DRAFT HAZARD IDENTIFICATION OF THE DEVELOPMENTAL AND REPRODUCTIVE TOXIC EFFECTS OF BENZENE

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PREFACE

The Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65, California Health and Safety Code 25249.5 *et seq.*) requires that the Governor cause to be published a list of those chemicals "known to the state" to cause cancer or reproductive toxicity. The Act specifies that "a chemical is known to the state to cause cancer or reproductive toxicity … if in the opinion of the state's qualified experts the chemical has been clearly shown through scientifically valid testing according to generally accepted principals to cause cancer or reproductive toxicity." The lead agency for implementing Proposition 65 is the Office of Environmental Health Hazard Assessment of the California Environmental Protection Agency. The "state's qualified experts" regarding findings of reproductive toxicity are identified as members of the Developmental and Reproductive Toxicant Identification Committee of the Office of Environmental Health Hazard Assessment's Science Advisory Board (22 CCR 12301).

During a public meeting held in Sacramento, California, on May 12, 1995 the Committee selected benzene as a candidate for evaluation and requested that OEHHA staff prepare a review of the scientific evidence relevant to the reproductive toxicity of this agent. This draft document, which was released to the Committee and the public on September 5, 1997, responds to that request. While this hazard identification document does not provide dose-response evaluation, exposure assessment, or determination of allowable or safe exposure levels, the document does provide information which may be useful in such appraisals.

A public meeting of the Committee will be held December 9, 1997, in Sacramento, California. Following discussion and Committee deliberation, the Committee may determine whether or not benzene "has been clearly shown through scientifically valid testing according to generally accepted principles" to cause reproductive toxicity, or may defer a decision and prescribe an action plan that will discuss further steps to be taken and indicate the timeline for reconsideration of the chemical.

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A. Abstract

Exposures to benzene occur in connection with auto exhaust, auto fueling, tobacco smoke, and, in occupational settings, through its use as a chemical intermediate and as a component of petroleum products. Known toxic effects of benzene in humans include induction of myeloid leukemia and aplastic anemia. Benzene metabolites are clastogenic and target hematopoietic precursor cells.

There are a number of studies of the consequences of benzene exposure during organogenesis in mice, rats and rabbits, many of which used the inhalation route, which is the most common route of exposure for humans. The animal studies have consistently found developmental retardation as reflected in fetal weight and skeletal ossification at term. These effects occurred in the absence of reported maternal toxicity at some benzene concentrations. In mice, benzene also caused clastogenic effects and altered populations of hematopoietic precursors in the fetus when administered to the dam.

Relevant human studies have examined pregnancy outcome in relation to maternal occupational exposure to benzene, usually as one of a number of organic solvents, or environmental exposure to benzene as one of a number of contaminants. In case-control studies investigating maternal exposure to benzene as one of a number of concurrent exposures, there were elevated odds ratios, though most were not statistically significant, associated with adverse effects on fetal growth (preterm delivery), fetal loss (stillbirth), and birth defects (neural tube and major cardiac defects), as well as childhood leukemia. More definitive studies with assessment of benzene-specific exposure are needed to evaluate the suggested associations.

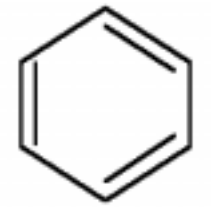
Female reproductive toxicity was not reported in the few relevant studies in the animal literature. However, in human studies, consistent reports of abnormal menstruation and excessive blood loss during childbirth in women occupationally exposed to benzene have been identified in 3 cross-sectional studies and in case series and case reports. More definitive studies with accurate assessment of benzene-specific exposure are needed to further evaluate the associations suggested by these studies.

Male reproductive toxicity studies in animals have reported benzene-induced damage to testes and sperm, including chromosomal damage. Dominant lethal effects were not reported in available rat and mouse studies. In humans, associations have been reported between paternal occupational benzene exposure and both fetal growth effects and fetal loss; a case-control study reported statistically significant elevated risks of small-for-gestational-age infants and stillbirth, while a cohort study found nonsignificant elevated risks of spontaneous abortion. Of 2 case-control studies of paternal benzene exposure and risk of childhood leukemia and non-Hodgkin’s lymphoma, the more recent study with better exposure assessment reported a statistically significant association while the earlier one failed to find such an association. Studies with accurate assessment of benzene-specific exposure are needed to evaluate the association between pre-conceptional paternal exposure to benzene and childhood leukemia.

Biological plausibility for some benzene developmental and male reproductive effects can be inferred from benzene effects on chromosomes and hematopoietic cells. There has been no direct inquiry into the mechanism of delayed intrauterine development effects. The data appear consistent with both direct effects of benzene and with effects of benzene metabolites.

B. Introduction

B.1. Chemical structure and main physical characteristics



Benzene (CAS # 71-43-2) is a clear, colorless liquid with a molecular weight of 78.11. It is highly volatile, with a vapor pressure of 95.2 mm Hg. Its solubility in water is 1,780 mg/L. It is also highly flammable. It occurs naturally as a product of pyrolysis, though most major releases are anthropogenic (ATSDR 1993).

B.2. Regulatory history

Benzene is listed as a carcinogen under Proposition 65 (22 CCR 12000) and has been identified as a toxic air contaminant by the California Air Resources Board. It has also been identified as a human carcinogen by the International Agency for Research on Cancer (IARC 1987) and by the US Environmental Protection Agency (USEPA) (IRIS 1994).

The legal airborne permissible exposure limit (PEL) established by the federal Occupational Safety and Health Administration (OSHA) is 1 ppm averaged over an 8-hour workshift. The National Institute of Occupational Safety and Health (NIOSH) has recommended an order of magnitude lower, with a Recommended Exposure Level (REL) of 0.1 ppm averaged over a 10-hour workshift (NJHSFS 1997).

The Office of Environmental Health Hazard Assessment (OEHHA) was asked to prepare a Hazard Identification Document on Benzene at the May, 1995 meeting of the Developmental and Reproductive Toxicant (DART) Identification Committee of the Science Advisory Board. Benzene was selected as a high priority candidate for consideration under Proposition 65 based on selection by a group of experts in reproductive toxicity combined with use, production and exposure data (Donald et al. 1992). Benzene was also one of 14 high priority agents chosen by a Delphi committee of experts organized by OEHHA to prioritize candidate DARTs.

B.3. Exposure information

Benzene's primary industrial use is as an intermediate in chemical manufacturing processes, including the production of ethyl benzene (55%), cumene (24%), cyclohexane (12%) and nitrobenzene (5%) (ATSDR 1993). The 1990 US Toxic Release Inventory identified 39 facilities in California that manufacture or process benzene (ranging from 0-99,999 in thousands of pounds). A total of 98% of benzene produced in the US is derived from petroleum-related industries (ATSDR 1993). Occupational exposures to benzene can occur at chemical manufacturing and petroleum refining facilities, during transport

and in automotive repair or service-related industries (if they involve exposures to gasoline or gasoline vapors).

Exposures among the general population usually occur via auto exhaust, auto fueling and tobacco smoke (ATSDR 1993). The dominant sources of benzene in the atmosphere are gasoline fugitive emissions and gasoline motor vehicle exhaust (Hammond 1996). The California Air Resources Board has tracked the amount of benzene in various air basins throughout California under the Toxic Air Contaminants Identification and Control Act. A trend analysis of the data indicated that the ambient amount of benzene in air samples in California has decreased over the past 5 years from an average of 2.7 ppb to 1.3 ppb, as shown in Figure B.3. There was a 49 percent decline between 1990 and 1995 (ranging from 35% to 68% depending on the location). It was noted in the report that the limit of detection is 0.5 ppb (Hammond 1996). In March of 1996, California began using "California Phase II reformulated gasoline", which limits the benzene content to 0.8 % by volume on average (a decrease from the Phase I gasoline, which allowed 1.7%) (ARB 1997). The benzene-containing aromatics (which are added to increase the octane rating) have been partially replaced by oxygenates like MTBE (Methyl Tertiary Butyl Ether) (Cal/EPA 1997).

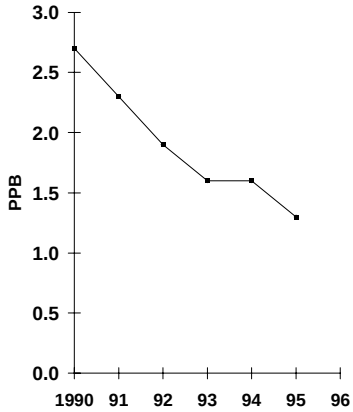


Figure B.3. Benzene in ambient air in California. (From Hammond 1996)

The other main route of exposure for the general population is via tobacco smoke. The benzene in tobacco smoke is a pyrolysis product. A USEPA Total Exposure Assessment Methodology (TEAM) study showed that a typical smoker (32 cigarettes/day x 55 µg/cigarette) takes in ~2 mg benzene/day, with 1.8 mg delivered by mainstream smoke. The benzene body burden for smokers was 6 to 10 times that of non-smokers. According to the TEAM study, non-smokers inhale an average of 0.2 mg benzene/day, depending on where they live, the amount of environmental tobacco smoke they are exposed to, the amount of time they spend driving, etc. (Wallace 1996).

Benzene in the environment is mainly anthropogenic, though some natural sources exist. The California Toxic Release Inventory shows a steady decline in the amount released by reporting sources, with 536,690 pounds released in 1987 and 136,582 pounds released in 1994. Most benzene is found in the atmosphere, though small amounts appear in surface and ground water due to hazardous waste sites, leaking underground gasoline storage tanks, industrial effluent and wet deposition. The volatilization half-life for benzene in surface water is 4.81 hours (ATSDR 1993). Benzene in groundwater does not volatilize and, due to anaerobic conditions, does not biodegrade either. Thus, the 2 major sinks for benzene are groundwater and the atmosphere. Bioconcentration does not appear to be a factor in aquatic species, a fact which is confirmed by its relatively low K_{ow} (2.13 - 2.15) (ATSDR 1993). It should be noted, however that it is ubiquitous in the environment and any specific exposures are in addition to this background level.

B.4. Pharmacokinetics

The pharmacokinetics of benzene have been extensively studied and recently reviewed (ATSDR 1993; Henderson et al. 1992; IPCS 1993; NTP 1986; Snyder et al. 1993; Snyder and Kalf 1994). Physiologically based, pharmacokinetic models have been developed for mice and rats (Medinsky et al. 1989a; Medinsky et al. 1989b), and for mice, rats, and humans (Travis et al. 1990).

B.4.1. Absorption

Absorption of benzene is highly efficient by the inhalation and oral routes, but occurs with low efficiency by the dermal route.

Inhalation studies in humans found initial absorption of 70% to 80% over the first 5 minutes, which dropped to around 50% after 1 hour. Retention (the amount absorbed but not excreted via the lungs) was around 30% (ATSDR 1993; IPCS 1993).

In rodents, the percentage of inhaled benzene retained decreased at higher exposures (ATSDR 1993; IPCS 1993). Rats (F344/N) and mice (B6C3F1) were exposed to benzene at concentrations ranging from approximately 10 to 1000 ppm for 6 hours. The resulting retention is shown in Table B.4.1. (below). The benzene retained from a 6 hour exposure dropped from 33% to 15% in rats and 50% to 9.7% in mice as the concentration increased. The amount of benzene inhaled by the mice per unit body weight was considerably greater than that inhaled by rats. The percentage retained by the mice was higher at all but the highest concentration (Sabourin et al. 1987).

Table B.4.1. Retention of inhaled benzene by rats and mice after 6 hours (Sabourin et al. 1987).

Exposure concentration (ppm)		Amount inhaled (mg/kg bw)		Percentage retained (%)	
rats	mice	rats	mice	rats	mice
13	11	9.9 ± 1.5[(1)]	15 ± 3	33 ± 6	50 ± 15
29	29	20 ± 2	31 ± 14	44 ± 4	52 ± 1
130	130	104 ± 21	159 ± 35	23 ± 4	38 ± 7
260	--	172 ± 5	--	22 ± 4	--
870	990	774 ± 543	1570 ± 340	15 ± 9	9.7 ± 1.8

[(1)] Values are mean ± standard deviation, with N = 3 for all values except N = 2 for mice at 130 ppm.

Oral exposure studies in animals found that 90% to 97% of the administered dose was absorbed (ATSDR 1993; IPCS 1993).

Dermal exposure has been demonstrated in humans, but the efficiency was low (on the order of 0.05% in vivo and 0.2% in *in vitro* tests). Dermal absorption in animals was less than 1% (ATSDR 1993; IPCS 1993).

B.4.2. Distribution

Benzene is rapidly distributed through the blood to most, if not all, tissues. Benzene is lipophilic, and reaches higher concentrations in lipid rich tissues. It is rapidly metabolized to hydrophilic compounds (see below), which also appear to be widely distributed (ATSDR 1993; IPCS 1993; Henderson et al. 1992).

Benzene has been found in the blood, brain, and liver of 1 human, and the blood, brain, liver, kidney, stomach, bile, abdominal fat, and urine of another human who died from benzene inhalation (ATSDR 1993). Benzene has also been found in maternal and umbilical cord blood (see discussion in Section C.3.1) (Dowty et al. 1976).

Male rats (F344) exposed to benzene at 500 ppm for 6 hours were found to have benzene widely distributed. Some of the results are shown in Table B.4.2.1 (below). Steady state concentrations of benzene in fat were considerably higher than other tissues tested. Steady state concentrations in bone marrow were somewhat higher than in other tissues tested, excluding fat. Both the approach to steady state and the elimination were rapid: half times ($t_{1/2}$s) were hours or fractions of hours (Rickert et al. 1979).

Table B.4.2.1. Distribution of benzene to tissues in rats (Rickert et al. 1979).

		Half-times ($t_{1/2}$ in hr)	
Sample	Steady state concentration (mg/g or mg/mL)	Approach to steady state	Elimination
blood	11.5 ± 0.7[(1)]	1.4	0.7
bone marrow	37.0 ± 2.2	<0.5	0.5
fat	164.4 ± 15.0	2.0	1.6
liver	9.9 ± 0.7	1.9	0.4
lung	15.1 ± 0.9	1.5	0.4
kidney	25.3 ± 1.3	1.3	0.6
spleen	4.9 ± 0.5	0.9	0.8
brain	6.5 ± 0.6	2.6	0.6

[(1)] Values are mean ± standard error, with N = 3.

The distribution of benzene and selected metabolites was examined in rats (F344/N) and mice (B6C3F1) exposed to 50 ppm ^{3}H-benzene by inhalation for 6 hours. Some of the results are presented in Table B.4.2.2 (below). ^{3}H-labeled phenol and hydroquinone were found in liver, lung, and blood of mice. ^{3}H-labeled catechol was found in the liver of mice, but was below detection limits in lung or blood. ^{3}H-labeled phenol, hydroquinone, and catechol were below detection limits in liver, lung, and blood of rats. The differences

between mice and rats were at least partly attributable to differences in metabolism between species (Sabourin et al. 1988). Note that these measurements would not include phenol, hydroquinone, or catechol derived from endogenous or dietary sources. See discussion in Section B.4.3 below.

Table B.4.2.2. Distribution of ^{3}H-benzene and selected ^{3}H-benzene-derived metabolites in rats and mice (Sabourin et al. 1988).

	Rats			Mice		
chemical	liver	lung	blood	liver	lung	blood
benzene	3.8 ± 1.4[(1)]	3.3 ± 0.7	0.9 ± 0.2	4.5 ± 0.9	1.5 ± 0.6	1.9 ± 0.5
phenol	ND[(2)]	ND	ND	0.3 ± 0.1	0.6 ± 0.2	1.3 ± 1.1
catechol	ND	ND	ND	0.3 ± 0.1	ND	ND
hydro-quinone	ND	ND	ND	2.1 ± 0.3	1.2 ± 0.2	4.3 ± 4.0

(1) nmol chemical/g tissue. Values are mean ± standard error with N = 3 to 4.
(2) ND: not detected. Limits of detection were 0.06 for benzene, 0.09 for phenol, 0.08 for catechol, 0.1 for hydroquinone.

The distribution of benzene metabolites can also be inferred from the presence of benzene-derived adducts in the blood. Rats and mice gavaged with labeled benzene were found to have labeled benzoquinone-hemoglobin adducts 24 hours later (McDonald et al. 1994). After benzene exposure, S-phenyl cysteine, which is believed to be produced by the binding of benzene oxide to cysteine, has been found in hemoglobin from rats and mice (but not humans), and albumin from rats, mice, and humans (Bechtold et al. 1992a; Bechtold et al. 1992b; McDonald et al. 1994).

B.4.3. Metabolism

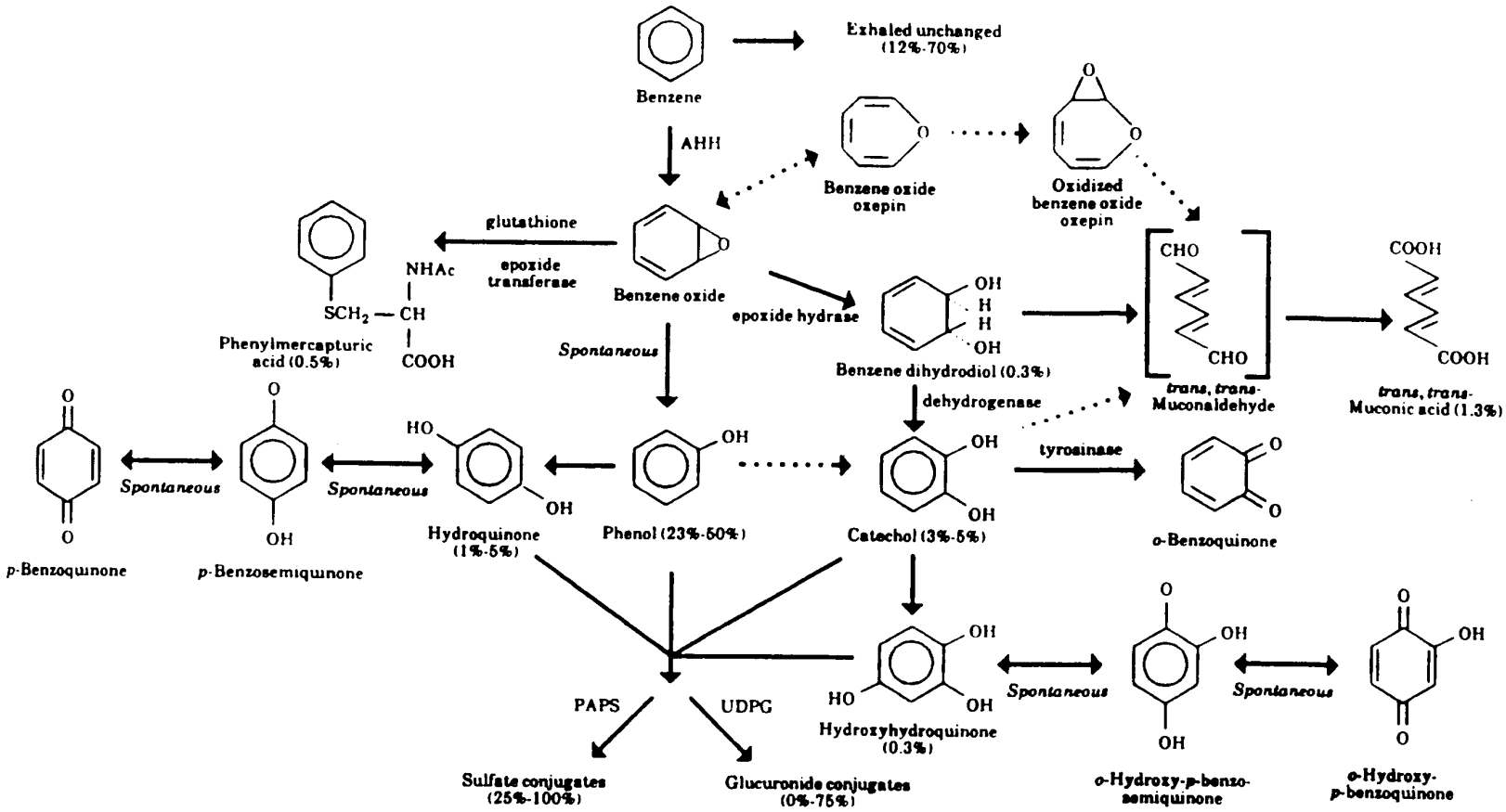
Benzene is metabolized to numerous other compounds in several steps (Figure B.4.3). Initial metabolism is by cytochrome P450s. The main P450 responsible appears to be CYP2E1. In knockout mice lacking CYP2E1 expression, total benzene metabolism was reduced to 13% of wild type mice (Valentine et al. 1996). This P450 is inducible by ethanol, acetone, and benzene (Johansson and Ingelman-Sundberg 1988; Koop et al. 1989; Seaton et al. 1994). The initial product is benzene oxide (an epoxide). Benzene oxide undergoes several reactions, producing phenol, benzene glycol (benzene dihydrodiol), muconaldehyde (muconic aldehyde, a ring-opening product), and pre-phenyl mercapturic acid. Phenol is the major metabolite. Phenol can be further hydroxylated (principally by CYP2E1) to hydroquinone, catechol, and trihydroxybenzene. Catechol is believed to be produced mainly by the dehydrogenation of benzene dihydrodiol. The hydroxylated derivatives can be conjugated to glucuronides or sulfates. Muconaldehyde oxidizes to muconic acid. Hydroquinone and catechol can be oxidized to the respective benzoquinones (ATSDR 1993; IPCS 1993; Snyder et al. 1993).

