepatic Effects. There is some evidence that the human liver is Damaged after chronic inhaltion of nitrobenzene. The liver was Enlarged and tender and the results of liver function tests were Abnormal in a woman who was occupationally exposed to nitrobenzene for 17 months (exposure levels not measured or estimated) (Ikeda and Kita 1964).

Liver lesions reported in animals studies include hepatocyte Necrosis in male Sprague-Dawley (CD) rats exposed to nitrobenzene at 35 ppm for 2 weeks (Medinsky and Irons 1985) and increased liver weight, hepatocyte hyperlasia, and multinucleated hepatocytes in male B6C3F1 mice exposed to nitrobenzene at 16 ppm for 90 days (Hamm 1984).

Renal Effects. No studies were located regarding renal effects in Human after inhalation exposure to nitrobenzene.

Dose-related increases in kidney weights were observed in Fischer-344 rats (both sexes), but not in Sprague-Dawley (CD) rats Exposed to nitrobenzene at 10 to 125 ppm for 14 days (Medinsky and Irons 1985). At 125 ppm, hydropic degeneration of the cortical tubular cells was observed only in Sprague-Dawley rats (20% of males; 90% of females), and hyaline nephrosis only in Fischer-344 rats (100% of males; 20% of females). Renal effects reported in B6C3F1 mice in this study included minimal to moderate multifocal degenerative changes in tubular epithelium of males exposed to 35 ppm for 2 weeks. However, neither hydropic degeneration of the cortical tubular cells nor hyaline nephrosis was seen in mice even at the highest exposure level (125 ppm). Using the same three animal models exposed to nitrobenzene at 5 to 50 ppm for 90 days, dose-related real lesions were observed in both rat strains but not in mice (Hamm 1984).

Difference in species and possibly strain susceptibility to the Renal effects of nitrobenzene exposure may exist, but their relevance to The potential renal effects in humans is not clear. The occurrence of Renal effects in male rats, but not female rats or mice of either sex, in response to exposure to chemical toxicants is not unique to nitrobenzene. These differences have also been found with exposure to 1,4,-dichlorobenzene, isophorone, and unleaded gasoline (Charbonneau and Wwenberg 1988) and have been attributed to the production of high Concentrations of the protein alpha-2µ-globulin in the kidneys of male Rats, but not in female rats, mice, or humans. These observations Suggest that the severe renal effects observed in male rats exposed to Nitrobenzene will probably not occur in exposed humans.

Other Systemic Effects. No studies were located regarding cardiovascular, respiratory, gastrointestinal, musculoskeletal, dermal or ocular effects in humans or animals after inhalation exposure to nitrobenzene.

Dose-related splenic lesions have been reported to occur in B6C3Fl mice exposed to nitrobenzene at 10 to 125 ppm for 14 days (Medinsky and Irons 1985) and in F-344 and Sprague-Dawley (CD) rats at 5 to 50 ppm for 90 days (Hamm 1984). These lesions were described as sinusoidal congestion, an increase in extramedullary hematopoiesis and hemosiderin-laden macrophages infiltrating the red pulp, and the presence of proliferative capsular lesions. The results of these studies suggest that the spleen may also be a sensitive organ in cases of human inhalation exposure to nitrobenzene.

2.2.1.3 Immunological Effects

No studies were located regarding immunological effects in humans or animals after inhalation exposure to nitrobenzene. Splenic lesions observed in studies in rats and mice (see Section 2.2.1.2) suggest that potential immunologic effects may warrant further attention.

2.2.1.4 Neurological Effects

Neurological effects have been noted in the case of a woman who was occupationally exposed to nitrobenzene for 17 months at an unknown level. These effects included headache, nausea, vertigo, confusion, and paresthesia (Ikeda and Kita 1964).

Neurologic signs were not observed in mice or rats exposed to 5, 16, or 50 ppm nitrobenzene in air for 90 days. These animals were observed twice daily for clinical abnormalities (Hamm 1984).

When Sprague-Dawley (CD) rats and B6C3Fl mice were exposed to nitrobenzene at 125 ppm daily for two weeks, damage to the hindbrain (cerebellar peduncle), including bilateral cerebellar perivascular hemorrhage and malacia (cell breakdown), was observed in 8/19 mice (both sexes) and in 14/19 rats (both sexes) (Medinsky and Irons 1985). No brain lesions were found in Fischer rats exposed to the same levels. The reason for these strain differences under similar conditions is not apparent.

2.2.1.5 Developmental Effects

No studies were located regarding developmental effects in humans after inhalation of nitrobenzene.

Studies in animals indicate that inhalation exposure to nitrobenzene does not result in fetotoxic, embryotoxic or teratogenic effects at concentrations up to 40 ppm in rats (Tyl et al. 1987) and up to 100 ppm in rabbits (Bio/dynamics Inc. 1984). While the mean numbers of resorption sites and percentage of resorptions/implants in rabbits were higher in the 100 ppm group than in concurrent controls, these parameters were within the historical control range. However, animal data from these studies indicate that nitrobenzene is maternally toxic. In rats, spleen weights increased in the mothers (dams) at 10 ppm, and there was transient reduction in body weight gain in the 40 ppm group (Tyl et al. 1987). In rabbits, maternal effects noted at 40 ppm and above were increased methemoglobin levels and increased liver weights (Bio/dynamics Inc. 1983). Since no developmental toxic effects occurred in animals even at doses producing some maternal toxicity, developmental toxicity may not be a major concern in humans.

2.2.1.6 Reproductive Effects

No studies were located regarding reproductive effects in humans following inhalation exposure to nitrobenzene.

In a two-generation study in rats, nitrobenzene exposure (10 weeks) resulted in a decrease in fertility indices at 40 ppm for F_0 and F_1 generations, while other reproductive parameters were unaltered (Dodd et al. 1987). The study data suggested that the decrease was caused by effects in males. Atrophy of seminiferous tubules, spermatocyte degeneration and reduced testicular and epididymal weights were reported in F_0 and F_1 generations. A five-fold increase (above levels during exposure) in the fertility index was reported after 9 weeks of recovery from inhalation exposure to nitrobenzene, but reversibility was not studied histologically. Maternal toxicity was not observed. Hamm (1984) reported that both F-344 and Sprague-Dawley (CD) rats exposed to nitrobenzene at 50 ppm for 90 days had testicular atrophy, bilateral degeneration of the seminiferous tubules, and a reduction in or absence of mature sperm in the epididymis. No testicular lesions were observed in B6C3Fl mice under the same exposure conditions.