The major site of benzene metabolism is the liver. However, the liver is not a major site of benzene toxicity. Metabolism likely occurs in other tissues as well, since P450 enzymes are widely distributed. Metabolism in bone marrow may be particularly important, as bone marrow is the major target of chronic benzene toxicity (see Non-DART Toxicities, Section B.5). Bone marrow contains relatively high levels of peroxidases, which can react with benzene metabolites to produce additional reactive, and potentially toxic, compounds. (Eastmond et al. 1986; Ross et al. 1996; Snyder et al. 1993; Snyder and Kalf 1994).

Metabolism appears to be qualitatively similar among mammals, including humans. However, there are important quantitative differences between species. Mouse, rat, monkey, chimpanzee, and human have been compared at various levels. None are identical (Henderson 1996; Henderson et al. 1989; Henderson et al. 1992; Medinsky et al. 1989a; Medinsky et al. 1989b; Sabourin et al. 1988; Sabourin et al. 1992; Seaton et al. 1994; Travis et al. 1990). In both mouse and rat, the fraction of benzene metabolized drops at higher exposure levels, indicating saturation of some pathway (Henderson et al. 1989; Sabourin et al. 1987). Relatively greater concentrations of muconic acid and hydroquinone conjugates have been found in the bone marrow of mice than of rats. It has been suggested that these metabolites are responsible for the greater sensitivity of mice than of rats to the hematotoxic effects and carcinogenicity of benzene (see below) (Henderson et al. 1989; Sabourin et al. 1987). Liver samples from 10 humans and mice and rats were assayed for CYP2E1 activity. It was found that human CYP2E1 activity varied by 13 fold. Mouse and rat CYP2E1 activities fell within the range of human activities. Mice had 2.5 fold greater activity than rats (Seaton et al. 1994).

Many benzene metabolites are present at substantial levels in humans and animals not exposed to benzene. Phenol, hydroquinone, catechol, and their conjugates have been found in the urine of unexposed humans, with highly skewed distributions (Carmella et al. 1982; Drummond et al. 1988; Inoue et al. 1986; Inoue et al. 1988; Roush and Ott 1977). The levels of these compounds were the equivalent to what would be produced by 1-3 ppm benzene for phenol and its conjugates, 1-2 ppm benzene for hydroquinone and its conjugates, and 20 ppm benzene for catechol and its conjugates (Inoue et al. 1986; Inoue et al. 1988). Food appears to be the major source of catechol and its conjugates (Carmella et al. 1982). In rats and mice singly gavaged with high levels of labeled benzene (up to 400 mg/kg), unlabeled, i.e. dietary and/or endogenous, benzoquinone-hemoglobin adducts greatly exceeded those formed from labeled benzene (2.7 to 473 fold). However, animals exposed multiple times to benzene would be expected to accumulate higher levels of adducts, possibly exceeding background levels in some cases (McDonald et al. 1994). In contrast to these results, in unexposed humans, urinary muconic acid and S-phenyl cysteine (a benzene oxide reaction product) were present at low levels (Boogaard and van Sittert 1996). These observations have implications for the mechanism of benzene toxicity: see Section B.5.3, Benzene metabolites and non-DART toxicities, below.

Figure B.4.3. Benzene metabolism (NTP 1986).



B.4.4. Elimination and excretion

Elimination of benzene and its metabolites is relatively rapid, with most eliminated in hours to days. The main route of elimination for unmodified benzene is by exhaled air. This is the case whether the benzene was initially absorbed or retained from inhalation, oral, or dermal routes. Benzene metabolites, especially the glucuronide and sulfate conjugates, are excreted mainly in the urine. A small amount of benzene and/or its metabolites is excreted in the feces. At lower exposures, urinary excretion predominates. At higher exposures, the fraction eliminated by exhaled air becomes progressively larger. It is likely that this is due to saturation of metabolic pathways (ATSDR 1993; IPCS 1993).

In humans, benzene excretion is multiphasic (ATSDR 1993). In 1 study, a human was exposed to benzene at 31 ppm for 8 hours, and elimination in breath followed for 125 hours. A 4 component exponential model was fit to the data, with $t_{1/2}$s of 19 minutes, 1.8 hours, 4.2 hours, and 27 hours. Further experiments on the same individual using different exposure conditions found similar $t_{1/2}$s (Sherwood 1988). In 3 humans who had inhaled 25 ppm benzene and 100 ppm toluene for 2 hours, 90-95% (estimated from graph) of the benzene was eliminated from blood in 300 minutes. Elimination was fit to a 3 component exponential model with rate constants corresponding to $t_{1/2}$s of 1.7, 25, and 219 minutes. Similar rate constants were found for benzene in exhaled air (Sato et al. 1974). As has been discussed above (Section B.4.3), there is considerable inter-individual variation in human CYP2E1 activity, which could affect the rate constants for elimination.

In rats and mice exposed to 100 or 300 ppm benzene for 6 hr/d, 5 d/wk, for 20 exposures, elimination of benzene from the blood was monitored after the 1st, 6th, and 20th exposures. Elimination could be fitted to a 1 component exponential model for all exposures except for mice after 20 exposures, which required a 2 component model. The rate constants corresponded to $t_{1/2}$s of 15.4-16.3 minutes for mice at 100 ppm, 21.1-37.5 minutes for mice at 300 ppm, 51-100 minutes for rats at 100 ppm, and 128-154 minutes for rats at 300 ppm. Thus, mice eliminated benzene from the blood more rapidly than rats. Elimination from both species was slower at the higher exposure level (Snyder et al. 1981b).

In rats, following exposure to 500 ppm for 6 hours, the elimination of benzene from various tissues was rapid. The $t_{1/2}$s ranged from 0.4 hours for lung and kidney to 1.6 hours for fat, with blood intermediate at 0.7 hours (see Table B.4.2.1 above) (Rickert et al. 1979). In rats and mice exposed to 50 ppm benzene for 6 hours, the elimination of several metabolites (muconic acid, hydroquinone glucuronide and phenyl sulfate) from blood, liver, and lung occurred with $t_{1/2}$s of less than 2 hours (estimated from graphs) (Sabourin et al. 1988).

In rats and mice exposed to benzene by gavage, it was found that the majority of elimination was in urine at lower doses (50 mg/kg or less), but in air at higher doses (150 mg/kg or higher). Elimination by air was primarily or exclusively benzene, whereas excretion by urine was predominantly or exclusively water soluble metabolites. The $t_{1/2}$s varied with dose and species, but appear to be on the order of less than 1 to a few hours (estimated from graphs) (Sabourin et al. 1987).

Little information was located concerning the excretion of benzene or benzene metabolites in milk. A report from Canada indicated that benzene had been "detected" in human breast milk. No reference or other information was reported (Giroux et al. 1992). A physiologically based, pharmacokinetic model was developed for predicting the transfer of volatile chemicals in breast milk. Experimental results for benzene blood/air and milk/air partition coefficients were incorporated. The model predicted that occupational exposure to benzene would result in the excretion of benzene in breast milk (Fisher et al. 1997).

B.5. Non-DART toxicities

The non-DART toxicities of benzene have been extensively studied and recently reviewed. In humans, death, neurological effects, hematotoxic effects, leukemia, and chromosomal aberrations have been associated with benzene exposure. In experimental animals, death, neurological effects, hematotoxic effects, multi-site carcinogenicity, and chromosomal aberrations have been found following benzene exposure (ATSDR 1993; IPCS 1993; NTP 1986; Snyder et al. 1993; Snyder and Kalf 1994).

B.5.1. Human non-DART toxicities

Acute, high level exposure to benzene has caused death in humans by inhalation (estimated at 20,000 ppm) and oral (estimated at 125 mg/kg) routes. Death was attributed to respiratory arrest, central nervous system depression, or cardiac collapse (ATSDR 1993; IPCS 1993).

Long term human occupational exposures (mainly by inhalation) have been associated with hematotoxic effects. These effects have been somewhat variable, ranging from deficiencies in specific blood elements (red blood cells {RBCs}, white blood cells {WBCs}, or platelets) to pancytopenia and aplastic anemia. In many cases these effects reversed after exposure ceased. However, deaths have also resulted. Exposures, when measured or estimated, were in the tens to hundreds of ppm for months to years (ATSDR 1993; IPCS 1993; Aksoy and Erdem 1978; Dosemici et al. 1996; Forni et al. 1971; Forni 1996; Goldwater 1941; Greenburg et al. 1939; Hunt 1979; Kipen et al. 1989; Linet et al. 1996; Rothman et al. 1996; Smith 1928; Yin et al. 1996). Reduction in RBCs was typically accompanied by an increase in mean corpuscular volume, i.e. typically a macrocytic anemia (Fishbeck et al. 1978; Goldwater 1941; Greenburg et al. 1939). Abnormal and excessive bleeding has often been found in the diseases associated with benzene exposure (e.g. pancytopenia/aplastic anemia or acute myeloid leukemia; see

below) (Aksoy and Erdem 1978; Linet et al. 1996). This is likely due to reduction in platelets (thrombocytopenia), which are involved in blood clotting (Smith 1986). Hemorrhage during childbirth has been observed in several cases of benzene poisoned women. In some cases, the woman and/or the infant died. However, in other cases, following elimination of exposure, hemorrhage was not a problem in subsequent births (Forni et al. 1971; Forni 1996; Hunt 1979; Messerschmitt 1972; Smith 1928). This manifestation of benzene toxicity, while not directly affecting the reproductive system, may also be considered to potentially adversely affect reproductive function in women and development of the offspring.

Long term human occupational exposures have been associated with increased incidence of leukemia (predominantly acute myeloid leukemia, AML). The exposure conditions were similar to those for hematotoxic effects. Some individuals developed leukemia subsequent to hematotoxicity. (ATSDR 1993; IPCS 1993; Aksoy and Erdem 1978; Linet et al. 1996; Rinsky et al. 1987; Yin et al. 1987; Yin et al. 1996). A group of rubber workers in the U.S. (the "Pliofilm cohort") has been extensively studied. A marked progressive increase of the incidence of leukemia with increasing cumulative exposure to benzene was found. The Standard Mortality Ratio (ratio of deaths among the exposed group to deaths expected; 100 means no increase) increased from 109 for <40 ppm-years to 6,637 for >400 ppm-years (Rinsky et al. 1987). It has been argued that the exposures in this analysis were underestimated (Paustenbach et al. 1992). None-the-less, subsequent reanalyses confirmed the exposure-response trend, although the magnitude of the effect was argued to be smaller (Crump 1996; Paxton 1996). A recent, very large cohort study of Chinese workers exposed to benzene also examined the relationship between leukemia and exposure levels. In this study, a substantial increase in risk for leukemia was found at the lowest cumulative exposure level (< 40 ppm-years, relative risk = 1.9, 95 % CI = 0.8-4.7). An increase in risk was found from the low to intermediate (40 - 99 ppm years), but not the intermediate to high ($\geq$ 100 ppm-years) cumulative exposure levels (Hayes et al. 1997). The same study of Chinese workers also found an association of benzene exposure with lung cancer and non-Hodgkin's lymphoma (Hayes et al. 1996; Hayes et al. 1997).

Long term human occupational exposures have been associated with chromosomal aberrations in peripheral lymphocytes and red blood cell precursors. The levels of exposure are similar to those associated with hematotoxicity and leukemia. In peripheral lymphocytes, chromosomal damage included aneuploidy, deletions, breaks, translocations, and unstable forms (ATSDR 1993; IPCS 1993; Forni et al. 1971; Forni 1996; Tunca and Egeli 1996; Zhang et al. 1996). Recently, an assay for protein (glycophorin A) variants in mature erythrocytes from heterozygous individuals has been used as an indicator of chromosomal damage in erythrocyte precursors. Results indicated an increase in gene translocations, but not inactivation, in a benzene exposed group (Rothman et al. 1996).

B.5.2. Experimental animal non-DART toxicities

Acute, high level exposures of experimental animals to benzene have resulted in death. In rats, an LC_{50} of 13,700 ppm for 4 hours of exposure was found. A group of rabbits exposed to 45,000 ppm for 30 minutes all died. In rats, LD_{50}s of 5,600 mg/kg for single gavage and 930 mg/kg in food for 1 day were found (ATSDR 1993; IPCS 1993).

Hematotoxic effects have been extensively studied in mice and rats. Mice appear to be more sensitive than rats to these effects (Snyder et al. 1978; Ward et al. 1985). In mice, numerous reports have found reductions in counts of white blood cells (WBCs) and red blood cells (RBCs). Typical exposures by inhalation resulting in reductions in RBCs or WBCs were at 100-300 ppm, 6 hr/d, 5d/wk, for 1 week or longer, although reductions have been found as low as 10 ppm. Reductions in WBCs have also been found at 25 mg/kg/d by gavage. Reduction of WBCs was somewhat more sensitive than RBCs, i.e. the magnitude of the reduction was greater, it occurred at lower concentrations, or after shorter periods of time (Aoyama 1986; Baarson et al. 1982; Baarson et al. 1984; Baarson and Snyder 1991; Cronkite et al. 1982; Dempster et al. 1984; Green et al. 1981a; NTP 1986; Rosenthal and Snyder 1984; Rozen et al. 1984; Seidel et al. 1989a; Seidel et al. 1989b; Snyder et al. 1978; Snyder et al. 1980; Snyder et al. 1981b; Snyder et al. 1982; Snyder et al. 1988; Vacha et al. 1990; Ward et al. 1985). In rats, most reports have found reductions in counts of WBCs, but not RBCs. Exposures where reductions were found were at 50 ppm, 8 hr/d for 7 days by inhalation, 300 ppm for 6 hr/d by inhalation, 25-50 mg/kg/d for several weeks by gavage, 5 mL/kg by injection (sc), and 0.5 mL/kg/d for 4 days by injection (sc) (Gill et al. 1979; Greenlee and Irons 1981; Li et al. 1986; NTP 1986; Ward et al. 1985). One report in rats found an equivocal and inconsistent reduction in RBCs, but no effect on hemoglobin or hematocrit (Snyder et al. 1978). In both mice and rats, males are generally more sensitive than are females (NTP 1986; Tice et al. 1989; Ward et al. 1985).

In addition to effects of benzene exposure on RBC counts, other aspects of effects on RBCs have been investigated in some studies. In 2 studies in mice by inhalation at 300 ppm for 2 weeks or longer, reduction in RBC counts were accompanied by increases in mean corpuscular volume and mean corpuscular hemoglobin. As a result, hematocrit and total hemoglobin were reduced to a lesser extent than were RBC counts (Vacha et al. 1990; Ward et al. 1985). Other long-term inhalation studies in mice have also found no reduction or a smaller magnitude of reduction in hematocrit or total hemoglobin than in RBC counts (Green et al. 1981a; Seidel et al. 1989b). A study of benzene exposed workers in China found similar results (Rothman et al. 1996). However, a shorter-term (1 week) study in mice, which used a wide range of benzene concentrations (1.1 to 4862 ppm), found a dose-responsive reduction of RBC counts but an erratic and non-dose-responsive effect on hematocrit (Green et al. 1981a). Thus, longer term exposures to benzene can produce macrocytic anemia, although the effects of short-term exposures may be more complex.

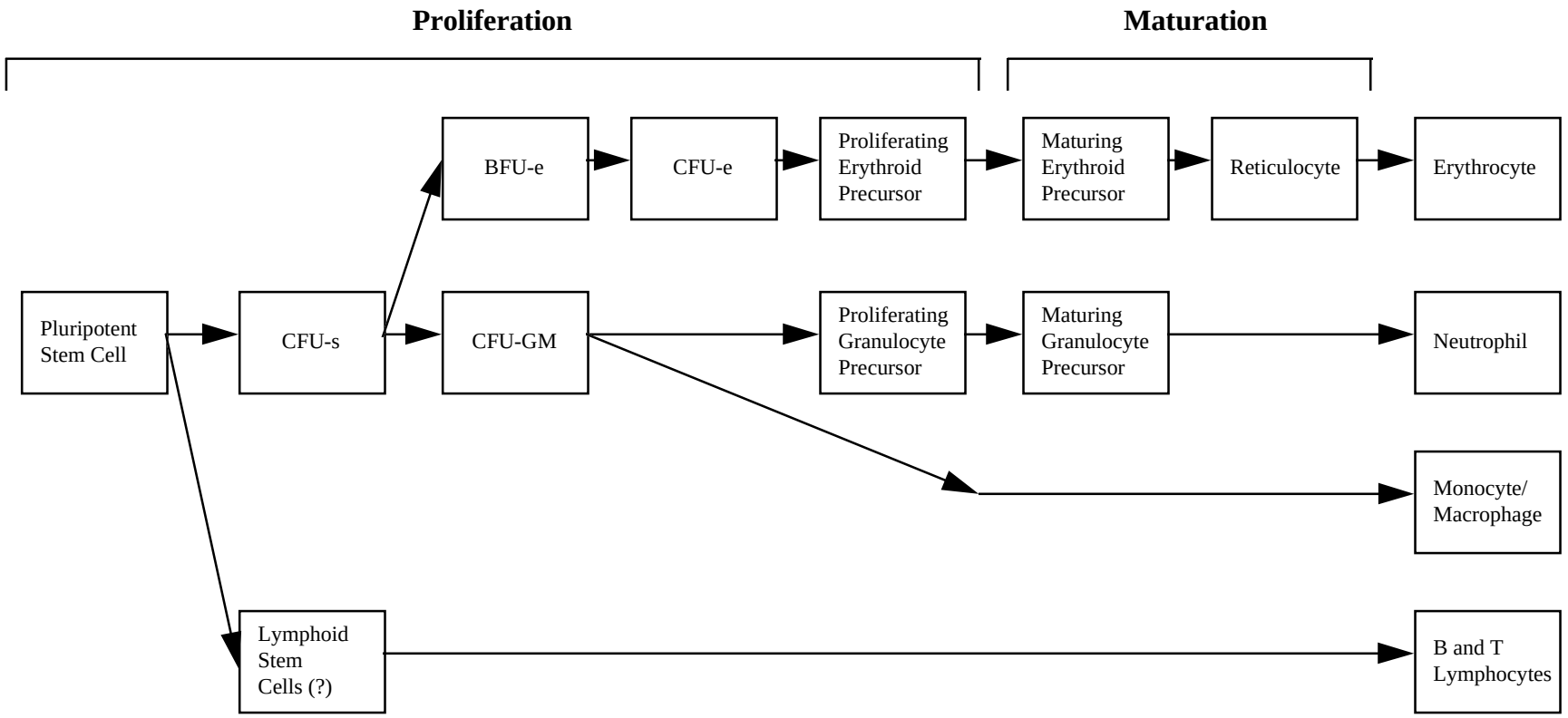
Functional aspects of the effects benzene exposure on the immune system have been examined in several studies. In a study of antibody response and contact sensitivity, mice

were exposed to 0, 50, or 200 ppm benzene for 7 or 14 days. Reduced B and T lymphocytes, and reduced ability to produce antibodies against sheep red blood cells was observed at exposures of 50 ppm for 14 days or 200 ppm. The 200 ppm group also showed increased contact sensitivity to picryl chloride, and reduced activity of suppresser T lymphocytes for this response (Aoyama 1986). In another study of antibody response, mice were exposed to 400 ppm benzene for up to 22 days. Reduced primary antitoxin responses to tetanus toxoid, but no effect upon secondary responses, was found (Stoner et al. 1981). In a study of splenic lymphocyte response, mice were injected (ip) with benzene at doses from 44 to 660 mg/kg for 3 days. Reduced *in vitro* proliferation of splenic lymphocytes in response to the mitogens Con A and LPS was found at all doses. Reduced anti-sheep red blood cell plaque forming response was consistently found at 264 mg/kg and higher doses (Wierda et al. 1981). In a study of resistance to infection, mice were exposed at 0, 10, 30, 100 or 300 ppm benzene for 5 days before infection, or 5 days before and 7 days after infection with *Listeria monocytogenes.* Increased bacterial counts in spleen were found on day 4, but not day 1 or 7 in the 300 ppm pretreated, and the 30 ppm and up pretreated plus posttreated groups (Rosenthal and Snyder 1985). In another study of resistance to infection, mice were exposed to 10 ppm benzene by inhalation for 3 hr/d for 1 or 5 days, and challenged with *Streptococcus zooepidemicus* or *Klebsiella pneumonia*. No increase in mortality from *S. zooepidemicus* was found. Bactericidal activity to *K pneumonia* was increased for 1 day exposure, but decreased for 5 day exposure (Aranyi et al. 1986). In a study of resistance to tumor cells, mice were exposed to 0, 10, 30, or 100 ppm benzene for 100 days, and challenged with 5×10^3 or 1×10^4 tumor cells. Mice exposed to 100 ppm benzene and challenged with 1×10^4 tumor cells had highly increased tumors and mortality compared to controls. No effects were seen in other treated groups (Rosenthal and Snyder 1987). Thus, under appropriate exposure conditions, benzene can reduce the ability of the immune system to produce antibodies, fight infections, and fight tumor cells.