The testicular effects observed in these studies suggest that reproductive toxicity may be an area of concern for occupationally exposed humans.

2.2.1.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans after inhalation exposure to nitrobenzene.

Cytogenetic analyses of lymphocytes in the peripheral blood or in splenic blood of rats exposed to nitrobenzene at 5 to 50 ppm for 21 days did not reveal an increase in sister chromatid exchange (SCE) or chromosome breakage (Kligerman et al. 1983).

2.2.1.8 Cancer

No studies were located regarding cancer in humans or animals after inhalation exposure to nitrobenzene.

2.2.2 Oral Exposure

Table 2-2 and Figure 2-2 describe the health effects observed in laboratory animals associated with oral exposure to nitrobenzene at varying levels and exposure durations.

2.2.2.1 Death

Although the early literature describes many "poisonings" and deaths that were attributed to nitrobenzene ingestion, the lack of reliable chemical identification makes it impossible to determine the actual cause of death in some of these cases. In early case studies that describe such events, nitrobenzene may have been identified only by its odor; in other cases, aniline may have been identified in the stomach contents. Because nitrobenzene is reduced to aniline by the microflora in the intestines, the presence of aniline in the stomach may more reasonably be attributed to the ingestion of aniline. In addition, due to prompt and aggressive medical attention when these incidents have occurred, most of the available case studies report that the victim has survived. Therefore, firm conclusions cannot be drawn about the potential lethal effects of nitrobenzene ingestion by humans.

An LD_{50} of 600 mg/kg in rats was reported by Smyth et al. (1969).

2.2.2.2 Systemic Effects

Hematological Effects. When nitrobenzene is ingested, the outstanding systemic effect is methemoglobin formation. In this condition, the blood releases less oxygen to the tissues and all general body functions tend to be slowed down (WHO 1986). A latency period (after ingestion and before any signs or symptoms occur) can be as short as 30 minutes or as long as 12 hours. Usually, the higher the dose, the shorter the latency period.

TABLE 2-2. Levels of Significant Exposure to Nitrobenzene - Oral

Figure Key	Species	Route	Exposure Frequency/ Duration	Effect	NOAEL (mg/kg/day)	LOAEL (Effect) Less Serious (mg/kg/day)	LOAEL (Effect) Serious (mg/kg/day)	Reference
ACUTE EXPOSURE								
Systemic								
1	Rat	(G)	1x	Hemato	100	200[a] (methemoglobinemia)		Goldstein et al. 1984
Neurological								
2	Rat	(G)	1x				550[b] (malacia/ hemorrhage)	Morgan et al. 1985
Reproductive								
3	Rat	(G)	1x				300[c] (testic. necrosis, decr. sperm)	Levin et al. 1988

[a]Converted to an equivalent concentration of 4,000 ppm in food for presentation in Table 1-4.
[b]Converted to an equivalent concentration of 11,000 ppm in food for presentation in Table 1-4.
[c]Converted to an equivalent concentration of 6,000 ppm in food for presentation in Table 1-4.

LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; mg = milligrams; kg = kilograms; (G) = gavage; x = time; hemato = hematological; testic. = testicular; decr. = decreased

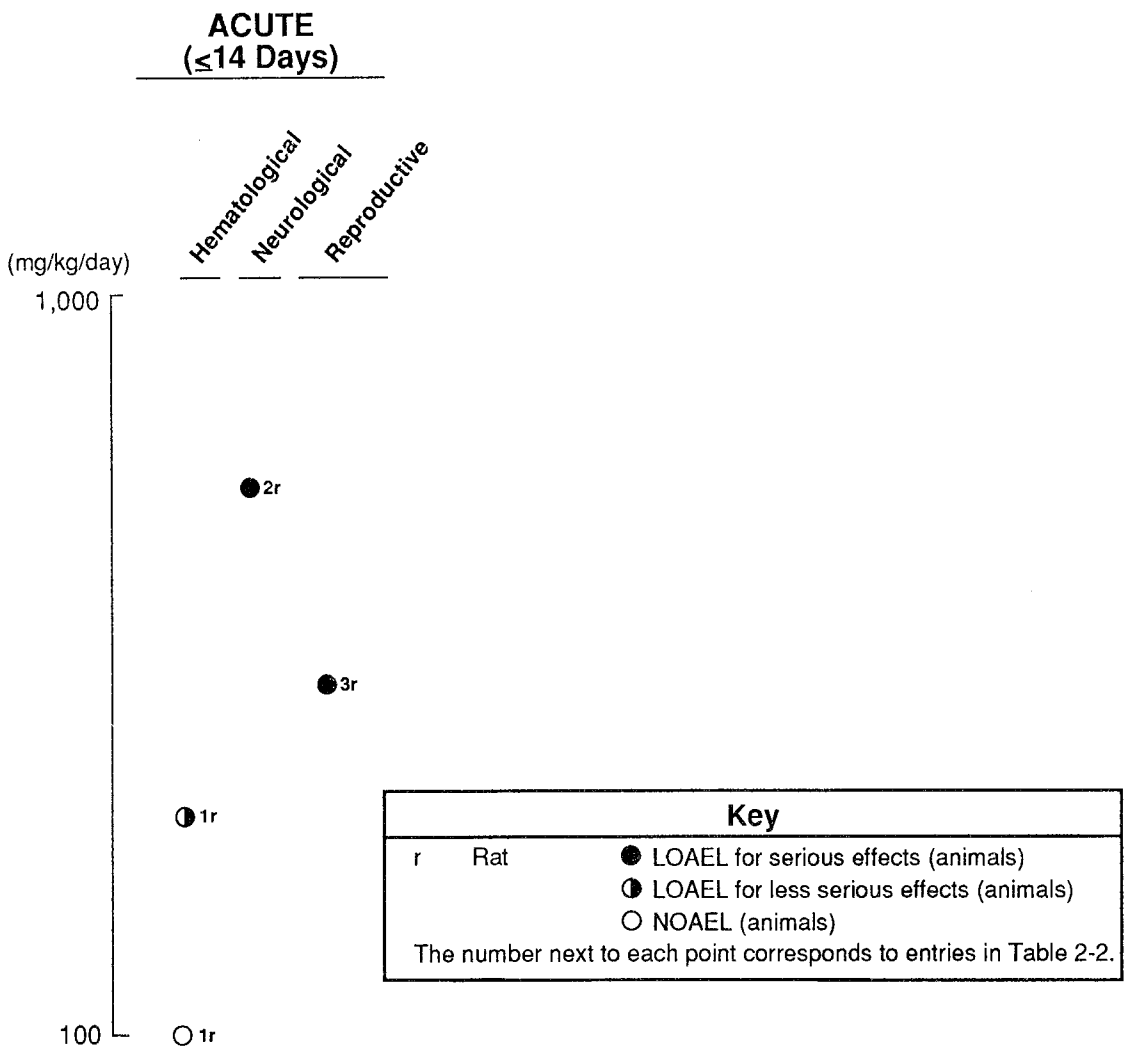

FIGURE 2-2. Levels of Significant Exposure to Nitrobenzene – Oral

No data are available to reliably estimate the level of human oral exposure to nitrobenzene that results in methemoglobinemia. Two of the case studies that were located indicate that some vague quantity, such as a few drops or a partial spoonful, was swallowed and part of that amount was vomited out before cyanosis and methemoglobinemia were observed (Carter 1936; Leader 1932). Another study (Myslak et al. 1971) estimated that a dose of 4.3 to 11 g was swallowed by a 19-year-old woman, based on the urinary levels of p-nitrophenol.

Oral administration of nitrobenzene to rats and mice results in methemoglobinemia (Goldstein et al. 1984a; Rickert 1984a).

The mouse is apparently more resistant to the methemoglobin forming properties of nitrobenzene than are other species (Shimkin 1939; Smith et al. 1967). The action of bacteria normally present in the small intestine of the rat is an important element in the formation of methemoglobin resulting from nitrobenzene exposure. Germ-free rats do not develop methemoglobinemia when orally administered nitrobenzene (Reddy et al. 1976). This observation leads to the speculation that a nitrobenzene metabolite such as aniline (which is formed by the bacterial reduction of nitrobenzene in the intestines of rats) may be involved in methemoglobin formation in this species. In addition, diet has been shown to play a role in the production of methemoglobin by influencing the intestinal microflora. The presence of cereal-based pectin in the diets of rats was shown to increase the ability of orally administered nitrobenzene to induce methemoglobinemia (Goldstein et al. 1984a).

Other Systemic Effects. No studies were located regarding hepatic, respiratory, cardiovascular, gastrointestinal, renal, musculoskeletal, dermal, or ocular effects in humans or animals after oral exposure to nitrobenzene.

2.2.2.3 Immunological Effects

No studies were located regarding immunological effects in humans or animals after oral exposure to nitrobenzene.

2.2.2.4 Neurological Effects

Neurological effects following nitrobenzene ingestion by humans have been reported as headache, nausea, vertigo, confusion, unconsciousness, apnea and coma (Carter 1936; Leader 1932; Myslak et al. 1971). Levels of nitrobenzene associated with these effects cannot be reliably estimated in most of the case studies from which these descriptions have been derived.

Brain pathology was reported after a single oral administration of nitrobenzene at 550 mg/kg to male rats (Morgan et al. 1985). Observations included petechial hemorrhages in the brain stem and cerebellum and malacia (cell breakdown) in the fourth ventricle.

2.2.2.5 Developmental Effects

No studies were located regarding developmental effects in humans or animals after oral exposure to nitrobenzene.

2.2.2.6 Reproductive Effects

No studies were located regarding reproductive effects in humans after oral exposure to nitrobenzene.

No single or multigeneration reproduction studies in animals were found. However, an acute systemic study in rats indicated that the testes are sensitive to the toxic effects of nitrobenzene. Typical signs included testicular degeneration and transiently decreased sperm production following a single oral dose of 300 mg/kg (Levin et al. 1988).

Although no human studies were found and animal reproduction studies have not been performed by oral administration, the testicular degeneration in rats reported by Levin et al. (1988) suggests that reproductive toxicity may be of concern in exposed humans.

2.2.2.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans after oral exposure to nitrobenzene.

Rats gavaged with nitrobenzene at 200 or 500 mg/kg were tested for unscheduled DNA synthesis in liver slices (Mirsalis et al. 1982). No significant increase in DNA synthesis was found.

2.2.2.8 Cancer

No studies were located regarding cancer in humans or animals after oral exposure to nitrobenzene.

2.2.3 Dermal Exposure

Cases of severe and nearly lethal toxic effects after dermal exposure to aniline-based dyes have been reported as early as 1886. These cases have involved mainly infants exposed to dye-stamped diapers and persons wearing freshly dyed shoes. The resulting condition was often termed "nitrobenzene poisoning", even though exposure to

nitrobenzene did not necessarily occur. Several conclusions and generalizations about the dermal absorption and toxic effects of nitrobenzene, especially in infants, seem to have been based on these studies which should more appropriately be considered as part of the data base for aniline.

2.2.3.1 Death

No studies were ocated regarding death in humans after dermal exposure to nitrobenzene.

Dermal applications of nitrobenzene to female C3H or male A-strain mice resulted in the death of 12 of 18 and 8 of 10 animals, respectively (Shimkin 1939). Although 2 or 3 applications were required for the C3H mice, most animals were in partial collapse within 15 minutes and dead by the third day. Most of the strain-A mice were dead within the first day. The dermal dosage was not stated.

2.2.3.2 Systemic Effects

Hematological Effects. No studies were located regarding hematological effects in humans after dermal exposure to nitrobenzene. Dermal painting of C3H female or strain-A male mice with nitrobenzene resulted in methemoglobinemia by 3 hours after application (Shimkin 1939).

Hepatic Effects. No studies were located regarding hepatic effects in humans after dermal exposure to nitrobenzene. In mice dermally exposed to nitrobenzene, the liver was the most severely affected organ. There was diffuse necrosis in the outer two thirds of the lobules of the liver (Shimkin 1939).

Renal Effects. No studies were located regarding renal effects in humans after dermal exposure to nitrobenzene.

When mice were dermally painted with nitrobenzene for 1 to 3 applications, there was slight swelling of the glomeruli and tubular epithelium upon histological examination (Shimkin 1939).

Other Systemic Effects. No studies were located regarding other systemic effects (respiratory, cardiovascular, gastrointestinal, musculoskeletal, splenic, dermal, ocular) in humans or animals after dermal exposure to nitrobenzene.