There have been numerous studies of the cells which develop into WBCs and RBCs (termed stem cells, progenitors and/or precursors) (see Figure B.5). Commonly measured parameters include bone (and/or spleen) cellularity (numbers of cells), Colony or Burst Forming Units (or Cells), and ^{59}Fe flux. Colony or Burst Forming Units involve assays for cells with the ability to form colonies under defined conditions *in vivo* or *in vitro*. The different assay conditions are believed to correspond to different stages of cell differentiation (Alberts et al. 1989). It is thought that there are numerous feedback loops among the different cell stages which serve to regulate hematopoiesis. A mathematical model has been constructed of these processes (Scheding et al. 1992). In general, in mice, bone and spleen cellularity and CFU-s, BFU-e, CFU-e, and CFU-GM (see Figure B.5) are found to be reduced to varying extents under different benzene exposure conditions. This is, however, an oversimplification. The changes in CFU and BFU assay numbers are complex and often involve both reductions and increases over time. Moreover, there is not a direct correlation between progenitor stages assayed and the levels of mature cells in the blood. Typical exposures which produce BFU and CFU effects are roughly comparable to those producing effects on peripheral WBCs and RBCs. (Baarson et al. 1982; Baarson et al. 1984; Baarson and Snyder 1991; Cronkite et al. 1982;

Cronkite et al. 1985; Cronkite et al. 1989; Green et al. 1981b; Hilderbrand and Murphy 1983; Seidel et al. 1989a; Seidel et al. 1989b; Seidel et al. 1990; Tice et al. 1989; Toft et al. 1982).

Figure B.5. Stages of hematopoiesis (adapted from Scheding et al. 1992, Alberts et al. 1989, and Male et al. 1996).



Development of blood cells from pluripotent stem cells. Pathways of 3 major lineages are shown: several other blood cell types and platelets are not shown. Neutrophils and monocytes/macrophages are considered “myeloid” cells. Note that the existence of a specific lymphoid stem cell is not clear. BFU-e (Burst Forming Unit-erythroid), CFU-e (Colony Forming Units-erythroid), CFU-GM (colony Forming Unit-Granulocyte Monocyte/Macrophage), and CFU-s (Colony Forming Unit-spleen) are stages which are commonly assayed.

An example of the complexity of the relationship between progenitors and peripheral blood cells can be found in the sequence BFU-e --> CFU-e --> --> RBC. Male mice were exposed to 10 ppm benzene for 6 hr/d, 5 d/wk. The effects on assays for femoral erythroid progenitors and on RBCs are presented in Table B.5.2.1 (below). After 32 days, BFU-e and CFU-e were reduced to about 63-67% of control, but RBC were not reduced. After 66 days, BFU-e were about 63% of control, CFU-e were about 41% of control, and RBCs were about 85% of control. After 178 days, BFU-e had increased to about 115% of control, CFU-e dropped to about 5% of control, and RBCs were about 89% of control (Baarson et al. 1984). Other reports have found that the magnitude of reductions of CFU-e are typically considerably greater than the magnitude of reductions of BFU-e or RBCs (Baarson and Snyder 1991; Seidel et al. 1989a; Seidel et al. 1989b). The significance of this divergence is not clear. The CFU-e is thought to have limited proliferative potential (about 6 cell divisions) (Alberts et al. 1989). It has been suggested that the reduction of CFU-e reflects a shorter transit time through this stage (Scheding et al. 1992; Seidel et al. 1989b), or that the *in vitro* assay becomes ineffective under benzene exposure conditions (Cronkite et al. 1989; Scheding et al. 1992).

Table B.5.2.1. Effects of exposure of mice to 10 ppm benzene on femoral erythroid progenitor cells and RBCs (Baarson et al. 1984).

Days of exposure	BFU-e	CFU-e	RBCs
32	67%[(1)]	63%	98%
66	63%	41%	85%
178	115%	4.7%	89%

[(1)] Value compared to control.

Iron is incorporated into heme in the final stages of RBC maturation. Studies of ^{59}Fe uptake into RBCs have found a transient reduction from single injections of benzene (Andrews et al. 1977; Lee et al. 1974). However, after prolonged inhalation of benzene, the ^{59}Fe uptake into RBC was found to be increased from controls (Seidel et al. 1989b; Vacha et al. 1990). This supports the concept that shorter transit times through cell stages are part of the physiological response to benzene (Seidel et al. 1989b).

Benzene has been found to be a multi-site carcinogen in mice and rats by inhalation and oral routes. The specific tissues/organs affected varied by route, species, and strain. By inhalation in mice, increased lymphomas, leukemias, Zymbal gland carcinomas, hepatomas, and lung adenomas have been observed, although not all were observed in all strains tested. By inhalation in rats, increased leukemia, Zymbal gland carcinoma, hepatoma, nasal carcinoma, and liver tumors have been observed (ATSDR 1993; IPCS 1993). An NTP study by gavage in mice found increased lymphoma, and neoplasms of the Zymbal gland, lung, Harderian gland, mammary gland (female only), preputial gland (male only), forestomach, ovary, and marginally in the liver (female only). In rats, increased neoplasms of the Zymbal gland, oral cavity, and skin (male only) were found. Reduced survival and weight gain, and hematotoxic effects were also found (Huff et al.

1989; NTP 1986). In these studies, mice were more sensitive than rats to the carcinogenic effects of benzene (Medinsky et al. 1989a; Medinsky et al. 1989b). Although increased lymphomas and/or leukemias have been observed in rats and mice, these were infrequent compared to other sites. Most neoplasms were of epithelial origin (IPCS 1993), whereas the hematopoietic system is of mesenchymal origin (Tavassoli 1991).

Benzene has consistently given negative results in assays for point mutations in bacterial cells. *In vitro* assays in human and experimental animal cells have been largely negative, although there have been some equivocal positive reports (IPCS 1993; ATSDR 1993). Recently, use of the sensitive ^{32}P-postlabeling technique has allowed the detection of increased DNA adducts *in vivo* following benzene exposure (Bauer et al. 1989; Bodell et al. 1996; Li et al. 1996; Snyder et al. 1989), although this has not been consistently observed (Reddy et al. 1989; Reddy et al. 1994). *In vivo* exposure to benzene in mice and rats has consistently been found to be clastogenic, i.e. to produce chromosomal changes. Numerous studies have found increases in chromosomal aberrations, sister chromatid exchanges, and micronuclei in bone marrow and blood cells of mice and rats exposed by inhalation. Similar results have been obtained in mice exposed by gavage, but not in Chinese hamsters. By the oral route, male mice are consistently more sensitive than are females or fetuses (ATSDR 1993; Ciranni et al. 1988; Harper et al. 1989; IPCS 1993). Recently, in rats gavaged with benzene, increased micronuclei in the Zymbal glands have been found. The Zymbal gland is a sebaceous gland in the region of the external ear canal of rodents, and was found to have increased neoplasms in carcinogenicity studies (see above) (Angelosanto et al. 1996). Two recent studies have found that topoisomerase II, a key enzyme involved in relieving torsional stress in DNA, is inhibited by benzene metabolites *in vitro*. It has been suggested that this could be the source of the clastogenic effects (Frantz et al. 1996; Hutt and Kalf 1996).

B.5.3. Benzene metabolites and non-DART toxicities

There is general agreement in the field that benzene metabolites, not benzene, are primarily responsible for the non-DART toxic effects. Evidence for this comes from the observations that alteration of benzene metabolism alters benzene toxicity, and that some benzene metabolites produce some similar effects. It is not clear, however, which metabolite(s) is (are) mainly responsible for the toxic effects. Some combinations of metabolites have greater than additive effects. It has also been proposed that a 2-step process is involved. In this proposal, metabolites produced by the liver are transported to the bone marrow, where further metabolism (by peroxidases) occurs and more or additional toxic species are produced. This is proposed as the basis for the selective toxicity of benzene to bone marrow cells (ATSDR 1993; IPCS 1993; Snyder et al. 1993; Snyder and Kalf 1994).

Several studies have indicated that modifications to benzene metabolism alter the toxic effects of benzene. In rats injected (sc) with benzene at 2200 mg/kg, partial hepatectomy (70-80%) reduced the rate of metabolism of benzene, and eliminated the reduction of

^{59}Fe incorporation into RBCs (Sammett et al. 1979). Toluene was found to reduce benzene metabolism, probably by competitive inhibition of P450s. Toluene was protective against a reduction of ^{59}Fe incorporation into RBCs resulting from benzene injection (Andrews et al. 1977). The main enzyme responsible for the initial metabolism of benzene, CYP2E1, is inducible by ethanol (Johansson and Ingelman-Sundberg 1988; Koop et al. 1989). Ingestion of ethanol enhanced the reduction of WBCs and RBCs resulting from benzene inhalation (Baarson et al. 1982; Snyder et al. 1981a). Ingestion of ethanol also enhanced the reduction in BFU-e, CFU-e, and CFU-GM resulting from benzene inhalation (Seidel et al. 1990). "Knockout" mice lacking CYP2E1 expression, and control mice, were exposed to 200 ppm benzene for 6 hr/d for 5 days. It was found that characteristic benzene toxicities, including reduced bone marrow cellularity and increased micronuclei in polychromatic erythrocytes, occurred in the control, but not the "knockout" mice (Valentine et al. 1996). Mice injected with prostaglandin synthase inhibitors (aspirin, meclofenamate, or indomethacin) were protected against reduced bone marrow cellularity and increased micronuclei in polychromatic erythrocytes resulting from benzene injection. The authors hypothesize that the peroxidase activity in prostaglandin synthase in bone marrow is involved in benzene toxicity (Kalf et al. 1989).

Relatively greater concentrations of muconic acid and hydroquinone conjugates have been found in the bone marrow of mice than of rats. Model calculations indicate that mice metabolize relatively more benzene to hydroquinone and muconaldehyde than do rats. This suggests that these metabolites are involved in the greater sensitivity of mice than rats to toxic effects of benzene (Henderson et al. 1989; Medinsky et al. 1989a; Medinsky et al. 1989b; Sabourin et al. 1988). As discussed in the Metabolism section (B.4.3, above), substantial amounts of phenol and the metabolites downstream from phenol occur in the absence of benzene exposure. The impact of these background concentrations on benzene-induced toxicity is not well understood. However, it has been suggested that toxic effects of the benzoquinones may only be important at high levels of benzene exposure (McDonald et al. 1994; Rappaport et al. 1996). An alternate hypothesis is that the background levels of quinone species are involved in disease in the general population and any addition to background, such as exposure to benzene, would add to the overall incidence.

C. Developmental Toxicity

The main focus of research and regulatory consideration of benzene has been its leukemogenic properties in humans. There are several previous reviews of benzene developmental and reproductive toxicity (Hunt 1979; Chatburn et al. 1981; Barlow and Sullivan 1982; Schreiner 1983; Schwetz 1983; Wyrobek et al. 1983; Davis and Pope 1986; Maronpot 1987; McDiarmid et al. 1991; Skalko 1993; IARC 1982; IPCS 1993; IRIS 1994; ATSDR 1993; anonymous 1984; ACGIH 1990), but none that contain all the studies reviewed in the present document.

C.1. Human developmental toxicity studies

Several studies have examined pregnancy outcomes in relation to exposure to benzene, usually as one of a number of concurrent chemical exposures; some but not all of these studies have included a separate analysis examining the effect in relation to a measure of benzene exposure. Outcomes examined in relation to exposure of the mother during pregnancy include: fetal growth, including decreased birthweight, growth retardation, or prematurity; fetal loss, including spontaneous abortion and perinatal mortality; congenital malformations; and childhood leukemia. Studies which were identified that addressed these outcomes are summarized below. Studies which have examined these outcomes in relation to paternal exposure are discussed in Section E (Male Reproductive Effects).

C.1.1. Fetal growth

Potential effects on development *in utero* include impacts on the growth of a fetus, as well as on the duration of gestation. Measurement of such effects commonly occurs at birth, at which time weight and gestational age can be determined. Measures of fetal growth include mean birthweight, low birthweight (<2500 grams), and intra-uterine growth retardation (IUGR), which is defined as less than the tenth percentile of weight for gestational age (also called small-for-gestational-age, SGA). Because studies of low birthweight in the aggregate combine determinants of fetal growth with influences on duration of gestation, and may thus dilute causative associations in either subgroup, some investigators examine birthweight in full term births only, and examine preterm births as a separate category.

All of the studies that have examined the relationship of benzene exposure to fetal growth effects had study populations with simultaneous exposure to a number of chemicals, and many did not assess the effects of benzene exposure specifically. Studies of occupational exposures to solvents in laboratories in Sweden and Finland (Axelsson et al. 1984; Taskinen et al. 1994), and in a range of occupations in the U.S. (Savitz et al. 1989), provide some data on fetal growth measures in relation to maternal exposure to benzene, usually as one of a number of organic solvent exposures. Although some of these studies found effects, the results found in the various studies are not consistent. Decreases in birthweight were seen in 1 case control study of laboratory workers exposed to multiple solvents (Taskinen et al. 1994) but not in a cross-sectional study of a similar population (Axelsson et al. 1984). One study that separately analyzed occupational benzene exposure in relation to fetal growth outcomes (Savitz et al. 1989) examined both risk of preterm delivery and small-for-gestational-age infants in association with maternal benzene exposure, but found no significant differences. These studies are discussed in detail below. In studies of populations exposed to multiple chemicals (including benzene) due to residence near hazardous waste sites and/or contaminated drinking water, an increased risk of some adverse fetal growth measures (*e.g.*, numbers of low birthweight babies) has been found in some studies (Goldman et al. 1985; Witkowski and Johnson 1992) but not in others (Bove et al. 1995); however, the mixture and variability of chemical exposures, as well as the study designs, limit the conclusions which can be made regarding benzene toxicity based on these studies, and they are not discussed

further in this section. Further research which includes more accurate assessment of benzene-specific exposure is needed to evaluate the possibility of an association with fetal growth effects.

Axelsson et al. (1984)

Axelsson et al. (1984) studied pregnancy outcome in women employed in laboratories in Sweden in a cross-sectional study (see Section C.1.2 for more details). In a study involving 556 subjects, information on birthweight obtained in a mailed questionnaire was available for 968 live births. Mean birthweights for pregnancies exposed to solvents were not significantly different from unexposed pregnancies. No benzene-specific results were reported. In a regression analysis which took into account several factors known to influence birthweight (e.g., parity, maternal smoking, infant gender), exposure to solvents was not related to birthweight ($r = 0.028$) in a model that also included work in a laboratory ($r = -0.015$).

Savitz et al. (1989)

Savitz et al. (1989) examined the effect of parental occupational exposures on risk of several adverse pregnancy outcomes using the National Natality Survey, a probability sample of 9,941 live births registered in the U.S. in 1980, in which low weight infants (<2500 grams) were oversampled by fourfold, and the National Fetal Mortality Survey (discussed in Section C.1.2). Birth certificate data were merged with data from questionnaires sent to married women approximately 6 months after delivery. After exclusion of nonrespondents, unmarried mothers, plural births, mothers who did not work within 12 months of delivery and women with missing or incomplete data, 3,668 live births were available for analysis of maternal occupation by industry.

Data were analyzed in 2 case-control studies of pregnancy outcome: stillbirth (see Section C.1.2), preterm and small-for-gestational-age (SGA) infants. Analysis for preterm delivery (defined as birth before 37 completed weeks of gestation) included 363 preterm births (cases) and 2,624 term births (controls) for maternal occupational exposure. When all infants with known birthweight and gestational age data were compared with norms specific to the week of gestation (with infants in the lowest tenth percentile classified as SGA), 218 SGA infants and 2,712 infants appropriate for gestational age were available for analysis of maternal occupation.

Occupational exposure of the parents was based on jobs held within 12 months of delivery. The exposure linkage system assigned exposure to specific agents based on industry and occupation, with linkages of none, low, medium and high or unknown assigned to each agent; these are “probably better interpreted as probabilities of exposure than of markers of exposure intensity” according to the authors. In this study, only medium or high linkages were considered ‘exposed’. Although subjective judgment was often required in translating occupational codes from the surveys to categories for the linkage system, assignment was blind with respect to pregnancy outcome. Low exposure

industries (e.g., sales, law, real estate) served as the referent (unexposed) category. For maternal exposure to benzene, work in the textile industry, especially sewers and tailors, were the majority (48% of benzene-exposed mothers); other major contributors were barbering and cosmetology (36%), chemical, drugs and paint industry (6%).

Expected associations of demographic, socioeconomic and lifestyle factors were observed (e.g., strong effects of late prenatal care, low education, and cigarette smoking) in a multivariate analysis developed from an extensive initial list of potential confounders. Maternal exposure to specific agents, in general, was unrelated to risk of preterm delivery. A small, statistically insignificant increase was seen in the analysis of benzene exposure (OR = 1.2; 95% CI = 0.7-2.3). Overall risk of SGA was lower for women with medium or high linkage levels than for other women, with reductions in risk associated with several exposures, including benzene (OR = 0.6; 95% CI = 0.3-1.3).

The most important limitation of this study is the quality of the available exposure information. Potential misclassification may have occurred due to variable work practices, use of protective equipment and environmental controls, given that job was accurately reported and coded. The survey's restriction to married women limits its generalizability, as it underrepresents teenage mothers and black women, and the sizable nonresponse would tend to exclude socioeconomically disadvantaged women. Strengths of the Savitz et al. (1989) study include its large, nationally representative sample of married mothers, analysis of specific exposures based on maternal occupation, and data on the most important potential confounders (e.g., maternal smoking, pregnancy history).

Taskinen et al. 1994

In a study of laboratory workers in Finland conducted by Taskinen et al. (1994) (described in more detail in Section C.1.2), the possible effects of occupational exposure on the birthweight of children was examined among the referents (n = 500) identified for the other portions of the study. Mother's employment in a laboratory was associated with lower birthweight; a mean decrease of 133 grams (95% CI = -246 to -20) was found. No data specific to benzene exposure were reported with respect to birthweight. Gainful employment in general had no influence on birthweight in this study. The authors noted that they did not have information on the height and weight of the mother, and suggested that the findings be interpreted with this in mind. Also, information on exposure was collected for the first trimester of pregnancy only, and events later in pregnancy which may have influenced birthweight (e.g., work in a standing position, smoking) were unknown.

C.1.2. Spontaneous abortion and perinatal mortality

Perinatal mortality is defined broadly as death in the period from 20 weeks gestation to 28 days post-delivery, and encompasses stillbirths (fetal death from 20 weeks to term) and neonatal deaths (death between birth and 28 days of life); not all investigators define

these terms in the same way. Spontaneous abortion (also called miscarriage) is usually defined as fetal loss up to 20 weeks gestation.