No studies were located regarding the following health effects in humans or animals after dermal exposure to nitrobenzene.

2.2.3.3 Immunological Effects

2.2.3.4 Neurological Effects

2.2.3.5 Developmental Effects

2.2.3.6 Reproductive Effects

2.2.3.7 Genotoxic Effects

2.2.3.8 Cancer

2.3 TOXICOKINETICS

2.3.1 Absorption

2.3.1.1 Inhalation Exposure

In humans, nitrobenzene was well absorbed through the lung in the one study located. During a 6-hour exposure of volunteers to nitrobenzene, Salmowa et al. (1963) found absorption to average 80% (73 to 87%) in 7 men breathing 6 ppm nitrobenzene. The efficiency of uptake was dose dependent, but showed considerable individual variation.

No studies were located regarding the uptake of nitrobenzene by animals after inhalation exposure.

2.3.1.2 Oral Exposure

No studies were located regarding the uptake of nitrobenzene by humans after oral exposure.

After oral administration of 250 mg/kg of nitrobenzene by stomach tube to rabbits, Parke (1956) recovered 0.5% (1.3 mg) of the administered dose of nitrobenzene from the exhaled air of the rabbit. The amount of nitrobenzene in the blood was not measured. Unchanged nitrobenzene in the urine was less than 0.1% (0.25 mg).

2.3.1.3 Dermal Exposure

The toxicokinetics of dermal exposure have not been well studied in either humans or experimental animals. Piotrowski (1967) found that approximately half of the dose of nitrobenzene was absorbed through the skin when volunteers were exposed to either 1 or 5.5 ppm nitrobenzene in air.

In animal studies, nitrobenzene appears to be absorbed after dermal application based on observations of toxic responses in the treated animals. Shimkin (1939) reported that dermal painting of mice with liquid nitrobenzene (dose not stated) resulted in the death of most test animals after 1 to 3 applications. Dermal exposure of rabbits to nitrobenzene (dose not stated) for 22 to 205 days resulted in greater neural damage than did intravenous exposure (Matsumaru and Yoshida 1959). Administration of alcohol (not further identified) by stomach tube following exposure to nitrobenzene resulted in neurotoxicity by both routes of exposure.

2.3.2 Distribution

2.3.2.1 Inhalation Exposure

No studies of the distribution of nitrobenzene or its metabolites after inhalation exposure by humans or animals were found in the literature.

2.3.2.2 Oral Exposure

Radiolabeled nitrobenzene has been followed after oral administration in a number of studies in rats and mice (Goldstein and Rickert 1984; Levin and Dent 1982a; Morgan et al. 1985). In summary, these studies have shown that nitrobenzene is reduced to nitrosobenzene, phenylhydroxylamine, and aniline by the bacteria of the intestine. Metabolism of nitrobenzene resulted on covalent binding to the hepatic microsomes. Nitrosobenzene and phenylhydroxylamine were bound to the hemoglobin. Unaltered nitrobenzene was recovered from the brain at a rate of 0.02% of the administered dose. The subcellular site of nitrobenzene metabolism was not found. The major urinary metabolites were p-aminophenol and p-nitrophenol together with the sulfate and glucuronide conjugates.

In a study of rats that received radiolabeled nitrobenzene by gavage, it became bound to the tissues after the first day according to the following indices [mmol/mol Hb/dose (mmol/kg)]: blood-229, liver-129, kidney-204, lung-62. BY day 7, the same indices were: 134, 26.5, 48, 29. After the first day, 50% of the dose (radioactivity) appeared in the urine and 4% in the feces. After the fifth day, 65% of the dose had appeared in the urine and 16% appeared in the feces (Albrecht and Neumann 1985). These studies confirmed the observation of Rickert et al. (1983), that the excretion of nitrobenzene is delayed. The binding indices also indicated that 4 to 5 times as much nitrosobenzene is formed from nitrobenzene as from an equal amount of aniline.

2.3.2.3 Dermal Exposure

No studies were located regarding the distribution of nitrobenzene or its metabolites after dermal exposure.

2.3.3 Metabolism

The covalent metabolism and binding of nitrobenzene to hemoglobin was studied by Albrecht and Neumann (1985). When Wistar rats were administered 25 mg/kg radiolabeled nitrobenzene by gavage, biotransformation was first seen in the intestine where nitrobenzene was sequentially reduced to nitrosobenzene, phenylhydroxylamine and aniline. These findings were also reported in Fischer-344 rats (Levin and Dent 1982a). The observation that germ-free rats do not develop methemoglobinemia when administered nitrobenzene (Reddy et al. 1976) has led to the speculation that a nitrobenzene metabolite such as aniline may be involved in methemoglobinemia formation in this species. The nitrobenzene metabolites, nitrosobenzene and phenylhydroxylamine have been found to bind with hemoglobin in the blood of orally exposed mice and rats (Goldstein and Rickert 1984).

2.3.4 Excretion

2.3.4.1 Inhalation Exposure

Urinary excretion rates of p-nitrophenol were found in 7 volunteers who had inhaled 6 ppm nitrobenzene for 6 hours (Salmowa et al. 1963). The rate of urinary elimination varied considerably from individual to individual, but showed a general dose dependence at 1 to 6 ppm nitrobenzene. In general, excretion was most rapid during the first two hours and then leveled off. In some cases, p-nitrophenol could be detected for as long as 100 hours after exposure to 6 ppm for 6 hours. In a 47-year-old woman who had been occupationally exposed to nitrobenzene for 17 months, p-nitrophenol and p-aminophenol were found in the urine (Ikeda and Kita 1964).

2.3.4.2 Oral Exposure

After oral exposure to nitrobenzene, the major route of excretion is the urine. In most cases of human poisoning, the metabolites excreted in the urine are p-aminophenol and p-nitrophenol (Myslak et al. 1971; Von Oettingen 1941). Five days after oral administration to rats, Albrecht and Neumann (1985) found 65% of the administered dose (25 mg/kg) in the urine and 16% in the feces.

2.3.4.3 Dermal Exposure

A unique apparatus was developed to measure skin absorption from the nitrobenzene vapor in the air without inhalation of nitrobenzene (Piotrowski 1967). At 1 ppm, about 8 mg is absorbed through the skin and about 20% is excreted in the urine the first day.