Results of several studies examining fetal loss, including spontaneous abortion, stillbirth and perinatal mortality in relation to exposure to benzene as one of a number of concurrent solvent exposures during pregnancy are not entirely consistent but indicate a need for further research. Of these studies, 3 performed separate analyses of outcome in relation to a measure of benzene exposure (Axelsson et al. 1984; Savitz et al. 1989; Taskinen et al. 1994) and 1 reported results for exposure to benzene and toluene together(Huang 1991). A cross-sectional study of occupational exposure of laboratory workers in Sweden (Axelsson et al. 1984) found a slightly increased but not statistically significant difference in miscarriage rates for those exposed to organic solvents during pregnancy, but no association with perinatal mortality. In the analysis of miscarriage in women who worked with benzene during their first trimester, these authors found no statistically significant elevated risks (Axelsson et al. 1984). Two case-control studies of occupationally exposed women with multiple exposures reported statistically significant elevated risks of spontaneous abortion (Huang et al. 1991; Taskinen et al. 1994). In the study by Taskinen et al. (1994), a significantly elevated risk of spontaneous abortion in laboratory workers exposed to multiple aromatic hydrocarbons (including benzene) during pregnancy was found for those exposed frequently, but not for those reporting less frequent exposure; in the analysis reported for any benzene exposure, the adjusted odds ratio was not elevated. A case-control study of maternal occupational exposure and risk of stillbirth (Savitz et al. 1989) found a positive association with occupations linked to benzene exposure; risk increased with increasing exposure, and was statistically significant in the highest exposure category. All of these studies are discussed in detail below. More definitive studies with accurate assessment of benzene-specific exposure are needed to evaluate the association suggested by some of these studies.

Axelsson et al. (1984)

Axelsson et al. (1984) conducted a cross-sectional study of laboratory workers on the payroll of a university in Sweden, who were born after 1935 and were employed between 1968 and 1979. The women were asked about solvent exposures during the first trimester and thereafter and pregnancy outcomes, as well as smoking habits, medicine and disease exposures during pregnancy. To verify questionnaire information, investigators used a Swedish registry of all births and a register of congenital malformations; when information reported by participants differed from registry accounts, hospital records were checked. Of the women who received the questionnaire, 745 (95%) responded. Among these women, 556 reported they had been pregnant, with 997 live births and 119 miscarriages resulting. Birth defects were examined in another analysis, described in Section C.1.3. The miscarriage rate was slightly increased for women exposed to multiple solvents during pregnancy but was not statistically significant (RR = 1.31, 0.89-1.91). In this study, risk factors for miscarriage (pregnancy number, age and shift work) were significantly negatively correlated with working with solvents ($p<0.0001$), and thus uncontrolled confounding by these factors may have biased the results toward the null.

Women who reported working with benzene during their first trimester had miscarriage rates comparable to women not engaged in laboratory work or studies during pregnancy (12.2% and 11.5%, respectively). No difference was found for perinatal death rates (1.2% and 1.0%) in those exposed to laboratory work or studies during pregnancy versus others.

Savitz et al. (1989)

In a study on the association of parental occupational exposures and risk of several adverse pregnancy outcomes in the U.S. (described above, see Section C.1.1), Savitz et al. (1989) conducted a case control analysis of risk of stillbirth. Using data from the National Fetal Mortality Survey, which included 6,386 stillbirths registered in 1980 (with losses at gestational age of 28 weeks or more or a weight of 1,000 grams or more considered eligible), the study included 2,096 stillbirth cases and 3,668 live birth controls for the analysis of maternal occupation. Savitz et al. (1989) found an elevated risk of stillbirth related to maternal occupational exposure to benzene (adjusted OR = 1.3; 95% CI = 1.0-1.8). This risk increased across "linkage levels" for benzene (ORs of 0.9, 1.2, and 1.4 for low, medium and high linkage levels, respectively), and was statistically significant for the highest level (95% CI = 1.1-1.9).

Huang et al. (1991)

A case-control study of women occupationally exposed to both benzene and toluene in China was conducted by Huang et al. (1991). These investigators found an elevated incidence of spontaneous abortion (5.7% in exposed vs. 2.4% in controls) and gestosis (any toxemic manifestation in pregnancy) (22.6 % in exposed vs. 10.5% in controls); these differences were statistically significant. The exposure levels were not reported.

Taskinen et al. (1994)

Taskinen et al. (1994) conducted a case-control study of laboratory workers in Finland. Women who worked in laboratories during 1973 to 1986 were identified by job title from the state payroll, a union list of laboratory assistants and from a registry of employees occupationally exposed to carcinogens. Cases (n = 206) had only 1 spontaneous abortion during the study period; referents (n = 329) had given birth to a baby but had no registered spontaneous abortion, and were matched to cases on age at the time of conception and year of the end of the pregnancy; all subjects were ages 20 to 34 at the beginning of the study pregnancy. Information on occupational exposure, tobacco use, alcohol consumption and other factors was self-reported in a mailed questionnaire. Reported exposure to each chemical was first classified according to frequency of exposure. Two industrial hygienists then constructed exposure indices to organic solvents, carcinogens and radiation, and constructed scales by considering reported frequency, intensity and duration of exposure as well as fume hood use. Benzene was included in both the organic solvent and carcinogen categories.

Taskinen et al. (1994) found a slight but not statistically significant increase in spontaneous abortions in women employed in a laboratory (OR = 1.4; 95% CI = 0.9-2.2), controlling for several factors including parity, alcohol consumption, and previous miscarriages. A significantly elevated risk of spontaneous abortion in laboratory workers exposed to aromatic hydrocarbons (including benzene) during pregnancy was found for those workers exposed frequently (for 2 to 5 days per week: adjusted OR = 2.7, 95% CI = 1.3-5.6; $p<0.01$), but not for those reporting less frequent exposure (1 to 2 days/week: adjusted OR = 0.8, 0.4-1.4). Matched analyses for frequent exposure to toluene, xylene or formalin all resulted in significantly elevated odds ratios; however, in the analysis reported for any benzene exposure, the adjusted odds ratio was not elevated (OR = 0.8; 95% CI = 0.4-1.7). While the study indicates an increased risk of spontaneous abortion related to exposure to organic solvents during pregnancy, the authors suggest that results concerning individual chemicals be interpreted cautiously, as the subjects were exposed to multiple chemicals.

C.1.3. Birth defects

All of the epidemiologic studies that have examined the relationship of benzene exposure to birth defects had study populations with simultaneous exposure to a mix of organic solvents. While some studies have found positive associations of maternal exposure to solvents during pregnancy and risk of birth defects, only an exploratory ecological study provided information on benzene-specific risks. Positive associations of occupational and other exposure to organic solvents and incidence of all birth defects were found in 2 (Holmberg 1979; Holmberg et al. 1986) of 3 studies based on a Finnish registry of malformations, but not in the third (Kurppa et al. 1983); no information on benzene-specific relationships was available in these studies. A cross-sectional study in Sweden (Axellson et al., 1984) did not find an association of malformations with exposure to multiple solvents, but had very small numbers of affected offspring. A case-control study of laboratory workers in Finland (Taskinen et al. 1994) found no association with reported solvent use during the first trimester. In an ecological study in New Jersey (Bove et al. 1995), nonsignificant elevated risks for specific defects were reported in association with estimated benzene exposure based on contaminated drinking water supplies. All of these studies are discussed in detail below. Two additional studies, not described here, examined the influence of residence near contaminated hazardous waste sites (containing benzene as well as other contaminants) on reproductive outcomes (Budnick et al. 1984; Goldman et al. 1985); both found positive associations with incidence of all birth defects, but did not provide information about benzene-specific relationships. Further studies are needed to adequately assess the association of benzene exposure and congenital malformations.

Finnish Population-Based Studies:
Holmberg (1976); Kurppa et al. (1983); Holmberg et al. (1986)

A Finnish registry of malformations was used to study the relationship of occupational and other exposure to organic solvents and birth defects in 2 separate studies. Holmberg

(1976) conducted a case-control study using the Finnish Register of Congenital Malformations. Information on occupational exposures as well as family history, prior pregnancies, complications and results of prenatal examinations was collected by an unblinded interviewer, with hospital records used for additional information on the pregnancy. In some cases, factories were visited to clarify reported exposures. Case mothers were exposed more often than control mothers to organic solvents during the first trimester of pregnancy ($X^2 = 8.07$, $p<0.01$). Anencephaly was the most frequently reported defect.

In 1983, Kurppa et al. used the same register to study the relationship of solvent exposure to birth defects. This much larger study consisted of 1047 case/control pairs. In this study, interviewers were blinded, and information similar to that above was collected from case mothers. Exposure was categorized into none, minor, substantial and strong, with only exposures during the first trimester considered. In some cases, quantitative exposure estimates were used, if available (e.g., employees of large factories). Exposure to organic solvents was associated with central nervous system (CNS) defects for the first 2 years of the study. However, in the following 2 year period, no association was observed. The authors attributed the initial association observed by the same group (Holmberg 1979) to either chance or the small number of women exposed.

A third report (Holmberg et al. 1986) discusses results from 1475 case-referent pairs. Relative to other studies, there were substantial numbers of cases in 4 categories of malformation: CNS defects, oral clefts, skeletal malformations and cardiac defects. Solvent exposures were divided into 4 categories: aromatic, halogenated hydrocarbons, aliphatic hydrocarbons and lacquer petrols (usually a mixture of 85% aliphatic and 15% aromatic hydrocarbons). The unadjusted relative risk estimate for first trimester solvent exposure was 1.6 (95% CI = 1.0-2.5) for all malformations pooled. Adjustment for mother's age, smoking habits and alcohol consumption during pregnancy did not change the point estimate or confidence interval.

These 2 Finnish studies, which are really updates of the same study, show a relationship between organic solvent exposure and birth defects. Although benzene exposure information was collected, no analysis was presented on the association of birth defects with benzene.

Axelsson et al. (1984)

In a cross-sectional study conducted Axelsson et al. (1984) described above (Section C.1.2), women employed as laboratory workers in Sweden were asked about solvent exposures during the first trimester and thereafter and pregnancy outcomes. Information obtained by questionnaire was verified using birth and congenital malformations registries. Among the women responding to the questionnaire, about half of the pregnancies resulting in live births (492) were reported for women who had worked with solvents in the first trimester, compared to 496 unexposed. Of the 39 children born with malformations, there was a nearly equal prevalence rate among children of solvent-

exposed (18) and nonexposed (21) mothers. There were not enough malformations to look at distinct patterns. In this study, women reported on exposures occurring up to 10 years earlier, so it is unclear if misclassification bias may have diluted the findings.

Taskinen et al. (1994)

Taskinen et al. (1994) conducted a study of pregnancy outcome in Finnish laboratory workers as described above (Section C.1.2). Cases who participated (n = 36), identified from the Finnish Registry of Congenital Malformations, completed a questionnaire on occupational exposure, health status, use of contraception as well as smoking and alcohol use. Logistic regression was used to relate solvent exposure to probability of birth defects. Overall, the odds ratio for solvent-exposed women was not increased, and all the ORs for specific classes of organic solvents were below unity (most of these had fewer than 4 cases available for analysis). Employed women in general had odds ratios significantly below unity; the authors mentioned selection bias resulting from women who quit their jobs due to difficult pregnancies or ill health later related to a negative pregnancy outcome, as well as the possibility of chance, to explain these results.

Bove et al. (1995)

Bove et al. (1995) examined the association of contaminated drinking water and birth defects in an ecological study of all live births (80,938) from 1985 to 1988 to residents of 75 selected towns in northern New Jersey. Contamination in the drinking water supplies of mothers of children born with birth defects (n = 669) was compared to that of mothers of live births that didn't fit a study case definition (n = 52,334) (i.e., were not low birthweight, small for gestational age, preterm, and were born without birth defects). Monthly exposure to each contaminant was estimated separately, based on biannual samples submitted to the state by the water companies serving these towns, and these estimates were assigned to each gestational month of each live birth. For the birth defect outcomes, exposures were averaged over the first trimester only.

In this exploratory study, an association was considered positive when the highest exposure level with at least 2 outcome cases achieved an odds ratio greater than unity; positive associations were found for benzene exposure and risk of neural tube defects (OR = 2.05, 90% CI = 0.61-5.81) and major cardiac defects (OR = 1.75, 90% CI = 0.72-3.93) (only 50, 90, and 99% CIs were reported). The estimated benzene exposure in this study was limited and extremely low: benzene was present in less than 2 percent of the towns studied, based on tap water sample data for each water company in the study area; where benzene was present, maximum estimated monthly exposure was 2 ppb.

C.1.4. Childhood leukemia

A study which examined childhood leukemia in relation to occupational exposure during pregnancy (Shu et al. 1988) found an elevated risk associated with benzene exposure.

More definitive studies with accurate assessment of benzene-specific exposure are needed to evaluate the association suggested by this study.

Shu et al. (1988)

Shu et al. (1988) examined the association between maternal occupational exposures during pregnancy and childhood leukemia in a well-designed matched case-control interview study in Shanghai, China. Using a population registry, 309 childhood leukemia cases in China were compared to 618 control children. These investigators found an association between childhood leukemia and maternal occupation in the chemical industry (chemical processors and related workers, rubber and plastic products makers, leather workers, painters, and chemical analysts) (OR = 3.3; 95% CI = 1.6-6.8). They found increased risks associated with self-reported occupational exposure to benzene (OR = 2.0; 95% CI = 0.9-4.3) and gasoline (OR = 1.6; 95% CI = 0.8-3.1). When childhood leukemia cases were separated by histopathological cell type, maternal benzene exposure was found to be associated with statistically significant increased risks of acute nonlymphocytic leukemia (OR = 4.0; 95% CI = 1.8-9.3) but not with acute lymphocytic leukemia (ALL); maternal gasoline exposure was associated with an increased risk of ALL (OR = 1.7; 95% CI = 1.0-3.0).

C.2. Animal developmental toxicity studies

Studies in rats, rabbits and mice report remarkably consistent effects of benzene administered during organogenesis. Fetal growth retardation, skeletal variations and delayed ossification were reported, sometimes in the absence of maternal toxicity. Malformations were rarely reported even at maternally toxic doses. Genotoxicity was seen in fetal as well as maternal tissues when benzene was administered during pregnancy to mice. Other studies in mice focused on fetal hematopoiesis and demonstrated benzene effects on fetal blood forming cells. Animal developmental toxicity studies are outlined in Tables C.2.1-C.2.3 at the end of Section C.

C.2.1. Inhalation exposure during embryonic development: fetal growth retardation

The majority of developmental toxicology studies have administered benzene during organogenesis via inhalation, the most common route of human exposure (see Table C.2.1). There are 6 such studies in rats, 2 in mice and 2 in rabbits. They are consistent in their findings, taking into account differences in group size and amount of exposure. (Studies in which benzene was administered both before and during gestation are reviewed in Section D.2. Animal Female Reproductive Toxicity Studies.)

Rats

In rats, continuous (24 h/day, 7 day/week) inhalation exposure at concentrations of 50 ppm and above (50, 123, 150, 313, 500, 1000 ppm) (Tatrai et al. 1980a; Tatrai et al.

1980b; Hudak and Ungvary 1978) led to significantly lower fetal weights in term fetuses exposed during organogenesis compared to sham controls. Maternal weight gain during pregnancy was also significantly lower at all these benzene concentrations. The dose-response relationship was not linear. A plateau in the dose-response curve was noted. At 50 ppm, fetal weights were 5% lower than controls, while at 123 ppm through 1000 ppm fetal weights were 20-30% lower than controls with no apparent dose response relationship. This plateau may be related to saturation of metabolism to active metabolites as discussed above (Section B.4.3). At concentrations of 150 ppm and above, effects also included significant fetal loss, including resorption of the entire litter, and instances of maternal death.

Two rat inhalation studies using shorter exposure times and/or lower benzene concentrations, noted statistically significant effects on fetal weight in the absence of reduced maternal weight gain. In a study with a large group size (N = 32-37 pregnancies/group), 6 h/day exposures to 100 ppm benzene, led to significantly lower fetal weights (7% lower than control) in the absence of effects on maternal weight, maternal weight gain, maternal survival, or resorptions (Coate et al. 1984). (Group differences were evaluated with 1-tailed t-tests, rather than the usual 2-tailed tests). The second study used a 7 h/day exposure with group sizes of 11-15 (Kuna and Kapp 1981) and demonstrated a significant reduction in term fetal weight (14 and 18% relative to controls at 50 and 500 ppm benzene). As regards maternal toxicity endpoints, total resorptions and maternal survival were not affected at these benzene concentrations and maternal body weight gain was lower than controls only during the exposure period (GD 6-15). Dams gained significantly more weight than controls after the exposure period (GD 15-20). Both of these studies included lower benzene concentrations (1, 10, 40 ppm in Coate et al. 1984, 10 ppm in Kuna and Kapp 1981) at which no significant effects on dams or fetuses were detected.

In contrast to these 2 studies (Coate et al. 1984; Kuna and Kapp 1981), no significant effect on fetal weight was found in third study (Green et al. 1978) that used benzene exposures of 100 and 300 ppm for 6 h/day. The fetal weights of the benzene groups were lower than controls in the Green et al. study (0.2 g (4%) for the 100 ppm group, and 0.3 g (5%) for the 300 ppm group), but the group size (N = 14-18 pregnancies) was about half that in the Coate et al. study. At a higher concentration, 2200 ppm, Green et al. did find a significant effect on fetal weight (10% lower than controls) accompanied by reduced maternal weights throughout the treatment and post-treatment periods.

Although fetal weight was the main index of intrauterine growth retardation, there is some indication that fetal length was also affected. Two studies included measures of fetal length which indicated that benzene-induced growth retardation is symmetrical (both weight and length affected). A significant 5% lower rat fetal length relative to controls was found at a 2200 ppm 6 h/day benzene exposure (Green et al. 1978). Fetal weight was 10% lower than controls. A significantly lower rat fetal crown-rump length (7%) accompanied a significantly lower (18%) fetal weight was reported for a 500 ppm, 7 h/day benzene exposure in rats (Kuna and Kapp 1981).

Mice

In mice, 12 h/day exposures to 133 or 333 ppm benzene led to a significant increase in percent "weight retarded fetuses" (from 7% in controls to 25% and 27% in the 2 exposed groups), with no effect on percent dead or resorbed fetuses (Ungvary and Tatrai 1985). Maternal toxicity was not discussed in this study. A second mouse inhalation study (Murray et al. 1979) used 500 ppm benzene for 7 h/day on GD 6-15 and found a statistically significant difference (6%) from control in fetal body weights on GD 18. There was no benzene effect on resorption, or on dam appearance, demeanor, body weight, body weight gain, food or water consumption. The ability to identify effects was enhanced by the group sizes (N = 35-37).

Low benzene concentrations (20 ppm or less) were used in a series of studies of fetal hematopoietic toxicity in mice (see Section C.2.7). These studies reported that various developmental toxicity parameters (litter size, fetal weight, number of dead, resorbed or malformed fetuses) in the benzene exposed groups (N = 5-13 pregnancies) were "unaffected" or "within control limits".

Rabbits

In rabbits, 24 h/day exposures to 333 ppm benzene on GD 7-20 led to significantly lower mean fetal weight accompanied by increased resorptions and reduced maternal weight gain (Ungvary and Tatrai 1985). A 133 ppm concentration in the same study had no apparent effect on maternal or fetal parameters (Ungvary and Tatrai 1985). In another rabbit study (Murray et al. 1979), 7 h/day exposure to 500 ppm produced no significant effect on fetal weight, resorption or maternal toxicity parameters. Group sizes in the rabbit studies were 11-19/group. No concentrations lower than 100 ppm have been studied in rabbits as they have in rats; however, data do not suggest that rabbits are more sensitive than rats to the growth retarding effects of inhaled benzene during organogenesis.

Maternal toxicity

Maternal toxicity is monitored in developmental toxicity because the presence of severe maternal toxicity may interfere with the interpretation of developmental effects (USEPA 1990). Benzene effects on fetal growth in rats were reported in the absence of effects on maternal toxicity. However, maternal toxicity is not described in detail in these studies. In particular, it is not clear whether reduced maternal weight gain at the higher benzene concentrations was associated with reduced food intake or general toxicity during the exposure period, or was simply reflecting reduced fetal growth. Two studies in rats (Kuna and Kapp 1981; Coate et al. 1984) reported maternal weight gain separately for the exposure period (during organogenesis) and the post exposure period. Reduced weight gain during exposure was compensated for in the post-exposure period. Maternal food intake data was not reported in any study, although 1 study (Murray et al. 1979) mentioned that food and water intake were not influenced by 500 ppm benzene in mice. In studies with exposure periods < 24 h/day, food was typically withheld during the benzene exposure, while 24 h exposures result in feeding taking place during exposure.