2.4 RELEVANCE TO PUBLIC HEALTH

Studies in animals, combined with observations in humans, indicate that the principal adverse health effects associated with short-term inhalation or oral exposure to nitrobenzene are methemoglobinemia, neurological effects, and liver injury. Data related to dermal exposure and to long-term exposure by any route are not considered sufficient to clearly assess the potential effects.

Death. Accidental poisonings and deaths in humans that were attributed to the ingestion of nitrobenzene have been reported; but as discussed in Section 2.2.2.1, these studies usually lack clear chemical identification of nitrobenzene as the ingested substance. In those inhalation case studies (Stevens 1928; Stevenson and Forbes 1942) and oral case studies (Carter 1936; Leader 1932; Myslak et al. 1971) in which the patients were apparently near death due to severe methemoglobinemia, termination of exposure and prompt medical intervention resulted in gradual improvement and recovery. Data relating to dermal exposure to nitrobenzene, as discussed previously in Section 2.2.3, are questionable since there may have been exposure to aniline-based dyes and little or no exposure to nitrobenzene. Data in animals indicate that nitrobenzene can be lethal via oral, inhalation or dermal exposure. Although human exposure to sufficiently high quantities of nitrobenzene can probably be lethal via any route of exposure, it is considered unlikely that levels of exposure high enough to cause death would occur except in cases of industrial accidents.

Systemic Effects. The chief systemic effect associated with human exposure to nitrobenzene is methemoglobinemia. However, it is difficult to locate clear evidence of this effect, since nitrobenzene was identified only by its odor in several early case studies. Methemoglobinemia was reported to occur in twin 3-week-old babies (Stevens 1928), in a 12-month-old girl (Stevenson and Forbes 1942), and in a 47-year-old woman (Ikeda and Kita 1964), all of whom were exposed to nitrobenzene via inhalation. However, levels of exposure were neither known nor estimated. In addition, the compound to which the 12-month-old girl was exposed also contained kerosene, turpentine, and oil of lilacine. The 3-week-old twins were exposed to nitrobenzene in a toilet deodorant called "Creco". No other ingredients were stated in the study. Oral exposure to nitrobenzene at unspecified amounts has

also resulted in methemoglobinemia (Carter 1936; Leader 1932; Myslak et al. 1971). There is no clear evidence that dermal exposure to nitrobenzene results in methemoglobinemia in humans. Reports of methemoglobinemia resulting in dermal contact with dyes allegedly containing nitrobenzene are complicated by the early confusion in nomenclature for aniline and nitrobenzene.

Methemoglobinemia has also been reported in mice and rats exposed to nitrobenzene via inhalation (Hamm 1984) and in rats and mice exposed orally (Goldstein et al. 1984a; Rickert 1984a). Dermal painting studies in mice resulted in the onset of methemoglobinemia within 3 hours after nitrobenzene application (level not stated) (Shimkin 1939). This finding suggests that methemoglobinemia may also occur in dermally exposed humans.

Liver effects have been reported in both humans and animals exposed to nitrobenzene. Hepatic enlargement and tenderness, jaundice, and altered serum chemistries were reported in a 47-year-old woman who had been occupationally exposed to nitrobenzene for 17 months (Ikeda and Kita 1964). The authors considered these changes to be related to increased destruction of hemoglobin and enlargement of the spleen. Liver effects observed in animals following nitrobenzene exposure are hepatocyte necrosis in rats (Medinsky and Irons 1985) and increased liver weight, hepatocyte hyperplasia, and multinucleated hepatocytes in mice (Hamm 1984). Hepatic effects have not been reported in oral studies. Dermal painting studies in mice resulted in diffuse necrosis in the outer two-thirds of the lobules of the liver (Shimkin 1939).

There are no data on renal effects in humans exposed to nitrobenzene by any route. In rats, strain-related differences in renal effects have been reported as a result of inhalation exposure to nitrobenzene (Hamm 1984; Medinsky and Irons 1985). Observed effects have included increased kidney weights, hydropic degeneration of the cortical tubules and hyaline nephrosis. Renal effects have not been reported in studies of animals that were orally exposed to nitrobenzene. In dermal painting studies in mice, slight swelling of the glomeruli and tubular epithelium were reported (Shimkin 1939). These findings suggest that renal damage may also occur in exposed humans.

Splenic lesions reported in inhalation studies in mice and rats have included sinusoidal congestion, an increase in extramedullary hematopoiesis and hemosiderin-laden macrophages invading the red pulp, and the presence of proliferative capsular lesions (Hamm 1984; Medinsky and Irons 1985). These findings suggest that the spleen may also be a target organ during human inhalation exposure to nitrobenzene.

2. HEALTH EFFECTS

Little information is available on the effects of inhalation, oral or dermal exposure of humans or animals to nitrobenzene on the respiratory, cardiovascular or musculoskeletal systems or on the skin or eyes.

Immunological Effects. No studies were located regarding immunologic effects in humans or animals after inhalation, oral, or dermal exposure to nitrobenzene. Splenic lesions reported in rodent inhalation studies (Hamm 1984; Medinsky and Irons 1985) suggest that this may be an area of potential concern.

Neurological Effects. Neurotoxic symptoms reported in humans after inhalation exposure to nitrobenzene have included headache, confusion, vertigo and nausea (Ikeda and Kita 1964); effects in orally exposed persons have also included those symptoms as well as apnea and coma (Carter 1936; Leader 1932; Myslak et al. 1971). Studies in animals exposed via inhalation have shown morphological damage to the hindbrain (cerebellar peduncle) (Medinsky and Irons 1985). Damage to the brainstem, cerebellum and fourth ventricle was observed in orally exposed animals. Thus, it is possible that similar neurological changes may occur in humans as a result of nitrobenzene exposure.

Developmental Effects. No studies of developmental effects in humans resulting from inhalation, oral or dermal exposure to nitrobenzene have been reported. Studies conducted via inhalation exposure did not result in fetotoxic or teratogenic effects in rats or rabbits (Bio/dynamics 1984; Tyl et al. 1987). No studies have been conducted using the oral or dermal routes. Developmental effects are not expected to be of concern to humans exposed to the typical levels in the environment or in occupational settings.