Maternal anemia could be a factor mediating adverse effects on the fetus (Carney 1997). Hematotoxicity is characteristic of benzene toxicity in mice, but is not likely to have been induced in the dams during the short term (8-10 day) exposure periods typical of the developmental toxicity studies. One mouse study (Green et al. 1978) examined hematological parameters (RBCs, hemoglobin, hematocrit) at the end of the 500 ppm exposure during organogenesis and found no statistically significant differences from controls. A rat study (Kuna and Kapp 1981) also failed to find a benzene effect on RBC and WBC at the end of gestation in rat dams exposed to 10, 50 or 500 ppm benzene 7h/day on GD 6-15. In a separate subchronic toxicity study in mice and rats (Ward et al. 1985) benzene produced anemia in female mice as evidenced by lower hematocrits, hemoglobin and RBCs at a 300 ppm exposure concentration (6 h/day, 5 day/wk). These effects were first seen after 14 days of exposure, but were not seen after 7 days of exposure. In the rats, 300 ppm benzene did not influence anemia-related hematology parameters.

C.2.2. Inhalation exposure during embryonic development: gross, soft tissue and skeletal findings

No benzene-induced increases in gross and visceral (soft tissue) malformations were reported in the inhalation teratology studies reviewed for this document. Anomalies reported in more than 1 study were: gastroschisis, 1 fetus each at 1, 10, 100 ppm exposures in rats (Coate et al. 1984), 1 fetus at 500 ppm exposure in rabbits (Murray et al. 1979); hypoplastic thymus, 1 rat fetus in each of 2 studies (Hudak and Ungvary 1978; Tatrai et al. 1980a). Dilated brain ventricles and urinary tracts were reported in several studies but were also found in control groups at a similar incidence. Kuna and Kapp (1981) considered dilated ventricles in 3 benzene-exposed rat fetuses to be sufficiently marked to be “clearly abnormal”.

Skeletal examinations were conducted as part of most of the inhalation teratology studies. Benzene effects on skeletal development were usually reported at concentrations which also produced fetal weight deficits. The three studies using 24 h/day exposures in rats defined skeletal retardation as poorly ossified vertebrae, bipartite vertebra centra and shortened 13th rib. A significantly higher incidence of this effect in benzene exposed rat fetuses than in controls was reported at 125, 150, 313, 500, and 1000 ppm (Tatrai et al. 1980a; Tatrai et al. 1980b). The incidence was also higher at the 50 ppm concentration than in controls, but not significantly so (Tatrai et al. 1980a). The magnitude of the effect was similar at 125 ppm and above. At a 313 ppm concentration a significantly higher frequency of irregular, fused sternebrae and extra ribs was reported along with an increased incidence of skeletal retardation (Hudak and Ungvary 1978).

In rat studies using shorter exposure periods (6,7 h/day), skeletal effects were reported at exposure concentrations below those producing fetal weight effects, and in the extremities as well as the axial skeleton and skull. Green et al. (1979) reported an increased incidence of missing sternebrae in litters exposed to benzene at 100 ppm and delayed ossification of sternebrae in female fetuses at 300 ppm although fetal weight

retardation was seen only at 2200 ppm. Kuna and Kapp (1981) reported a dose related decrease in the number of metacarpals and phalanges per forefoot as determined by regression analysis across exposures of 0, 10, 50 and 500 ppm benzene. In the growth retarded groups (50 and 500 ppm exposures) they described "lagging" ossification in the skull, vertebral column, rib cage pelvic girdle and extremities of rat fetuses exposed to 500 ppm benzene; a greater incidence of fetuses with skeletal and/or visceral variations in the 50 and 500 ppm benzene groups; and a significantly lower number of caudal vertebrae in the 500 ppm group.

In 1 rat study (Coate et al.), there were no statistically significant or apparent differences across exposure groups (0, 1, 10, 40, 100 ppm) in average number of sternebrae, caudal vertebrae, metacarpals, metatarsals and phalanges per litter. These results are not necessarily inconsistent with those of Green et al. and Kuna and Kapp, since the latter studies used higher benzene concentrations and evaluated delays in ossification as well as skeletal variants (absence of ossified bones).

Skeletal effects have also been reported in mice, but not rabbits, exposed to benzene during organogenesis. Ungvary and Tatrai (1985) using 12 h/day exposures in mice, found % skeletal retarded fetuses were significantly greater than controls at 2 concentrations (153 and 307 ppm) that also led to weight retardation. In rabbits, however, 24 h/day exposure to 307 ppm led to lower fetal weights than controls, but no increase in skeletal retardation. Similarly, Murray et al. (1979), using 6 h/day exposures to 500 ppm benzene in mice and rabbits found a significant increase in delayed ossification of sternebrae and skull bones and in unfused occipital skull bones in mice at benzene concentrations that also led to reduced fetal weights, but found no significant decrease in fetal weights or increase in skeletal variants in rabbits.

C.2.3. Oral administration during embryonic development

Two briefly reported studies administered benzene orally during organogenesis to mice (see Table C.2.2). An abstract (Nawrot and Staples 1979) reported increased maternal lethality, increased embryonic resorption and reduced fetal weights in mice given 1.5 or 3.0 mL/kg benzene by gavage on GD 6-15. At a lower dose (0.9 mL/kg) the effect on fetal weight was seen in the absence of effects of maternal lethality or resorption. No effect on malformations was reported at any dose. Skeletal evaluations were not mentioned. The same results were found with a shorter (GD 12-15) treatment period with 1.0 mL/kg. The second study (Seidenberg et al. 1986; Seidenberg and Becker 1987) administered 1300 mg/kg/day by oral intubation on GD 8-12. Of 30 pregnancies, there was 1 maternal death and 1 total resorption. Maternal weight gain, litter size and pup survival to PND 3 were not affected in the remaining pregnancies, but pups' weights were significantly lower than those of controls on PND 1 (0.07 g, 4.1%) and PND 3 (0.1 g, 4.2%). Litters delivered spontaneously in this study and pups were not examined for visceral or skeletal abnormalities.

C.2.4. Injection during embryonic development

Two other briefly reported studies administered benzene subcutaneously to mice. These studies are outlined in Table C.2.3. When benzene (3 mL/kg) was injected on either GD 11,12,13,14 or 15 (Watanabe and Yoshida 1970) cleft palate, agnathia and micrognathia were reported after exposure on day 13 and 14. However, there was no control group in this study. A second study using subcutaneous injection (0, 2, or 4 mL/kg, GD 8, 9, 12, or 13) examined skeletal as well as gross malformations (Matsumoto et al. 1975) as described in Barlow and Sullivan (1982). There were no effects on gross malformation, fetal or placental weight, fetal death or resorption (Barlow and Sullivan 1982); "a small degree of retarded ossification" was seen at 4 mL/kg. (1 mL benzene /kg by gavage is approximately 880 mg benzene/kg; for comparing intake via inhalation see Table B.4.1).

C.2.5. Interaction of benzene with other agents during embryonic development

Benzene has been studied in combination with toluene and xylene because exposure to these 3 chemically similar organic solvents often occurs together. These studies were conducted via inhalation in rats. Toluene (1000 mg/m^3, GD 7-14, 24 h/day) was reported to ameliorate the effects of benzene (400 mg/ m^3) on maternal endpoints (weight gain, organ weights), and also fetal growth retardation (Tatrai et al. 1980b; Ungvary 1985). However, an effect on fetal skeletal abnormalities (extra ribs) and embryolethality (postimplantation loss) was seen with toluene + benzene, but not with benzene alone. When benzene was combined with xylene (600 mg/ m^3), an effect on postimplantation loss emerged, but other parameters were similar to the benzene-alone exposure (Ungvary 1985).

Benzene was also studied in rats in combination with aspirin (acetylsalicylic acid, ASA) because of common metabolic pathways. Benzene inhalation (900 mg/m^3) for 3 days prior to ASA treatment (500 mg/kg, oral) resulted in higher maternal and fetal plasma salicylate concentrations and was reported to enhance maternal toxicity and fetal toxicity, including malformation rate (no data were presented) (Ungvary and Donath 1984).

C.2.6. Transplacental genotoxicity and carcinogenicity

As discussed in a previous section of this document (Section B.5), benzene is genotoxic and produces various forms of genetic damage, especially clastogenicity, in human and other mammalian cells. Transplacental genotoxicity has also received attention. Studies are outlined in Table C.2.6.1 and C.2.6.2 below. Induction of micronuclei in erythrocytes (polychromatic erythrocytes, PCE) was the major endpoint. A particular goal of these studies was to determine whether fetuses were more or less sensitive than adults to induction of genotoxicity.

All the available studies used mice and most administered benzene on GD 13-15. In mice, hematopoiesis in the fetal liver is initiated on GD 10, and peaks on GD 12/13 (Kale and Rao 1992). Subsequently, seeding of bone marrow begins and onset of bone marrow

hematopoiesis is initiated. Thus GD 13-15 can be considered a sensitive period for induction of hemaptopoietic genotoxicity in the fetal liver.

Benzene-induced micronuclei were found in fetal liver erythrocytes (polychromatic erythrocytes, PCE) in 3 studies (Ciranni et al. 1988; Ning et al. 1991; Xing et al. 1992). A significant increase in fetal liver PCE micronuclei, peaking at 21 h after benzene administration, was found when mice were given 1 mL/kg benzene by gavage on GD 13 (Ciranni et al. 1988). Also, a significant increase in fetal PCE micronuclei was found at similar doses (437-1318 mg/kg, approximately 0.5-1.5 mL/kg) administered i.p. on GD 14 and/or 15 (Ning et al. 1991; Xing et al. 1992). In contrast to these studies, no increase in fetal micronuclei formation was found when 880, 1320 or 1760 mg/kg (approximately 1, 1.5 and 2.0 mL/kg) benzene was administered by gavage (Harper et al. 1989). This lack of identification of effects in this study may have been due to the later time of administration (GD 16-17 vs GD 13-15) which may have missed the peak of fetal hematopoiesis. In addition to studies of micronuclei induction, 2 studies also reported increases in sister chromatid exchange in fetal cells when dams were given benzene i.p. at doses of 1.0 and 1.5 mL/kg (Sharma et al. 1985; Xing et al. 1992).

Most studies of transplacental genotoxicity compared effects in the fetus to those in the dam. Two studies found effects in the fetus (liver) and the dam (bone marrow) at similar doses (Sharma et al. 1985; Xing et al. 1992). Two other studies, both using i.p. administration, (Ning et al. 1991; Xing et al. 1992) reported an effect in the fetus at a lower dose than in the dam. Thus, mouse fetuses appear to be susceptible to the genotoxic effects of benzene, but sensitivity relative to dams is unclear. Additionally, 2 studies using oral administration compared benzene-induced genotoxicity in the fetus, dam, nonpregnant female and adult male. Cirrani et al. (1988) found a similar effect on PCE micronuclei in virgin females as in pregnant dams and fetuses but a larger effect in males. Harper et al. (1989) also reported a larger effect in males, a smaller effect in virgin females and, as mentioned above, no effect in pregnant dams or their fetuses. Greater sensitivity of males than females is characteristic of benzene hematotoxic effects in rodents. Because there is no good animal model for benzene-induced leukemia, no implications can be drawn concerning relative sensitivity of the fetus to carcinogenesis.

Some information on transplacental carcinogenicity has also come from studies in which rats were exposed to benzene (200 ppm), throughout gestation and lactation, and for an additional 8 weeks after weaning (Maltoni et al. 1985; Maltoni et al. 1989). They were compared to controls, and also to their dams, who were exposed to the same concentration of benzene for the same period. No statistical analysis of this experiment was presented. However, the authors stated that “an enhanced carcinogenic effect of benzene was observed in animals on which treatment was started during embryonal life” and that animals whose exposure began *in utero* appeared to have a higher incidence of some tumor types (Maltoni et al. 1985). No studies of benzene as a transplacental carcinogen (exposure limited to pregnancy) were located.

C.2.7. Transplacental hematopoietic toxicity

Because of benzene's well known hematopoietic toxicity, attention has been directed at potential effects on blood forming cells *in utero*. Taken together, the available evidence indicates that all 3 hematopoietic precursor lines (i.e., lymphocytes, erythrocytes and granulocytes) are affected by *in utero* benzene exposure in mice. Consequences of this *in utero* exposure to benzene can be detected in cell population numbers and functional properties into adulthood. However, the precise nature and functional consequences of benzene transplacental hematopoietic toxicity have not been defined. Damage during the initial *in utero* stages of hematopoiesis could have lasting effects as has been demonstrated for a number of other toxicants (Holladay and Luster 1994). Some of the studies of developmental hematopoietic toxicity of benzene found effects with very low benzene inhalation concentration exposures (5 and 10 ppm), so that hematopoietic effects may be the most sensitive developmental toxicity endpoint.

A series of 3 papers (Corti and Snyder 1996; Keller and Snyder 1986; Keller and Snyder 1988) have investigated this topic in Swiss-Webster mice exposed during organogenesis (GD 6-15) to benzene via inhalation at concentrations of 20 ppm or less. At these low doses, no maternal toxicity or general developmental toxicity (mortality, growth retardation) was seen. Hematopoietic organs (liver in fetuses and neonates, bone marrow and spleen in adults) and peripheral blood were sources of hematopoietic cells for evaluation. Hematopoietic progenitor cells were studied with an *in vitro* clonal expansion assay; also, immature and mature RBCs and WBCs from peripheral blood (differential blood counts) were enumerated from stained slides.

Table C.2.6.1 Animal studies of the transplacental genotoxicity of benzene by gavage

Reference	Study Design	Reported effects[(1)]
(Ciranni et al. 1988)	mouse gd 13 0, 880 mg/kg	Non-pregnant female bone marrow micronuclei in polychromatic erythrocytes (MN PCE) increased (SS) at 880 mg/kg. Maternal bone marrow MN PCE increased (SS) at 880 mg/kg. Fetal liver MN PCE increased (SS) at 880 mg/kg.
(Harper et al. 1989)	mouse gd 16 or 17 0, 880, 1320, 1760 mg/kg	Non-pregnant female bone marrow micronuclei in polychromatic erythrocytes (MN PCE) increased (SS) at 880, 1320, 1760 mg/kg. Maternal bone marrow MN PCE not increased at any dose tested. Fetal liver MN PCE not increased at any dose tested.

[(1)] Effects reported by authors to be statistically significant (SS) at $p < 0.05$ or biologically noteworthy.

Table C.2.6.2 Animal studies of the transplacental genotoxicity of benzene by injection

Reference	Study Design	Reported effects[(1)]
(Harper et al. 1989)	mouse i.p. gd 16 or 17 0, 880 mg/kg	Non-pregnant female bone marrow micronuclei in polychromatic erythrocytes (MN PCE) increased at 880 mg/kg (statistical significance not addressed). Maternal bone marrow MN PCE not increased at dose tested. Fetal liver MN PCE not increased at dose tested.
(Ning et al. 1991)	mouse i.p. gd 14 0, 109, 219, 437, 874 mg/kg	Maternal bone marrow micronuclei in polychromatic erythrocytes (MN PCE) increased (SS) at 437, 874 mg/kg. Fetal liver MN PCE increased (SS) at 219, 437, and 874 mg/kg. Fetal peripheral blood MN PCE increased (SS) at 437, 874 mg/kg.
(Sharma et al. 1985)	mouse i.p. gd 13 0, 0.125, 0.25, 0.50, 1.0, 1.5 mL/kg (0, 0.11, 0.22, 0.44, 0.88, 1.32 g/kg)[2]	Maternal bone marrow sister chromatid exchange increased (SS) at 1.5 mL/kg. Fetal liver sister chromatid exchange increased (SS) at 1.5 mL/kg.
(Xing et al. 1992)	mouse i.p. gd 14 -15 0, 439, 878, 1318 mg/kg/d	Maternal bone marrow micronuclei in polychromatic erythrocytes (MN PCE) and sister chromatid exchange increased (SS) at 1318 mg/kg/d. Fetal liver (MN PCE) increased (SS) at 878, 1318 mg/kg/d, and sister chromatid exchange increased (SS) at 1318 mg/kg/d.

[(1)] Effects reported by authors to be statistically significant (SS) at $p < 0.05$ or biologically noteworthy.
[(2)] Benzene doses reported in mL/kg were converted to g/kg using density = 0.87865 g/mL (CRC 1976).

The first 2 inhalation studies used 3 concentrations of benzene (5, 10, and 20 ppm), and evaluated offspring as fetuses (GD 16), immediately after the exposure, as neonates (PND 2) and adults (6 weeks of age) to evaluate persisting effects. In the first study (Keller and Snyder 1986), the effect on hematopoietic progenitor cells (colony forming units, CFU) was investigated. The endpoint was the ability of cell suspensions from liver (in fetuses and neonates) or spleen and bone marrows (adults) to form colonies of erythrocytes (CFU-e) or granulocyte/macrophages (GM-CFU-C). (A diagram of hematopoiesis is provided in Figure B.4.3.) Both increases and decreases in colony forming ability were reported when various treated groups were compared with controls. The authors suggest that decreases in colony forming ability are related to damage to progenitor cells and increases represent a compensatory response to this damage. Statistically significant differences from control in CFU-e were seen at all doses in both sexes as fetuses and some differences persisted in neonates and in adult offspring. For GM-CFU-C, statistically significant treatment effects were noted only in neonates. This study included a group in which mice exposed to 10 ppm *in utero* were re-exposed to benzene as adults at 10 weeks of age; decreased numbers of bone marrow CFU-e were measured in males, and decreased number of spleen GM-CFU were recorded in both males and females under these conditions compared to controls exposed to air *in utero*. The suggestion that in utero exposure has postnatal effects on the hematopoietic system receives additional support from an earlier mouse study (Iwanaga et al. 1970) in which a single benzene injection (4 mL/kg, s.c.) was given on GD 6, 9 or 12. Spleen and thymus weights, hematocrits and WBC counts were examined in adult offspring both before and after reexposure to benzene (5 daily injections, 0.1 mL/kg). Female offspring injected on GD 9 and 12 had reduced WBC counts as adults compared to controls, and also showed less of a reduction in WBCs in response to reexposure to benzene. In the females previously treated on GD12, hemoglobin concentrations were lower than controls after the benzene reexposure. There were no effects on WBCs or hemoglobin in adult male offspring. while those injected on GD6 had elevated WBCs. In addition, after the benzene reexposure, thymus weights were lower in male and female offspring in the GD 9 and GD 12 groups than in controls. Spleen weights were also lower in male offspring only.

The second inhalation study (Keller and Snyder 1988) used the same design but investigated the *blood cell differentials*, the number of various immature and mature granulocyte and erythrocyte cells in circulation as well as in hematopoietic organs. No effects of any benzene exposure on these parameters were reported in the fetuses. The authors attributed this to adequate compensation during this early period of rapid hematopoiesis. Neonates' (2 days old) differential cell counts were most affected by embryonic exposure to benzene, and adults demonstrated some of the same effects. Specifically, there were fewer erythrocyte precursors and more granulocyte precursors in neonates after *in utero* benzene exposure than in controls. Notably, benzene causes a similar, reciprocal effect on GM-CFU and CFU-e in adult mice after a 5 day 10 ppm exposure (Dempster and Snyder 1990). The reduction in erythrocyte precursors (early nucleated red cells) was particularly striking, with 0 cells of this type detected per 100 cells counted in the 20 ppm group. However, there was no decrease in RBCs in the peripheral blood of either neonates or adults; in fact, RBCs were significantly higher in

neonates and adults exposed to 5 ppm benzene *in utero*. In addition, there were more nondividing granulocytes in peripheral blood of neonates exposed to 20 ppm benzene than in controls. In liver of neonates, dividing and nondividing granulocytes, as well as lymphocytes, were elevated. Adult spleens demonstrated a similar pattern of elevated numbers of dividing and nondividing granulocytes.

In the third study in this series (Corti and Snyder 1996), comparisons were made between the effects of fetal exposure and those of adult exposure. Only the 10 ppm benzene exposure was used; adults were exposed for 10 days to correspond to the GD 6-15 *in utero* exposure. Cell differentials were not affected in the liver of fetuses, or in the bone marrow of adults exposed either *in utero* or as adults. CFU-e proved a more sensitive index of benzene effects, but marked sex differences were noted. Adult males exposed either *in utero* or as adults had lower numbers of bone marrow CFU-e than controls. CFU-e of *in utero* exposed males was also lower in fetal liver. The size of these effects in males were similar (about 30% lower than in controls) whether the exposures occurred *in utero* or as adults. Adult females exposed either *in utero*, as adults, or as pregnant adults, failed to show significant differences from controls in numbers of CFU-e. The authors suggest that estrogen has an antioxidant effect that protects progenitor cells in females. This experiment included subgroups of benzene exposed mice that were also treated with ethanol, but ethanol did not alter the benzene effect.