Reproductive Effects. The effects of nitrobenzene on reproduction have not been studied in humans by inhalation, oral or dermal routes of exposure. In rats, inhalation of nitrobenzene has resulted in testicular degeneration and decreased sperm levels (Dodd et al. 1987; Hamm 1984). Cessation of spermatogenesis, followed by a slow and incomplete recovery, was observed in rats following a single oral dose of nitrobenzene (Levin et al. 1988). These findings suggest that reproductive effects may also be an area of concern for men exposed to nitrobenzene in occupational settings.

Genotoxic Effects. The genotoxicity of nitrobenzene has been investigated in both in vitro and in vivo studies. The results of in vitro studies are presented in Table 2-3. In vivo studies are described in Sections 2.2.1.7 and 2.2.2.7. The results of these studies are generally negative and do not suggest potential human health concerns.

TABLE 2-3. Genotoxicity of Nitrobenzene In Vitro

End Point	Species (Test System)	Results: With Activation	Results: Without Activation	Reference
Prokaryotic organisms:				
Gene mutation	Salmonella typhimurium	-	-	Garner and Nutman 1977
Gene mutation	S. typhimurium	-	-	Shimizu et al. 1983
Gene mutation	S. typhimurium	-	ND	Ho et al. 1981
Gene mutation	S. typhimurium	-	-	Haworth et al. 1983
Gene mutation	S. typhimurium	-	ND	Anderson and Styles 1978
Gene mutation	S. typhimurium	-	-	Hughes et al. 1984

- = negative result; ND = no data.

Cancer. No studies were located regarding carcinogenic potential in humans or animals after inhalation, oral, or dermal exposure to nitrobenzene.

2.5 BIOMAREERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have been eliminated from the body by the time biologic samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc and selenium). Biomarkers of exposure to nitrobenzene are discussed in Section 2.5.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are often not substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by nitrobenzene are discussed in Section 2.5.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic. It can be an intrinsic genetic or

other characteristic or a preexisting disease that results in an increase in absorbed dose, biologically effective dose, or target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 2.7, "POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE."

2.5.1 Biomarkers Used to Identify and/or Quantify Exposure to Nitrobenzene

The presence of p-nitrophenol in the urine can be used to indicate exposure to nitrobenzene (Ikeda and Kita 1964). Measurement of p-nitrophenol, however, cannot be used to determine the level of nitrobenzene exposure or if harmful effects can be expected to occur. The nitrobenzene metabolites, nitrosobenzene and phenylhydroxylamine, have been found to bind with hemoglobin in the blood of orally exposed mice and rats (Goldstein and Rickert 1984). The presence of these hemoglobin adducts in human blood may also serve as a potential biomarker of exposure to nitrobenzene.

2.5.2 Biomarkers Used to Characterize Effects Caused by Nitrobenzene

The presence of methemoglobinemia can indicate exposure to nitrobenzene as well as to any of several other toxic substances. Therefore, this condition in itself cannot be used as a biomarker of effect for nitrobenzene.

2.6 INTERACTIONS WITH OTHER CHEMICALS

Synergism between orally administered nitrobenzene and six other common industrial compounds was demonstrated in rat studies using dea as the end point (Smyth et al. 1969). The combinations of chemicals showed increased lethality that varied from 20 to 47%. The compounds were: formalin, 20%; butyl ether, 28%; aniline, 32%; dioxane, 39%; acetone, 47%; and carbon tetrachloride, 47%.

Alcohol also has the potential for enhancing the toxicity of nitrobenzene; however the toxicokinetic mechanism is not known. It is clear, however, that alcohol does not simply enhance the absorption of nitrobenzene. When alcohol was given orally and nitrobenzene is given intravenously, there was increased toxicity in rabbits. Alcohol also enhanced the neural toxicity of nitrobenzene in rabbits when nitrobenzene was applied to the skin (Matsumaru and Yoshida 1959).

2.7 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

Populations that are considered unusually susceptible to nitrobenzene toxicity are those groups that are susceptible to methemoglobinemia. The newborn infant is especially vulnerable to methemoglobinemia due to the following factors (Goldstein et al. 1969; Von Oettingen 1941):

1. Fetal hemoglobin, which remains in the blood for some time after birth, is more prone to conversion to methemoglobin than is adult hemoglobin.

2. Umbilical cord blood is deficient in the enzyme glucose-6-phosphate dehydrogenase and thus cannot readily convert the methemoglobin that is formed "naturally" back to hemoglobin as is readily done in adults.

A condition described as "hereditary methemoglobinemia" may result from a genetic defect (Goldstein et al. 1969). The enzyme methemoglobin reductase is absent and persons are hypersensitive to any substances such as nitrite or aniline derivatives capable of producing methemoglobinemia. The trait is inherited as an autosomal recessive allele. Thus either sex may exhibit the trait which is ordinarily detected by the presence of cyanosis at birth. Such individuals would be extremely sensitive to the effects of nitrobenzene.

A more common genetic defect was also described in which the enzyme glucose-6-phosphate dehydrogenase has decreased activity (Goldstein et al. 1969). The pattern of inheritance of this trait is linked to one of several alleles on the X chromosome. The phenotype is expressed as an incomplete dominant trait. Thus, female heterozygotes are not known to have severely depressed enzyme levels and males may have a wide range of activity. These phenotypes express a wide range of levels of glucose-6-phosphate dehydrogenase enzyme in the red blood cell. This defect is ordinarily without adverse effects. It is only when these individuals are challenged with compounds that oxidatively stress erythrocytes (such as primaquine) that there is a hemolytic response. Reactors to primaquine (and fava beans) are found predominantly among groups that live in or trace their ancestry to malaria-hyperendemic areas such as the Mediterranean region or Africa. The incidence of "primaquine sensitivity" among Kurds, a Middle Eastern population, is 53%. Among American blacks, the incidence is 13%. Thus, individuals already exhibiting primaquine sensitivity would be expected to be more vulnerable to the additional hemolytic crisis that often follows 5 to 6 days after nitrobenzene exposure (Gosselin et al. 1984; Von Oettingen 1941).

The presence of susceptible populations in the workplace is obviously of great concern since chronic and potentially high levels of exposure to nitrobenzene combined with a genetic predisposition toward methemoglobinemia can put certain individuals at very high risk (Linch 1974).