In addition to hematopoiesis of erythroid and myeloid cells (erythrocytes and granulocytes), B lymphocyte development has been studied (Wierda et al. 1989). Benzene (i.p. injection of 100 mg/kg) was given twice daily to pregnant BALB/C mice on GD 12.5-19.5. At the end of this treatment period, there was a reduction, relative to controls, in the number of B cells and pre-B cells in maternal bone marrow and also in fetal liver. In an assay of the ability to generate B cells, cell cultures were derived from maternal bone marrow and fetal liver, depleted of B cells, and then incubated for 2 days and examined for the presence of new B cells. Both maternal and fetal cell cultures demonstrated a reduced ability to regenerate B cells. An accumulation of immature B cells indicated that regulatory factors needed for final maturation step were missing, suggesting benzene toxicity to supportive macrophages and stromal cells. In a direct test of this hypothesis, fetal liver cell cultures from benzene-exposed mice demonstrated a reduced ability to produce adherent stromal cell colonies *in vitro*. Further work demonstrated that these changes in fetal B cell populations in response to benzene had lasting functional consequences. The proliferative response of neonatal splenocytes to LPS (lipopolysaccharide, a B cell mitogen) was lower in benzene-exposed neonates than controls. Also, on postnatal day 8, pre-B cells were elevated in offspring of benzene-exposed dams and when liver cells were cultured and depleted of B cells, pre B cells accumulated to higher levels during the first 24 h in culture.

The longterm functional consequences of benzene transplacental hematopoietic effects are not known. In adults, anemia, immunotoxicity and leukemia are associated with benzene hematopoietic toxicity. None of these outcomes has been studied after intrauterine benzene exposure. However, 2 studies (Keller and Snyder 1986; Iwanaga et

al. 1970) have reported postnatal changes in precursor cells, WBCs, hemoglobin, and spleen and thymus weights after prenatal benzene exposure. Other research supports an association with intrauterine hematopoietic toxicity and immunotoxicity. In adult female mice exposed prenatally to chlordane, there was a reduced number of GM-CFU in bone marrow, but no change in bone marrow cellularity or peripheral blood WBC counts (Barnett et al. 1990; Blyler et al. 1994). At the functional level, the delayed type hypersensitivity response was significantly depressed (Barnett et al. 1985b; Barnett et al. 1985a). As regards leukemia, no animal models are available, but evidence from human studies suggests that childhood leukemia, particularly with onset in infancy, may originate from genetic damage to hematopoietic cells acquired *in utero* (Greaves 1993). As discussed above, children of mothers occupationally exposed to benzene during pregnancy have been reported to demonstrate an increased risk of acute nonlymphocytic leukemia (mainly acute myeloid leukemia) (Shu et al., 1988), although a link to *in utero* damage to hematopoietic cells has not been investigated.

C.3. Developmental toxicity: Other relevant data

C.3.1. Distribution and metabolism in pregnant females and conceptuses

Some information has been located concerning distribution and metabolism of benzene in pregnant females and conceptuses. There is 1 study in humans touching on distribution, and 3 studies in mice of distribution and/or metabolism.

Volatile organic compounds in human maternal and cord blood samples were studied by gas chromatography-mass spectroscopy (Dowty et al. 1976). The subjects were 11 normal term deliveries at a hospital in New Orleans. Numerous volatile organics were identified in maternal and cord blood. The authors stated that benzene, carbon tetrachloride, and chloroform were present in cord blood in concentrations equal to or greater than those in maternal blood. No quantitative data was presented. In 2 chromatographic profiles, benzene was identified in peaks which coincided with carbon tetrachloride in one instance, and cyclohexane in the other instance. If and/or how benzene was independently quantified was not stated in the article.

The distribution of ^{14}C-benzene and its metabolites in pregnant mice was studied by liquid scintillation counting and autoradiography (Ghantous and Danielsson 1986). Mice were exposed to ^{14}C-benzene at about 2000 ppm by inhalation on GD 11 or 17 for 10 minutes. Samples for liquid scintillation counting were either frozen immediately on solid CO_2 or kept at 50^{o} C for 24 hours to evaporate volatile substances. No determination was made of which benzene metabolites would volatilize under these circumstances.

Table C.3.1.1A. Tissue concentrations of volatile[(1)] radioactivity in mouse after ^{14}C-benzene inhalation on GD 17(Ghantous and Danielsson 1986).

Tissue	0 hours	0.5 hours	1 hour	4 hours
lung	219 ± 100[(2)]	33 ± 32	30 ± 30	5.9 ± 3.0
liver	161 ± 58	22 ± 5.6	16 ± 7.8	9.4 ± 5.2
kidney	152 ± 52	12 ± 5.9	9.1 ± 4.7	0.4 ± 0.4
brain	315 ± 179	3.3 ± 2.1	1.8 ± 1.1	0
fat	204 ± 103	121 ± 29	116 ± 21	1.4 ± 1.4
plasma	-[(3)]	-[(3)]	-[(3)]	-[(3)]
placenta	61 ± 12	7.9 ± 1.6	5.2 ± 1.6	0.7 ± 0.3
fetus	24 ± 6.9	3.5 ± 0.8	2.5 ± 0.8	0.1 ± 0.1

(1) Volatile radioactivity = total radioactivity - non-volatile radioactivity (see footnote to Table C.3.1.1B, below).
(2) Radioactivity determined by liquid scintillation counting. Values in dpm/mg or dpm/μL, mean ± standard error, n = 4.
(3) Sample not taken.

Table C.3.1.1B. Tissue concentrations of non-volatile[(1)] radioactivity in mouse after ^{14}C-benzene inhalation on GD 17(Ghantous and Danielsson 1986).

Tissue	0 hours	0.5 hours	1 hour	4 hours
lung	40 ± 30[(2)]	32 ± 5.6	27 ± 7.2	5.9 ± 1.3
liver	55 ± 44	86 ± 16	89 ± 25	17 ± 4.9
kidney	15 ± 9.9	61 ± 9.1	76 ± 21	13 ± 3.5
brain	2.8 ± 0.9	5.1 ± 1.0	5.7 ± 1.8	3.3 ± 0.6
fat	5.2 ± 1.5	30 ± 10	23 ± 7.9	10 ± 3.0
plasma	193	91 ± 20	54 ± 21	18 ± 13
placenta	4.6 ± 1.8	15 ± 0.7	15 ± 1.7	4.3 ± 0.4
fetus	0.7 ± 0.2	2.7 ± 0.3	5.8 ± 0.6	4.3 ± 0.5

(1) Non-volatile radioactivity is that radioactivity remaining after heating samples to 50^{o} C for 24 hours.
(2) Radioactivity determined by liquid scintillation counting. Values in dpm/mg or dpm/μL, mean ± standard error, n = 4.

Selected results of liquid scintillation counting of tissue samples from GD 17 treated animals are presented in Table C.3.1 (above). It can be seen that the highest concentrations of volatile compounds were found immediately after exposure. Concentrations of volatile compounds dropped substantially after 0.5 and 1 hours, and were reduced to very low levels after 4 hours. Non-volatile compounds generally peaked at 0.5 to 1 hour after exposure, except in the maternal lung and plasma. The concentrations dropped substantially after 4 hours. The concentrations of both volatile and non-volatile compounds in the placenta and fetus were substantially lower than in maternal tissues.

In the same study, autoradiography of tissue sections was performed after exposures to ^{14}C-benzene on GD 11 and GD 17. The results qualitatively confirmed the liquid scintillation results. The autoradiographic studies found that radioactivity in GD 11 embryos was widely distributed. Radioactivity in GD 17 fetuses was also broadly distributed, but liver contained more than other tissues. In addition, "firmly tissue bound metabolites" were found in maternal liver and kidneys, but not in fetal tissues.

Another study examined the distribution of ^{14}C-benzene-derived radioactivity in mouse fetuses. Maternal mice were injected (i.p.) with small amounts (5 μL /mouse) of ^{14}C-benzene for 1, 2, or 4 days starting on gd 15.5. The tissue concentrations of radioactivity (i.e. including benzene and all metabolites) for were measured for placenta, whole fetus, and fetal liver. Radioactivity was found in all tissues measured, with differences between tissues less than a factor of 2. Fetal liver concentrations were similar to whole fetus for the 1 or 2 day administrations, but approached twice as much for 4 day administrations. No data for maternal tissues other than placenta was presented (Wierda et al. 1989).

One study compared the production of urinary metabolites among male, non-pregnant female, and pregnant female mice (see Table C.3.1.2). The mice were administered 880 mg benzene/kg by gavage. The mice were 6-8 weeks old; the pregnant females were treated with benzene on GD 16 or 17. In general, it was found that the males metabolized a substantially larger fraction of the benzene than did the females. There were some quantitative variations in the amounts of individual metabolites produced, with no obvious simple pattern. This study also examined the production of micronuclei in polychromatic erythrocytes (a clastogenic effect), and found no single metabolite or combination of metabolites which correlated with micronuclei over all 3 groups (Harper et al. 1989).

Table C.3.1.2. Production of urinary metabolites by mice after a single oral dose of benzene (880 mg/kg) (Harper et al. 1989).

	Male	Female	Pregnant female
Total phenols[(1)]	13.01	8.04	11.73
Total hydroquinones[(1)]	2.10	1.10	1.08
Total catechols[(1)]	0.55	0.71	0.61
Free muconic acid[(1)]	0.20	0.12	0.10
Percent of total dose present as major urinary metabolites	58%	38%	30%
Average mouse weight	34 g	26 g	42 g
Total dose	30.8 mg	22.9 mg	37.0 mg

[(1)] Total phenols, hydroquinones, catechols and muconic acid expressed as amount per 30g of mouse weight.

There are several studies in mice which indirectly indicate that benzene and/or its metabolites are able to reach the embryo or fetus. In these studies, benzene was administered at high levels (up to 2 mL/kg) once or twice by gavage (Ciranni et al. 1988; Harper et al. 1989) or i.p. injection (Ning et al. 1991; Sharma et al. 1985; Xing et al. 1992). Elevated frequencies of micronuclei or sister chromatid exchange were found in embryonic or fetal liver (the site of fetal hematopoiesis). There were statistically significant increases in all studies except that of Harper et al. 1989. These results support the interpretation that, at least at high doses, benzene and/or its metabolites reach the embryo or fetus in sufficient quantities to affect cellular processes.

No studies of the metabolism of benzene or its metabolites by embryonic or fetal tissue were located. However, numerous other xenobiotics have been found to be metabolized in embryonic tissues (Juchau et al. 1992). A number of P450 activities have been found in mammalian species, including humans. Some of these are inducible, although many appear to have different characteristics than the adult P450 isoforms. Other activities, including peroxidase activity and reduction activity have also been identified. It is possible, then, that embryonic and/or fetal metabolism of benzene and/or its metabolites occurs.

In summary, the available information indicates that benzene and its metabolites are widely distributed in pregnant mammals, and are able to reach the placenta and fetus. In mice, under short exposure conditions, the concentrations of benzene and benzene metabolites in the fetus was lower than in most maternal tissues. Late in gestation, the fetal liver had somewhat higher levels of benzene and/or benzene metabolites than the

whole fetus. Metabolism in mice was found to be qualitatively similar among males and pregnant and non-pregnant females, although males metabolized substantially more than females.

C.3.2. Mechanism(s) of benzene developmental toxicity.

C.3.2.1. Active agent

Although it is widely agreed that benzene metabolites are responsible for the non-reproductive toxicities (see Section B.4), it is not clear whether this extends to developmental endpoints. There are *in vitro* and *in vivo* studies which contain potentially relevant information. Comparison of benzene to toluene and xylene is potentially informative because toluene and xylene have similar physical-chemical properties to benzene (all are volatile aromatic hydrocarbons). However, toluene and xylene are metabolized primarily by hydroxylation of the methyl side groups, rather than the benzene ring (ATSDR 1994; ATSDR 1995). Thus, the metabolic products of toluene and xylene are distinctly different from those of benzene. Studies of the developmental effects of benzene metabolites are also potentially informative.

In 1 study, GD 10 rat embryos were removed and cultured *in vitro*. The embryos were exposed from GD 10.5 to 12 to benzene (0 - 3.14 mM), toluene (0 - 4.06 mM), xylene (0 - 2.70 mM), stryrene (0 - 5.56 mM), or styrene oxide (0 - 0.175 mM). Each of these compounds caused a dose-dependent growth retardation of the embryos. Statistically significant effects were found at 1.56 mM for benzene, 2.25 mM for toluene, 1.89 mM for xylene, 1.0 mM for styrene, and 0.053 mM for styrene oxide. The concentrations tested for the different compounds were not the same: as a consequence, the apparent differences in effective concentrations between benzene, toluene, and xylene may not be meaningful. Styrene and styrene oxide, however, clearly had effects at lower concentrations. The authors suggest that the observed growth retardation was a result of the common property of benzene, toluene, and xylene being solvents. In this interpretation, benzene was regarded as affecting the embryo directly (Brown-Woodman et al. 1994).

In another study, rat embryos were exposed *in vitro* to benzene or its metabolites from GD 10 - 11 for 30 hours. At concentrations up to 1.6 mM benzene, no statistically significant effects on viability, dysmorphogenesis, or growth were found. Addition of rat liver S9 (i.e. a metabolic activating system) to the benzene exposure had no effect on any of these parameters, although total protein (mg/embryo) was reduced at 1.6 mM. Several metabolites of benzene were also tested. In the absence of S9, phenol had no effect up to 1.6 mM. However, in the presence of S9, when exposed to phenol all embryos died at 0.2 mM, reduced embryo length was observed at 0.05 mM, and reduced prosencephalic index was observed at 0.01 mM. The enzyme(s) in the S9 fraction involved in producing these effects appeared to be a P450(s), based upon microsomal localization, NADPH dependence, and variations in effectiveness depending upon which inducers were used. Several other benzene metabolites were also tested without S9. Hydroquinone,

benzoquinone, and catechol all induced 100% embryonic death at 0.1 mM. T,t-muconaldehyde induced 100% embryonic death at 0.05 mM. The authors suggest that a combination of maternal and fetal metabolism of benzene is responsible for the developmental effects (Chapman et al. 1994).

Several studies which examined the effects of benzene exposure on developmental endpoints in experimental animals also examined the effects of toluene and/or xylene. These studies include exposure of rats by inhalation (Hudak and Ungvary 1978; Tatrai et al. 1980b; Ungvary 1985), mice and rabbits by inhalation (Ungvary and Tatrai 1985) and mice by gavage (Nawrot and Staples 1979; Seidenberg et al. 1986; Seidenberg and Becker 1987). The experimental conditions and results relevant to benzene have been discussed above (Section C.3). A comparable discussion of toluene and xylene is beyond the scope of this document. Briefly, however, the experimental animals were exposed to toluene and/or xylene at the same or higher doses or concentrations than the exposure to benzene. In the studies cited above, benzene had the most consistent effects on fetal weight. Toluene reduced fetal weight in mice by inhalation (Ungvary and Tatrai 1985) and in 1 study by gavage (Nawrot and Staples 1979). Xylene reduced fetal weights in rats in 1 study by inhalation (Ungvary 1985) and mice by inhalation (Ungvary and Tatrai 1985). The comparison of results of benzene to toluene and xylene is somewhat difficult; the effects found appear to be influenced by species, route, and length of exposure. While benzene, toluene, and xylene all have been found to reduce fetal weight, benzene did so under a broader range of circumstances than did toluene or xylene.

Metabolic products of benzene have been observed to affect developmental endpoints in experimental animals. Phenol was administered by gavage to rats (0, 30, 60, 120 mg/kg/d) and mice (0, 70, 140, 280 mg/kg/d) on GD 6-15 (RTI 1983a; RTI 1983b). Reductions in fetal weight were found at the high dose in both species. Reduced viability and increased cleft palate were found in the mice, although neither was statistically significant. Reduced maternal weight gain and increased maternal death were observed in the mice, but not in the rats. Hydroquinone was administered by gavage to rats (0, 30, 100, 300 mg/kg/d) on GD 6-15 (Eastman Kodak Co. 1985; Krasavage et al. 1991; Krasavage et al. 1992). Reduced fetal weight and maternal weight gain were found at the high dose. 1,2,3-Trihydroxybenzene was administered orally to pregnant rats at up to 300 mg/kg/d. Increased resorptions, reduced fetal weight, and reduced maternal weight gain were found (Picciano et al. 1983)). It should be noted, however, that this isomer may not be the predominant isomer of trihydroxybenzene formed by metabolism in mammals (see Section B.4).

One study examined the clastogenic effects of several benzene metabolites on mouse fetuses. Benzene metabolites were administered by gavage on gd 13. The quantities administered, in most cases, were similar to the quantities estimated to be produced metabolically by administration of 1 mL/kg (880 mg/kg) of benzene. It was observed that hydroquinone produced a relatively large (statistically significant) increase in fetal liver micronucleated polychromatic erythrocytes (MN PCE). However, catechol and p-benzoquinone produced modest increases, and no statistically significant increases were

observed with either phenol or 1,2,4-benzenetriol. In contrast, all these metabolites produced statistically significant increases in MN PCE from maternal bone marrow (Ciranni et al. 1988).

Overall, it appears that the data point to effects on developmental endpoints of benzene and of its metabolites. Toluene and xylene have similar physical-chemical properties but different metabolites then benzene. Toluene has been found to produce reduced fetal weight in animal models, and xylene have been found to produce similar effects in some studies. However, toluene and xylene produce effects under a more limited set of circumstances than does benzene. Three metabolites of benzene have been shown to produce reduced fetal weight. However, these studies were by gavage at relatively high doses. The data appear consistent with both direct effects of benzene and with effects of benzene metabolites.

C.3.2.2. Biological mechanisms of action

The mechanisms by which benzene is thought to produce its characteristic chronic toxicity (leukemia, anemia) in adults may also apply to its developmental toxicity. There is an obvious link between potential benzene effects on hematopoietic progenitor cells of the adult to those of the fetus. Benzene has been shown to have clastogenic effects and to disrupt differentiation and proliferation of these cells under the influence of growth factors (particularly GM-CSF) (Irons et al. 1992) These proposed mechanisms, excepting those specific to the bone marrow site of hematopoiesis, would potentially apply to fetal liver hematopoiesis.

Although not discussed in the developmental toxicology literature, mechanisms of benzene hematopoietic effect involving response to growth factors and cytokines could also be responsible for intrauterine growth retardation. In particular, GM-CSF is produced in large amounts by uterine epithelial cells under the regulatory control of gonadal hormones (Giacomini et al. 1995; Robertson et al. 1996). It has been identified as an important trophoblast growth factor in supporting placental and fetal growth (Drake and Head 1994; Guilbert et al. 1993). If benzene-induced changes in the response to GM-CSF occur in trophoblast, as they do in hematopoietic cells, this could potentially contribute to a placental insufficiency that would limit fetal growth.

C.4. Integrative evaluation

Studies in rats, rabbits and mice were consistent in reporting fetal growth retardation and delayed ossification when benzene was administered during organogenesis. In some of these studies, but not all, maternal toxicity was reported to occur concurrent with exposures that produced adverse fetal effects. This pattern of effects is consistent with delayed development. Malformations were rarely reported. The study quality was generally good, and studies using protocols meeting federal regulatory testing guidelines were included. Benzene is known to be genotoxic and several studies demonstrated that chromosome abnormalities were seen in fetal as well as maternal tissues when benzene was administered during pregnancy to mice. Also, a series of studies presented a detailed description of fetal hematopoietic effects of benzene administration to mice during organogenesis. Studies on retarded development and disrupted fetal hematopoiesis used an inhalation route, the route most common in humans. Studies with intrauterine exposure and postnatal evaluation were limited to hematopoietic endpoints.

There are very few animal studies using exposure before or throughout pregnancy and lactation and examining endpoints related to developmental toxicity. There are no available multigeneration studies. Scattered information from less recent and less completely reported studies have suggested benzene effects on pregnancy outcome variables but confirmatory work is lacking.

Compared to the results of toxicity testing in animals, human data are more limited and do not clearly support or contradict the more extensive animal data base. Many human studies were of multiple solvent or contaminant exposure including benzene and did not report separate findings for benzene. The largest case-control study of fetal loss which performed separate analyses in relation to a measure of benzene exposure (inferred from occupational category) found a positive association with stillbirth (OR = 1.3, 95% CI = 1.0-1.8); risk increased with increasing exposure (for the highest exposure category, OR = 1.4; 95% CI = 1.1-1.9). Corresponding endpoints in animal studies (dead fetuses, litter size) were not influenced by benzene exposure. In another study, self-reported maternal occupational benzene exposure was found to be associated with statistically significant increased risks of acute nonlymphocytic leukemia (OR = 4.0; 95% CI = 1.8-9.3). Reviews of the clinical literature suggests that leukemias occurring early in childhood may result from genotoxicity to hematopoietic cells *in utero*. Since benzene has been shown in animals to cause genotoxicity to the fetus when administered during pregnancy, there is a some indication of a biologically plausible link between benzene genotoxicity and childhood leukemia findings.

Benzene is lipid soluble and readily crosses the placenta. Reactive metabolites of benzene have been identified in the fetus, but direct information about transformation to reactive metabolites by the fetus is lacking. Developmental toxicology studies with other organic solvents (toluene, xylene) and with benzene metabolites appear consistent with involvement of both direct action of benzene and with actions of benzene metabolites. Effects of benzene on chromosomes and hematopoietic precursors demonstrated in adults

could be responsible for similar effects in the fetus. There has been no direct investigation of mechanism whereby benzene exposure could produce delayed intrauterine development.

Table C.2.1. Animal studies of the developmental effects of benzene by inhalation
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Reference	Study Design	Reported effects[1]
(Coate et al. 1984; Hazelton Laboratories 1982)	rat gd 6-15, 6 hr/d 0, 1, 10, 40, 100 ppm	Maternal death not increased, weight gain not reduced at any concentration. Fetal weight reduced (7%) (SS) at 100 ppm. Embryonic or fetal death or malformations not increased, litter size not reduced at any concentration.
(Corti and Snyder 1996)	mouse gd 6-16, 6 hr/d 0, 10 ppm	Maternal hematological parameter (CFU-e) not changed. Fetal and postnatal hematological parameter (CFU-e) reduced (SS) in males only at 10 ppm. Embryo or fetal death not increased, litter size, fetal or birth weight not reduced at concentration tested.
(Dobrzanska-Tatarczuch and Starek 1991; Starek et al. 1991) [Full articles in Polish, not available to OEHHA; abstract only available in English.]	rat gd 8-15, 6 hr/d 0, 1500, 3000 mg/m^3 (0, 460, 920 ppm)[2]	Maternal weight gain reduced, hematological parameters altered (leukocyte and lymphocyte counts reduced) (effective concentration not clear). Delayed ossification (effective concentration not clear). (Statistical significance not addressed.)
(Gofmekler 1968; Pushkina et al. 1968)	rat 10-15 d (20d?) + mating(?) + gestation, 24 hr/d 0, 1.0, 5.6, 20.4, 47.3, 56.6, 63.3, 670 mg/m^3 (0, 0.31, 1.7, 6.2, 14.5, 17.4, 19.4, 205 ppm)[2]	No pregnancies at 670 mg/m^3. Litter size reduced at 20.4, 47.3, [not 56.6], 63.3 mg/m^3. Malformations not increased, birth weight not reduced at any concentration. (Statistical significance addressed only for birthweight.)
(Green et al. 1978)	rat gd 6-15, 6 hr/d 0, 100, 300, 2200 ppm	Maternal weight gain reduced (SS) at 2200 ppm. Fetal weight reduced (10%) (SS), crown-rump length reduced (SS) at 2200 ppm. Missing sternebrae increased (SS) at 100, [not 300], 2200 ppm. Delayed ossification increased (SS) only in female fetuses at 300, 2200 ppm. Embryo or fetal death or malformations not increased, litter size not reduced at any concentration.
(Hudak and Ungvary 1978)	rat gd 9-14, 24 hr/d 0, 1000 mg/m^3 (0, 307 ppm)[2]	Maternal weight gain reduced (SS) at 1000 mg/m^3. Maternal death not increased at concentration tested. Fetal weight reduced (SS), retarded skeletal development (SS), fused sternebrae and extra ribs increased (SS) at 1000 mg/m^3. Embryo or fetal death or malformations not increased at concentration tested.

Table C.2.1. Animal studies of the developmental effects of benzene by inhalation
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Reference	Study Design	Reported effects[(1)]
(Keller and Snyder 1986)	mouse gd 6-15, 6 hr/d 0, 5, 10, 20 ppm	Increased and/or reduced fetal and/or postnatal hematological parameters (BFUe, CFUe, GM-CFU) (some, but not all SS: see text) at 5, 10, 20 ppm. Embryo or fetal death or malformations not increased, litter size or fetal weight or birth weight not reduced at any concentration.
(Keller and Snyder 1988)	mouse gd 6-15, 6 hr/d 0, 5, 10, 20 ppm Offspring examined at GD 16, PND 2, PNWK 6	Maternal death not increased, weight gain not reduced at any concentration. Peripheral blood early nucleated red cells reduced (SS) on PND 2 (but not GD 16 or PNWK 6) at 5, 10, 20 ppm. Peripheral blood late nucleated red cells reduced, non-dividing granulocytes increased (SS) on PND 2 (but not GD 16 or PNWK 6) at 20 ppm. Embryo or fetal death or malformations not increased, litter size or fetal weight or birth weight not reduced at any concentration tested. Peripheral blood RBC, total nucleated cells not reduced at any concentration.
(Kuna and Kapp 1981)	rat gd 6-15, 7 hr/d 0, 10, 50, 500 ppm	Maternal weight gain reduced (SS) from GD 5-15 (but not GD 0-20) at 50, 500 ppm. Maternal death not increased, maternal RBC or WBC counts not reduced at any concentration. Malformations increased at 500 ppm (1/98 exencephaly, 1/98 angulated ribs, 2/98 forefeet ossification out of sequence). Fetal weight reduced (14%, 18%) (SS), delayed ossification increased, skeletal and visceral variants increased (SS) at 50, 500 ppm. Crown-rump length reduced (SS) at 500 ppm. Embryo or fetal death not increased, litter size not reduced at any concentration.
(Kuna et al. 1992); (Bio/dynamics Inc. 1980a)	rat 10 wks at 5d/wk, then GD 0-20 and PND 5-20 0, 1, 10, 30, 300 ppm	Maternal death not increased, weight gain not reduced at any concentration. Postnatal weight gain reduced on PND 21 (SS, females only), but not PND 4 or 14 at 300 ppm. Litter size not reduced, postnatal death not increased, malformations not increased, birth weight not reduced at any concentration.
(Maltoni et al. 1983; Maltoni et al. 1985; Maltoni et al. 1989)	rat starting on GD 12: 0 ppm or: 200 ppm, 4 hr/d, 5d/wk for 7 wks, then 200 ppm, 7 hr/d, 5d/wk for 12 wks, then 300 ppm, 7 hr/d, 5 d/wk for 85 wks	Maternal weight gain reduced, cancer increased, WBC reduced, at 200/300 ppm. Maternal death not increased, RBC not reduced, at concentration tested. Postnatal death increased, cancer increased (multiple sites), weight gain reduced, WBC reduced, at 200/300 ppm. Postnatal RBC not reduced at concentration tested. (Statistical significance not addressed)

Table C.2.1. Animal studies of the developmental effects of benzene by inhalation
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Reference	Study Design	Reported effects[1]
(Maltoni et al. 1983; Maltoni et al. 1985; Maltoni et al. 1989)(continued)	rat starting on GD 12: 0 ppm or: 200 ppm, 4 hr/d, 5d/wk for 7 wks, then 200 ppm, 7 hr/d, 5d/wk for 8 wks (Endpoints monitored at 26-118 weeks)	Postnatal death not increased, weight gain not reduced at concentration tested. Postnatal cancer increased (multiple sites) at 200 ppm. (Statistical significance not addressed)
(Murray et al. 1979)	mouse gd 6-15, 7 hr/d 0, 500 ppm	Maternal weight gain not reduced, hematological parameters (RBC, PCV, Hgb, WBC) not changed at concentration tested. Fetal weight reduced (6%) (SS), delayed ossification increased (SS) at 500 ppm. Embryo or fetal death or malformations not increased, litter size not reduced at concentration tested.
	rabbit gd 6-18, 7 hr/d 0, 500 ppm	Maternal weight gain not reduced, hematological parameters (RBC, PCV, Hgb, WBC) not changed at concentration tested. Embryo or fetal death or malformations not increased, litter size not reduced, fetal weight not reduced, fetal hematological parameters (RBC, PCV, Hgb, WBC) not changed at concentration tested.
(Tatrai et al. 1980a); (Hudak et al. 1980) [Hudak et al. full article in Hungarian, not available to OEHHA: abstract only available in English]	rat gd 7-14, 24 hr/d 0, 150, 450, 1500, 3000 mg/m^3 (0, 46, 138, 460, 920 ppm)[2] (Author's calculation; equivalent to: 0, 50, 150, 500, 1000 ppm)	Maternal death increased: 0/48 controls, 0/20 at 150 mg/m^3, 3/20 at 450 mg/m^3, 1/22 at 1500 mg/m^3, 3/22 at 3000 mg/m^3. Maternal weight gain reduced (SS) at 150, 450, 1500, 3000 mg/m^3. Embryo or fetal death increased (SS) at 450, 1500, 3000 mg/m^3. Fetal weight reduced (5%, 29%, 20%, 22%) (SS) at 150, 450, 1500, 3000 mg/m^3. Delayed ossification increased (SS) at 450, 1500, 3000 mg/m^3. Malformations not increased at any concentration.
(Tatrai et al. 1980b; Ungvary 1985)	rat gd 7-14 (as reported in Tatrai et al. 1980b), gd 7-15 (as reported in Ungvary 1985), 24 hr/d 0, 400 mg/m^3 (0, 123 ppm)[2]	Maternal weight gain reduced (SS) at 400 mg/m^3. Maternal death not increased at concentration tested. Fetal weight reduced (20%) (SS), skeletal retardation increased (SS) at 400 mg/m^3. Embryo or fetal death or malformations not increased at concentration tested.

Table C.2.1. Animal studies of the developmental effects of benzene by inhalation
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Reference	Study Design	Reported effects[1]
(Ungvary and Tatrai 1985)	mouse gd 6-15, 3 times 4 hr/d 0, 500, 1000 mg/m^3 (0, 153, 307 ppm)[2]	Weight retarded (SS), skeletal retarded (SS) fetuses increased at 500, 1000 mg/m^3. Embryo or fetal death or malformations not increased at any concentration.
	rabbit gd 7-20, 24 hr/d 0, 500, 1000 mg/m^3 (0, 153, 307 ppm)[2]	Maternal death increased: 0/60 control, 0/11 at 500 mg/m^3, 2/15 at 1000 mg/m^3. Maternal weight gain reduced (SS) at 1000 mg/m^3. Embryo or fetal death increased (SS), fetal weight reduced (SS), minor anomalies increased (SS) at 1000 mg/m^3. Malformations, skeletal retardation not increased at any concentration tested.

[1] Effects reported by authors to be statistically significant (SS) at $p < 0.05$, or biologically noteworthy.
[2] Benzene concentrations reported in mg/m^3 were converted to ppm using the relationship 1.0 ppm = 3.26 mg/m^3 (ATSDR 1993).

Table C.2.2. Animal studies of the developmental effects of benzene by gavage

Reference	Study Design	Reported effects[1]
(Nawrot and Staples 1979)[Abstract]	mouse gd 6-15 0, 0.9, 1.5, 3.0 mL/kg/d (0, 0.78, 1.32, 2.64 g/kg/d)[2]	Maternal death increased (SS) at 1.5, 3.0 mL/kg/d. Embryo or fetal death increased (SS) at 1.5, 3.0 mL/kg/d. Fetal weight reduced (SS) at 0.9, 1.5, 3.0 mL/kg/d. Malformations not increased at any dose.
	mouse gd 12-15 0, 3.0 mL/kg/d (0, 2.64 g/kg/d)[2]	Maternal death increased (SS) at 3.0 mL/kg/d. Embryo or fetal death increased (SS), fetal weight reduced (SS) at 3.0 mL/kg/d. Malformations not increased at dose tested.
(Seidenberg et al. 1986; Seidenberg and Becker 1987)	mouse gd 8-12 1300 mg/kg/d	Maternal death not increased, weight gain not reduced at dose tested. Birth weight reduced (4%) (SS) at 1300 mg/kg/d. Fetal death not increased, litter size not reduced, postnatal weight gain not reduced at dose tested.

[1] Effects reported by authors to be statistically significant (SS) at $p < 0.05$ or biologically noteworthy.
[2] Benzene doses reported in mL/kg were converted to g/kg using density = 0.87865 g/mL (CRC 1976).

Table C.2.3. Animal studies of the developmental effects of benzene by injection

Reference	Study Design	Reported effects[1]
(Iwanaga et al. 1970) (Japanese, with abstract, tables and graphs in English)	mouse (specific route not known) maternal: 0 or 4 mL/kg on GD 6, 9, or 12. followed by: offspring: 0 or 0.1 mL/kg/d for 5 days, starting at 70 days of age	Subsequent to maternal treatment: Maternal deaths: control 0/7, GD 6 at 4 mL/kg 1/7, GD 9 at 4 mL/kg 0/5, GD 12 at 4 mL/kg 0/7. Maternal weight at term reduced (SS) at 4 mL/kg for GD 6, but not GD 9 or 12 treatment compared to controls. No effects on maternal weight gain. Percent live pups smaller at birth (SS) compared to controls for GD 6 and GD 12 groups. No effects on litter size. WBCs reduced (SS) on PND 70 in female offspring after 4 mL/kg on GD 9 or 12, but elevated after GD 6 treatment compared to controls. No treatment effects on postnatal weight PND 7-70. Subsequent to maternal and offspring treatment: Body weight lower (SS) in male offspring treated on PND 9, compared to controls, after treatment with 0.1 mL/kg/d. Hemoglobin reduced (SS) in female offspring treated on GD 12, compared to controls, after treatment with 0.1 mL/kg/d. Reduction in WBCs smaller (SS) in female offspring treated on GD 9 or 12, compared to controls, after treatment with 0.1 mL/kg/d. Thymus weight lower (SS) in males and females treated on GD 9 or 12, compared to controls, after treatment with 0.1 mL/kg/d. Spleen weights lower (SS) in males treated on GD 12, compared to controls, after treatment with 0.1 mL/kg/day.
(Watanabe and Yoshida 1970)	mouse s.c. 1x on GD 11-15 3 mL/kg (2.6 g/kg/d)[2]	Malformation frequency varied by day of treatment: gd frequency 11 1/113 12 0/102 13 10/127 14 2/98 15 0/38 (Statistical significance not addressed.)
(Wierda et al. 1989)	mouse i.p. gd 12.5-19.5, 2x/d 0, 100 mg/kg/treatment (0, 200 mg/kg/d)	Maternal hematological parameters altered (B and pre-B lymphocytes in bone marrow reduced) (SS) at 200 mg/kg/d. Fetal and neonatal hematological parameters altered (pre-B lymphocytes reduced, altered LPS response) (SS) at 200 mg/kg/d.

[1] Effects reported by authors to be statistically significant (SS) at $p < 0.05$ or biologically noteworthy.
[2] Benzene doses reported in mL/kg were converted to g/kg using density = 0.87865 g/mL (CRC 1976).

D. Female Reproductive Toxicity

D.1. Human female reproductive toxicity studies

All of the studies that have examined the relationship of benzene exposure and female reproductive toxicity had study populations with simultaneous exposure to other chemicals, and did not assess the effects of benzene exposure specifically (Michon 1965; Michon and Pilat 1968; Huang 1991). Elevated incidence of abnormalities of menstruation (prolongation of bleeding and abnormal intensity) (Michon 1965) and statistically significant differences in blood lost during labor (Michon and Pilat, 1986) were reported in cross-sectional studies of women exposed in a factory producing leather and rubber shoes in Poland. In China, a study of reproductive function of female workers in a leather shoe factory reported statistically significant differences in the incidence rate of menstrual disorders in women exposed to benzene and toluene; the authors also reported an increase in incidence rates with the length of employment. However, none of these studies provided information on the method for identification of the comparison group or indicated any matching of exposed and unexposed study subjects on important potential confounders such as age. These studies are discussed in more detail below.

Information regarding female reproductive toxicity in relation to benzene exposure in the absence of exposure to other organic solvents is provided by 2 published case series (Smith 1928; Vara and Kinnunen 1946). Smith (1928) reported on benzene toxicity in women occupationally exposed to benzene (n = 79) in 6 factories in New York in the mid 1920s, of whom 30 had blood counts indicating chronic poisoning; of these, 10% reported menstrual irregularities (profuse bleeding and/or more frequent menstruation) since starting to work in the factory. In addition, Smith (1928) reported on a death due to severe uterine hemorrhage following birth of a premature child in a woman who had shown signs of benzene poisoning after becoming pregnant. Vara and Kinnunen (1946) reported female reproductive toxicity in 12 of 30 women occupationally exposed to benzene, including ovarian hypoplasia, abnormal menses and reduced fertility. In addition, case series and case reports describing female reproductive toxicity resulting from exposure to mixed aromatic hydrocarbons or other mixtures including benzene were identified (Messerschmitt 1972; Mikulandra et al. 1993). Messerschmitt (1972) reviewed 5 case reports of severe anemia following exposure to benzene solvents and arseno-benzenes, which led to the death of 4 of 5 mothers at parturition, and 3 of 5 infants. Mikulandra *et al.* (1993) reported irregular menstrual cycles among 20 women workers in a factory in Croatia following a change in the composition of paints and glues used there; subsequent measurements found benzene at 5 times the permissible limit (not specified), as well as 3 and 10 times the permissible limits of toluene and cyclohexane, respectively. The lack of information on exposure levels and comparison groups in these case series and studies make it difficult to draw conclusions based on their results

Michon (1965)

Michon (1965) compared disturbances of menstruation in 500 women aged 20 to 40 years who worked in a factory producing leather and rubber shoes and were exposed to benzene, toluene, and xylene to 100 unexposed women. The unexposed women did not work in shoe production, but the method of identifying the comparison group was not described, nor was any indication made of matching to exposed women on the basis of age or other characteristics. Exposure levels in the factory were noted as being below the allowable limits (0.1 mg/liter previously, 0.25 mg/liter of air at the time of the study) for benzene and toluene. Although 5 different subgroups of exposed subjects were identified based on job activities, the relationship of job category to exposure levels was not discussed. Regularity, length and intensity of menstruation were recorded for all study subjects. In the exposed women 12.6% had prolonged menstruation compared to 6% in the unexposed, with "pathologically long" menstruation in 4.4% and 3%, respectively. Abnormal intensity of menstruation was found in 3.8% of exposed women compared to 2% of controls.

Michon and Pilat (1968)

A later cross-sectional study by Michon and Pilat (1968) investigated blood loss during the third stage of labor among 329 women exposed during pregnancy to benzene and toluene while working in a factory producing rubber and leather goods (the same factory investigated by Michon, 1965) in comparison to 286 women working in other industries. As above, the method of identifying the comparison group was not described, nor was any indication made of matching to exposed women on the basis of age or other characteristics. The exposure levels were not described. The authors found statistically significant differences in the mean blood lost during the third stage of labor by women giving birth for the first time (350 mL, exposed; 310 mL, unexposed) as well as by women who had previously given birth (270 mL, exposed; 230 mL, unexposed). The authors noted that these blood losses did not exceed the limits accepted as normal. They concluded that the type of employment, specifically the exposure to aromatic hydrocarbons, accounted for the differences found in this study.

Huang et al. (1991)

Huang et al. (1991) studied the reproductive function of 223 women exposed to benzene and toluene while working in a leather shoe factory in China compared to 327 unexposed women. No information on the method for selecting the comparison group, any matching of the exposed and unexposed on age or other characteristics, or on the exposure levels was provided in the English language abstract of this cross-sectional study. Statistically significant differences were found in the incidence rate of menstrual disorder (48.88% exposed, 16.21% unexposed), with a tendency for increases in rates of mense-blood anomaly and dysmenorrhea with length of employment. As discussed in Section C.1.2, these authors also reported statistically significant increased rates of spontaneous abortion (5.7% exposed, 2.4% unexposed) and gestosis (22.6% exposed, 10.5% unexposed). OEHHA is attempting to obtain the full article (published in a Chinese journal) for more complete information about this study.

D.2. Animal female reproductive toxicity studies

Information on female fertility is limited to 1 fully reported single generation study using rats and several older studies in non-English-language journals. In addition, reproductive organ toxicity data are available from chronic and subchronic toxicity studies. These studies are described below and are outlined in Tables D.2.1-D.2.5 at the end of Section D.