2.8 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of nitrobenzene is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of nitrobenzene.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that, if met would reduce or eliminate the uncertainties of human health assessment. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

2.8.1 Existing Information on Health Effects of Nitrobenzene

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to nitrobenzene are summarized in Figure 2-3. The purpose of this figure is to illustrate the existing information concerning the health effects of nitrobenzene. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not imply anything about the quality of the study or studies. Gaps in this figure should not be interpreted as "data needs" information.

2.8.2 Identification of Data Needs

Acute-Duration Exposure. Case studies of acute-duration human exposure to nitrobenzene via inhalation and the oral route indicate that methemoglobinemia is the major adverse effect found in humans. No data are available on human dermal exposure. Acute-duration studies conducted in rats via the inhalation and oral routes and in mice via the dermal route have also resulted in methemoglobinemia as well as various other systemic, neurological, and testicular effects. The data are not considered to be appropriate to use in calculating an MRL by any route because species- and strain-related differences in sensitivity have been noted in intermediate-duration inhalation studies in mice and rats, and

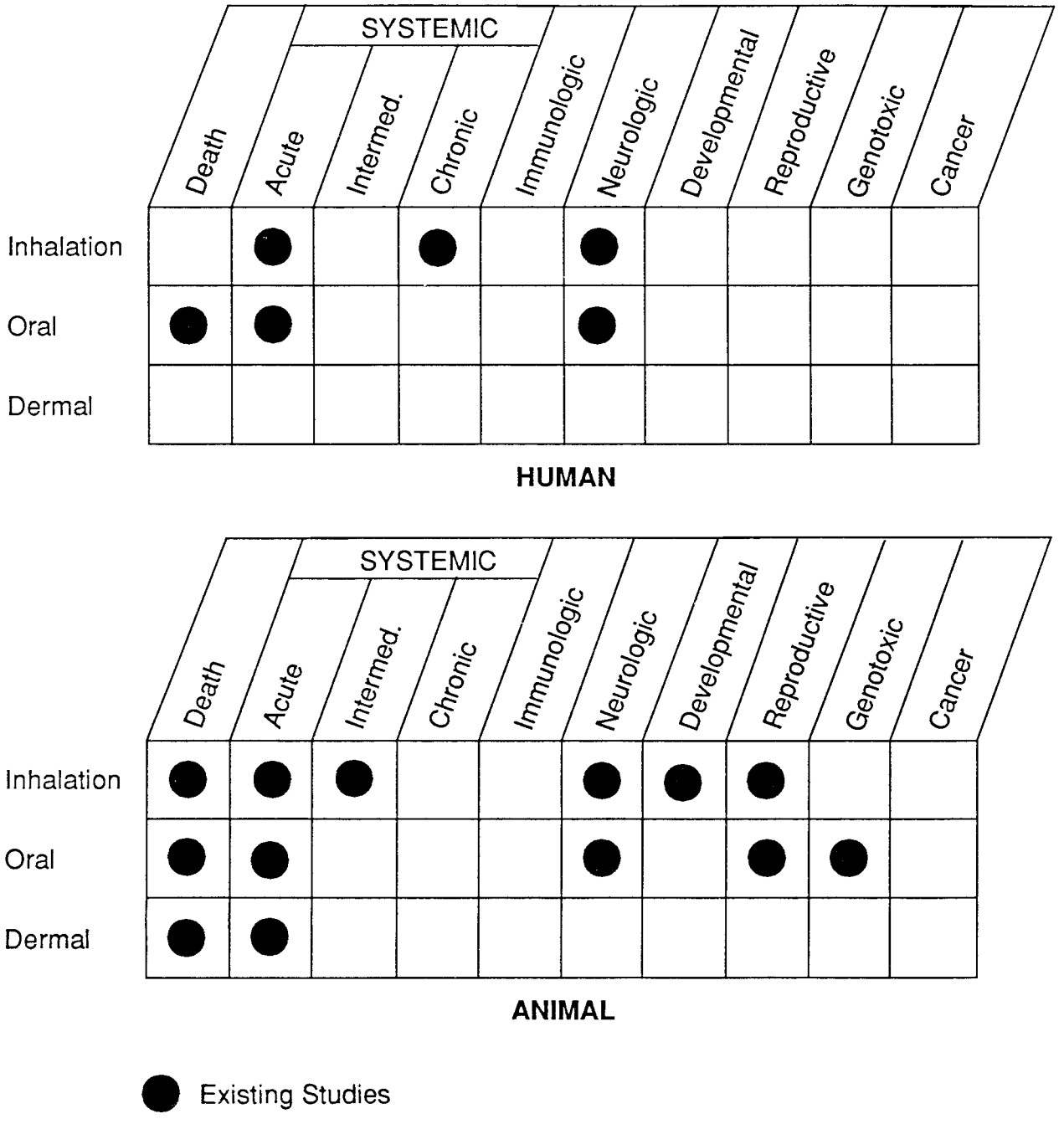

FIGURE 2-3. Existing Information on Health Effects of Nitrobenzene

it is possible that human sensitivity to methemoglobin formation may greatly exceed that of the test animals used in these studies. The available toxicokinetic data do not provide insight as to the possible reasons for the observed differences in rodent studies. However, there is no apparent need for further animal studies by any route of exposure for this duration period.

Intermediate-Duration Exposure. No data are available on human exposure to nitrobenzene by any route for this duration period. Data in animals are limited to two inhalation studies in rodents. Effects in rats included methemoglobinemia, renal and hepatic necrosis, splenic lesions, and testicular necrosis. Methemoglobinemia and adrenal lesions were reported in mice. The available data were not considered to be adequate to use in calculating an MEL for this route, because the database is very limited and because the different levels of sensitivity observed in mice and rats suggest that relative human sensitivity should be closely studied before calculations of MELs are attempted. There are no toxicokinetic data that provide a potential explanation for these differences. However, there is no apparent need for further inhalation studies for this duration period. A 90-day study via the oral route may provide useful information. However, the available information does not clearly establish that oral or dermal exposure to nitrobenzene are likely to occur in humans.

Chronic-Duration Exposure and Cancer. Available chronic-duration studies in humans are limited to a case report of a woman who was occupationally exposed to nitrobenzene and developed methemoglobinemia and hepatic and neurological effects. Chronic duration data in animals are limited to a two-generation inhalation study in rats that resulted in testicular lesions. No studies using the oral or dermal routes have been located and the available data are not considered appropriate to use in calculating an MRL by any route. The results of a Chemical Industry Institute of Toxicology (CIIT) inhalation bioassay using rats and mice which was completed in 1987 should provide useful information on the potential risks associated with chronic exposure to nitrobenzene in the vicinity of hazardous waste sites and, to a greater extent, in the workplace. Although nitrobenzene appears to be well absorbed via the oral route, there are no data to suggest that long-term oral exposure would be of concern in these populations. Dermal absorption of nitrobenzene in the air has been demonstrated in toxicokinetic studies in humans. Based on the results of the CIIT two-year inhalation study, chronic-duration dermal application studies may be useful in assessing the possible effects of dermal contact with nitrobenzene in the workplace.

There are no available carcinogenicity data in humans or animals using any route of exposure. Data from the CIIT inhalation bioassay should provide valuable information on the carcinogenic potential of airborne nitrobenzene. There is currently no apparent need for studies using the oral or dermal route. However, as stated above, the results of the CIIT bioassay may provide insight into the possible need for dermal application studies.

Genotoxicity. There are no data on the genotoxic potential of nitrobenzene in humans exposed via any route. The results of in vivo tests in rats exposed via inhalation or orally and in vitro tests have generally been negative and do not suggest a potential concern for exposed humans. Further studies in this area do not appear to be needed.

Reproductive Toxicity. There are no data on the potential reproductive effects in humans exposed to nitrobenzene via any route. Data in animals include a 90-day inhalation study in rats that resulted in testicular degeneration, a two-generation inhalation study in rats that resulted in testicular lesions and decreased fertility, and a single-dose gavage study in rats that resulted in testicular necrosis and temporarily decreased sperm levels. These data suggest that similar information on men exposed to nitrobenzene in the area of hazardous waste sites or in the workplace would be extremely useful. In addition, in any further animal studies conducted by any route and for any duration period, data on reproductive organ histopathology resulting from nitrobenzene exposure as well as toxicokinetic data on the distribution of nitrobenzene to the reproductive organs would be valuable information.

Developmental Toxicology. There are no available data on the developmental effects of nitrobenzene in humans exposed via any route for any duration period. The results of inhalation studies in rats and rabbits have been negative. There is no apparent need for further studies in this area. However, if any further developmental studies are conducted, it would be useful to have data on animals exposed earlier in the gestation period than day 6 (which was the earlier gestation day in the two available inhalation studies).

Immunotoxicity. No studies were located relating to immunotoxic effects in humans or animals exposed to nitrobenzene via any route. However, splenic lesions have been reported in mice and rats exposed to nitrobenzene via inhalation in both acute- and intermediate-duration studies. These results suggest that a battery of immune function tests in animals exposed via inhalation and orally would provide useful information. The results of the CIIT bioassay, however, may provide some information on this end point.

Neurotoxicity. Neurological effects including headache, nausea, vertigo, and confusion have been reported in case studies of humans exposed to nitrobenzene by inhalation. In orally exposed persons, apnea and coma have additionally been reported. No data are available in humans exposed via the dermal route. In animal studies, brain lesions have been observed in mice and rats exposed by inhalation and in rats that received a single oral dose. No data are available in animals exposed via the dermal route. Toxicokinetic studies in mice and rats provide evidence that nitrobenzene is distributed to brain tissue. Both the human and animal data provide clear evidence that nitrobenzene is a neurotoxic substance. Further studies in this area do not appear to be needed. In addition, results of the CIIT two-year bioassay may provide further information on this end point.

Epidemiological and Human Dosimetry Studies. No epidemiological studies were located regarding human health effects from nitrobenzene exposure. Studies of occupationally exposed populations would probably provide useful information. Areas of major interest would include methemoglobin levels, effects on reproductive function, immunological status, and neurobehavioral function.

Biomarkers of Exposure and Effect. The presence of p-nitrophenol in urine serves as a satisfactory biomarker of nitrobenzene exposure. Because nitrobenzene metabolites, nitrosobenzene and phenylhydroxylamine, bind to hemoglobin in the blood of rats and mice, the presence of these hemoglobin adducts in human blood may also serve as biomarkers of nitrobenzene exposure. More information on this possibility would be useful.

The presence of increased levels of methemoglobin can indicate exposure to nitrobenzene as well as to any of several other toxic substances. Therefore, methemoglobinemia by itself would not serve as a satisfactory biomarker of effect for nitrobenzene. Further study in this area does not appear to be potentially useful.

Absorption, Distribution, Metabolism, Excretion. Absorption data for humans exposed to nitrobenzene via inhalation and the dermal route indicate that it is efficiently absorbed by these routes. Although absorption studies using the oral route have not been located for humans, the available case studies suggest that it can also be absorbed via ingestion. However, quantitative data are lacking. Similarly, in animals, quantitative absorption studies using inhalation or dermal application are not available, but the available toxicity data using these routes suggest that absorption does take place. This does not appear to be a priority area for further research.

No distribution data are available for humans exposed to nitrobenzene via any route. Data in animals are limited to oral studies in rats and mice that indicate that there is some distribution to the blood, liver, brain, kidney, and lung. Not all tissues have been analyzed in these studies. Comprehensive distribution studies for nitrobenzene administered to mice and rats via all three routes would be very helpful in predicting the organ systems at potential risk in exposed humans.

Metabolism data available for nitrobenzene suggest that species and/or strain differences in toxicity may be related to the metabolic activities of intestinal bacteria that convert it to its toxic metabolite aniline. This is an area in which further study may be helpful in making comparisons of human sensitivity with that of other animals and thus may aid in the interpretation of the currently available animal studies and their relevance to humans.

Excretion data are available for humans exposed to nitrobenzene via the inhalation, oral, and dermal routes. The available animal studies have used the oral route. Urine appears to be the major route of excretion, although this has not been clearly established. There is no apparent need for further studies in this area.

Comparative Toxicokinetics. Species and strain differences in response to nitrobenzene exposure have been noted in inhalation studies using mice and rats. The reason for these differences and the toxicokinetics involved are not understood. Additional toxicokinetic studies in species other than rodents and attempts to estimate the sensitivity of humans relative to these test species would be valuable aids in interpreting the results of available toxicity studies and in understanding individual differences noted in response to nitrobenzene exposure.

2.8.3 On-going Studies

The CIIT has been preparing a final report on their two-year carcinogenicity studies of nitrobenzene administered to mice and rats via inhalation. No other research activities on nitrobenzene are known to be currently in progress.