D.2.1. Fertility

In the most complete study relevant to fertility, rats were exposed via inhalation (1, 10, 30, 300 ppm, 6 h/day) for 10 wks prior to mating and throughout pregnancy and lactation (Kuna et al. 1992). The group size for this experiment was 21-25 pregnancies/group and appropriate statistics were used. Fertility parameters (percentage pregnant, gestation length, litter size, gender ratio, conceptus loss, viability index and lactation index) were remarkably similar among the groups, with no indication of treatment related effects. Similarly, no effects on maternal body weight were identified. The protocol called for evaluation of estrous cycles via vaginal smears, but no data were reported. The only parameter influenced by benzene in this study was postnatal growth. Pups from the highest concentration group (300 ppm) were smaller than those of the other groups throughout lactation, although the effect was statistically significant only for the female pups on PND21. Pups may have been exposed to benzene vapors along with their dams during lactation, although this is not explicitly stated. Thus postnatal weight retardation may have been due to postnatal exposure of the pups rather than toxic effects on the dam which interfered with lactation. Liver weights on PND 21 were also lower in the 300 ppm female pups than in other groups.

In an early Russian study with an English translation, fertility and reproductive toxicity were evaluated in female rats exposed to relatively low concentrations of benzene (5 concentrations ranging from 1 to 63.3 mg/ m^3, 24 h/day, with an additional 670 mg/ m^3 group) for 10-15 days prior to mating and, presumably, continuing through gestation (Gofmekler 1968). The authors reported “a complete absence of pregnancy” in the 670 mg/ m^3 (205 ppm) group when each female was mated with a single male for 6-10 days (2 estrous cycles). Although this concentration (205 ppm) was lower than that found by others (Kuna et al. 1992) to have no effect on fertility, the daily exposure was longer (24 h vs 6 h), and the protocol provided less of an opportunity for mating (10 days vs 21 days). In addition, mating occurred during benzene exposure; introducing the possibility of interference with mating by benzene-induced CNS effects, and the possibility of male mediated effects on fertility. The authors also stated that the number of offspring per female was related to the benzene exposure concentration, although no statistical analysis was presented. A statistical analysis demonstrated no adverse effect of benzene exposure on newborn body weights, although both increases and decreases in relative organ weight were reported, depending on the organ and the benzene concentration. Analysis of ascorbic acid, nucleic acid and DNA concentration in offspring in another publication also reported inconsistent effects across doses (Gofmekler et al. 1968). The lack of detail

in the report and the inconsistent findings across doses make this information difficult to integrate into hazard identification (Barlow and Sullivan 1982).

A series of early studies published in the French language investigated the effect of pregnancy on benzene-induced hematotoxicity in rats, rabbits and guinea pigs (Desoille et al. 1963; Desoille et al. 1965; Desoille et al. 1967). Pregnancy appeared to enhance the reduction in WBCs caused by benzene in rats, but to reduce it in rabbits (no WBC effect was seen in guinea pigs) (Barlow and Sullivan 1982). Some information on pregnancy outcome was also presented. Benzene was administered subcutaneously at a dose of 100 mg/kg/day throughout gestation. For rats, there was no effect of benzene on maternal weight gain, gestation length, litter size, neonatal mortality or postnatal weight gain. For rabbits, there was no effect on gestation length, pregnancy loss, neonatal mortality or gross malformations. For guinea pigs, there was no effect on maternal weight gain, gestation length, number of offspring, neonatal body weight or neonatal mortality.

An early Eastern European study of female reproduction in rats (Avilova and Ulanova 1975) has been described by Barlow and Sullivan (1982). Reported inhalation concentrations were low (1.6 or 9.4 ppm). Barlow and Sullivan described "small but inconsistent" changes in the oestrus cycle, no effect on pregnancies, implantation or resorption, a significant increase in preimplantation loss in the 1.6 but not the 9.4 ppm group, an increase in body weight and incidence of hemorrhage in 9.4 ppm offspring at birth, and a decrease in body weight of 9.4 ppm offspring at 1 month of age. Limited detail in reporting of methods, results and statistical analysis make interpretation of this study difficult.

D.2.2. Reproductive organ toxicity

No reproductive tract pathology or histopathology was noted in rats in a subchronic toxicity study using inhalation exposure of 0, 1, 10, 30 and 300 ppm (Ward et al. 1985).

In the same study, 4 of 20 female mice exposed to the highest inhalation dose (300 ppm, 6 h/day, 5 d/wk, 13 wks) had bilateral ovarian cysts. The authors state that this finding was compound related and that "similar lesions of doubtful biological significance" were seen at lower doses. No effect on body weight or other absolute or relative organ weights (with the exception of an increased liver to body weight ratio) were seen with the exposure to 300 ppm in female mice. However, there were significant effects on a number of hematology parameters (RBCs, WBCs, hematocrit and hemoglobin) as well as on histopathology of the thymus, spleen and lymph node in the female mice. Female rats (n = 10/group) did not demonstrate changes in hematology or histopathology, but the 10 ppm group had greater thyroid weight and thyroid/body weight ratio than controls.

In a subchronic toxicity study in rabbits, i.p. injections were given at a dose of 350 mg/kg/d for 30d or 700 mg/kg/d for 15 days (Dikshith et al. 1980). No effects on ovarian weight or histopathology were reported. Another study in rabbits was briefly summarized in English (Vara and Kinnunen 1946). Five rabbits were given s.c. injections of 1 g

"benzol" until abnormal hematology was identified. Ovaries were described as reduced in size and demonstrating atresia and abnormal chromosome division, but no incidence data or other information on general toxicity was presented.

In a chronic (2 y) NTP bioassay using gavage exposures of 0, 25, 50 and 100 mg/kg/d (NTP 1986; Huff et al. 1989), reproductive tract tumors (uterus, ovary, mammary gland) were noted in both rats and mice, along with related histopathological observations such as hyperplasia and adenoma. An apparently unrelated observation of "senile atrophy" of the ovaries was reported in mice at all 3 doses. Reproductive organ weights were not reported. However, inferences about reproductive organ toxicity from these studies are limited because of carcinogenesis of the reproductive tract and other organs.

In a series of early experiments, benzene was applied directly to the surface of surgically exposed ovaries of mice (Batra 1959; Batra 1966; Solomon and Batra 1964; Sridharan et al. 1963). Benzene was used as a vehicle control for the treatment of interest, methylcholanthrene. Minimal reporting of methods, data and analysis make interpretation of these older studies difficult. See Table C.2.2. Damage to ova, reduced fertility, increased tumor incidence and malformations in offspring were described with either methylcholanthrene dissolved in benzene, or benzene alone.

D.3. Female reproductive toxicity: Other relevant data

D.3.1. Distribution and metabolism in females

No data was located regarding the distribution or metabolism of benzene in ovaries or other female reproductive organs. For a discussion of general metabolism, see Section B.4. In general, the observations that benzene and its metabolites are widely distributed suggest that they would reach the female reproductive organs.

D.3.2. Chromosomal aberrations and related effects of benzene metabolites

Several metabolites of benzene have been found to produce chromosomal aberrations and related effects *in vivo* or *in vitro*. Phenol, catechol, hydroquinone, p-benzoquinone, and 1,2,4-trihydroxybenzene have been found to produce increased micronuclei in polychromatic erythrocytes from pregnant mouse bone marrow *in vivo* (Ciranni et al. 1988). These metabolites have also been found to produce increases in sister chromatid exchanges in human peripheral lymphocytes *in vitro* (Erexson et al. 1985; Knadle 1985; Morimoto and Wolff 1980; Morimoto et al. 1983). Hydroquinone had been found to produce chromosome aberrations and increased sister chromatid exchanges in Chinese hamster ovary cells *in vitro*. Interestingly, lower concentrations of hydroquinone were required to produce increases in sister chromatid exchanges in the absence of metabolic activation (S9) than in the presence of it. Metabolic activation was required to produce increased chromosomal aberrations (NTP 1989). Use of the sensitive ^{32}P-postlabeling technique has allowed the detection of increased DNA adducts *in vivo* following benzene

exposure (Bauer et al. 1989; Bodell et al. 1996; Li et al. 1996; Snyder et al. 1989), although this has not been consistently observed (Reddy et al. 1989; Reddy et al. 1994). It is believed that these adducts result from the binding of reactive benzene metabolites to DNA (Bauer et al. 1989; Bodell et al. 1996; Snyder et al. 1989). Additionally, recent studies have found that topoisomerase II, a key enzyme involved in relieving torsional stress in DNA, is inhibited by benzene metabolites *in vitro* (Frantz et al. 1996; Hutt and Kalf 1996). Since benzene metabolites are able to produce chromosomal aberrations and related effects in multiple cells types *in vivo* and *in vitro*, it may be that the same effect would occur with germ line cells. This could adversely affect reproduction. However, no evidence has been located which indicates that benzene metabolites would selectively target (or selectively avoid targeting) female reproductive organs.

D.3.3. Effect of benzene on noradrenergic nerves of ovaries and uterus

Pregnant rats were exposed to benzene at 0 or 1,500 mg/m^3 (460 ppm) for gd 8-10. The abundance of fluorescent noradrenergic nerve fibers decreased in the ovary and uterus of exposed animals. Decreased density of fibers was also seen when benzene was injected into the iris of the eye. The authors concluded that there was a selective effect of benzene (also of toluene and p-xylene) on noradrenergic nerve fibers, which could disturb ovarian and uterine blood flow (Ungvary and Donath 1984).

D.4. Integrative evaluation

Endpoints relevant to female reproductive toxicity include estrus/menstrual cycles, fertility, resorption/spontaneous abortion, and damage to ova or reproductive organs. In animals, an appropriately designed, well conducted and adequately reported study using the inhalation route failed to demonstrate benzene effects on estrus cycles, fertility, early pregnancy loss, or damage to reproductive organs. In other studies, effects on conception, and damage to ova or the ovary were mentioned but could not be adequately evaluated. No data were located concerning benzene accumulation in reproductive organs or the production of active metabolites in reproductive organs. Thus, a clear picture of benzene as a female reproductive toxicant did not emerge from the available animal data. In human studies, consistent reports of abnormal menstruation and excessive blood loss during childbirth in women occupationally exposed to benzene were identified in 3 cross-sectional studies and in case series and case reports. All 3 of the cross-sectional studies are limited: the comparison groups were not exposed to the factory environment under study but were not otherwise described; the studies had no apparent matching and poorly described methods. All 3 cross-sectional studies and most of the case series and case reports involve women working in leather and/or rubber factories, and many of the women had benzene-associated toxicity; concurrent exposure to other solvents is likely to have occurred in most cases. More definitive studies with accurate assessment of benzene-specific exposure are needed to further evaluate the associations suggested by these studies.

Table D.2.1. Animal studies of the female reproductive effects of benzene by inhalation

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Reference	Study Design	Reported effects[1]
(Avilova and Ulanova 1975) (Russian: as summarized in Barlow and Sullivan 1982)	rat 4 months ("juveniles" begin at 1.5-2 months of age, "adults" begin at 4 months of age) 0, 5, 30 mg/m^3 (0, 1.6, 9.4 ppm)[2] Mated at end of exposure.	Estrous cycle length reduced (SS) at 1.6 ppm, increased (SS) at 9.4 ppm. Increased pre-implant loss (SS) in adults exposed at 1.6 ppm (not 9.4 ppm). Increased postnatal mortality, reduced body weight (SS) in offspring from juveniles exposed at 9.4 ppm. Increased body weight (SS) and hemorrhage in offspring from adults exposed at 9.4 ppm. Number of pregnancies, implants/dam, and resorptions/dam not affected at any concentration tested.
(Gofmekler 1968; Pushkina et al. 1968)	rat 10-15 d (20d?) + mating(?) + gestation, 24 hr/d 0, 1.0, 5.6, 20.4, 47.3, 56.6, 63.3, 670 mg/m^3 (0, 0.31, 1.7, 6.2, 14.5, 17.4, 19.4, 205 ppm)[2]	Fertility reduced: no pregnancies at 670 mg/m^3. Time from introduction of males until birth tended to increase (concentrations not clear). Litter size reduced at 20.4, 47.3, [not 56.6], 63.3 mg/m^3. Malformations not increased, birth weight not reduced at any concentration. (Statistical significance addressed only for birth weight.)
(Kuna et al. 1992); (Bio/dynamics Inc. 1980a)	rat 10 wks at 5d/wk, then mating, gestation, and lactation, 6 hr/d 0, 1, 10, 30, 300 ppm	Fertility not reduced at any concentration. Postnatal weight gain reduced on PND 21 (SS females only), but not PND 4 or 14, at 300 ppm. Litter size not reduced, postnatal death not increased, malformations not increased, birth weight not reduced at any concentration. Maternal weight gain not reduced at any concentration.
(Ungvary and Donath 1984)	rat gd 8-10, 8 hr/d 0, 1500 mg/m^3 (0, 460 ppm)[2]	Reduced abundance of fluorescent noradrenergic nerve fibers in the ovary and uterus at 1500 mg/m^3. (Statistical significance not addressed.)
(Ward et al. 1985)	mouse 13 wks, 5 d/wk, 6 hr/d 0, 1, 10, 30, 300 ppm	Ovarian histopathological effects (bilateral cysts) at 300 ppm. "No exposure related mortality." Female weight gain not reduced. Hematological effects: RBC (SS), hemoglobin (SS), hematocrit (SS), WBC (SS) reduced at 300 ppm.

Table D.2.2. Animal studies of the female reproductive effects of benzene by inhalation

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Reference	Study Design	Reported effects[1]
(Ward et al. 1985) (continued)	rat 13 wks, 5d/wk, 6 hr/d 0, 1, 10, 30, 300 ppm	Ovarian histopathological effects not found at any concentration. Female death not increased, weight gain not reduced at any concentration. Hematological effects: WBC (SS) reduced at 300 ppm; RBC, hemoglobin, hematocrit not reduced at any concentration.

[1] Effects reported by authors to be statistically significant (SS) at $p < 0.05$ or biologically noteworthy.
[2] Benzene concentrations reported in mg/m^3 were converted to ppm using the relationship 1.0 ppm = 3.26 mg/m^3 (ATSDR 1993).

Table D.2.3. Animal studies of the female reproductive effects of benzene by gavage

Reference	Study Design	Reported effects[1]
(Huff et al. 1989; NTP 1986)	rat 103 wks, 5 d/wk 0, 25, 50, 100 mg/kg/d	No ovarian, uterine, or mammary histopathological effects at any dose. Female death increased: 4/50 at 0 mg/kg/d, 10/50 at 25 mg/kg/d, 14/50 at 50 mg/kg/d (SS), 20/50 at 100 mg/kg/d (SS). Female weight gain reduced at 100 mg/kg/d. Hematological effects: WBC (SS) reduced at 25, 50, 100 mg/kg/d. RBC not reduced at any dose.
	mouse 103 wks, 5 d/wk 0, 25, 50, 100 mg/kg/d	Ovarian histopathological effect ("senile atrophy") frequency increased at 25, 50, 100 mg/kg/d. Uterine, mammary histopathological effects not found at any dose. Female death increased: 16/50 at 0 mg/kg/d, 19/50 at 25 mg/kg/d, 26/50 at 50 mg/kg/d, 31/50 at 100 mg/kg/d (SS). Female weight gain reduced at 100 mg/kg/d. Hematological effects: WBC (SS) reduced at 25, 50, 100 mg/kg/d. [No data on RBC.]

[1] Effects reported by authors to be statistically significant (SS) at $p < 0.05$ or biologically noteworthy.

Table D.2.4. Animal studies of the female reproductive effects of benzene by injection

Reference	Study Design	Reported effects[(1)]
(Desoille et al. 1963) (French, also summarized in Barlow and Sullivan 1982)	s.c. rabbit gestation 0, 0.25 mL/kg/d (40% benzene solution, approximately 0.1 g benzene/kg/d)	Maternal RBC (SS) and WBC (SS) reduced. Gestation length, abortion rate, neonatal mortality, and anomaly rate not affected at dose tested. (Statistical significance not addressed for these endpoints.)
(Desoille et al. 1965) (French, also summarized in Barlow and Sullivan 1982)	s.c. rat gestation 0, 0.1 g/kg/d	Maternal WBC (SS) reduced. Maternal body weight, length of pregnancy, average litter size, and neonatal mortality not affected at dose tested. (Statistical significance not addressed for these endpoints.)
(Desoille et al. 1967) (French, also summarized in Barlow and Sullivan 1982)	s.c. guinea pig gestation 0, 0.1 g/kg/d	Maternal body weight, length of gestation, number of offspring, neonatal mortality, and neonatal body weight not affected at dose tested. (Statistical significance not addressed for these endpoints.)
(Dikshith et al. 1980)	i.p. rabbit 30 d: 0, 0.4 mL/kg/d 15 d; 0.8 mL/kg/d (0, 0.35, 0.70 g/kg/d)[(2)]	Ovary weight, ovarian histopathological effects not found at any dose. Female death increased: 0/4 at 0 mL/kg/d, 0/4 at 0.4 mL/kg/d, 2/4 at 0.8 mL/kg/d. (Statistical significance not addressed for this endpoint.) Female weight gain reduced: 7% at 0.4 mL/kg/d, 28% at 0.8 mL/kg/d. (Statistical significance not addressed for this endpoint.) Hematological effects: RBC (SS) and hemoglobin (SS) increased at 0.8 mL/kg/d, WBC (SS) increased at 0.4 mL/kg/d, reduced at 0.8 mL/kg/d.
(Vara and Kinnunen 1946) (German with English summary)	s.c. rabbit injected until toxic effects were observed in blood (not clear how long or often) 1g/animal	Reduced ovarian weight, increased aneuploidy of ova at 1 g/animal (statistical significance not addressed). Hematological effect: “Blood picture showed clear symptoms of toxication.”

[(1)] Effects reported by authors to be statistically significant (SS) at $p < 0.05$ or biologically noteworthy.
[(2)] Benzene doses reported in mL/kg were converted to g/kg using density = 0.87865 g/mL (CRC 1976).

Table D.2.5. Animal studies of the female reproductive effects of benzene by direct application to ovary

Reference	Study Design	Reported effects[(1)]
(Batra 1959)	mouse (Strong A, C57BL, or C3H) Controls: not clear if completely untreated or sham operated. Treated: 1 paint brush stroke of benzene on ovaries (before mating).	Malformations increased in Strong A (SS) but not in C57BL or C3H strains at "1 stroke". Tumor incidence in progeny increased in Strong A (SS) but not C3H or C57BL strains at "1 stroke".
(Batra 1966)	mouse (Swiss) Controls: not clear if completely untreated or sham operated. Treated: 1 paint brush stroke of benzene on ovaries (before mating).	Ovarian histopathological effects observed, litter size reduced, postnatal death increased at "1 stroke". (Statistical significance not addressed.)
(Solomon and Batra 1964)	mouse (Swiss) Controls: not clear if completely untreated or sham operated. Treated: 1 paint brush stroke of benzene on ovaries (before mating).	Increased degenerate ova after fertilization at "1 stroke". (Statistical significance not addressed.)
(Sridharan et al. 1963)	mouse (C3H) Controls: not clear if completely untreated or sham operated. Treated: 1 paint brush stroke of benzene on ovaries (before mating).	Abnormalities increased for 4 generations at "1 stroke". (Statistical significance not addressed)

[(1)] Effects reported by authors to be statistically significant (SS) at $p < 0.05$ or biologically noteworthy.

E. Male Reproductive Toxicity

In accord with current Federal guidelines (USEPA 1996) and the warning provisions of Proposition 65, developmental endpoints are included under Male Reproductive Toxicity if effects are thought to be mediated by exposure of the father (male-mediated effects).

E.1. Human male reproductive toxicity studies

E.1.1. Fetal growth

One study that separately analyzed occupational benzene exposure and fetal growth outcomes (Savitz et al. 1989) found a significantly elevated risk of small-for-gestational-age infants associated with paternal exposure to benzene; the risk increased with increasing 'linkage level', reflecting an increasing probability of benzene exposure in the subject's occupation, in this study in which exposure was categorized based on job and industry codes. This study is discussed in detail below. More definitive studies with accurate assessment of benzene-specific exposure are needed to evaluate the association suggested by this study.

Savitz et al. (1989)

Savitz et al. (1989) examined the effect of parental occupational exposures on risk of several adverse pregnancy outcomes as described above (Section C.1.1). In the analysis of paternal occupation by industry, 5,669 live births were included. Analysis for paternal occupational exposure and preterm delivery included 552 preterm and 4,038 term infants, and for SGA, 371 SGA infants and 4,732 infants appropriate for gestational age. An exposure linkage system was used to assign exposure to specific agents based on industry and occupation, with only medium or high linkages considered 'exposed'; paternal exposure to benzene was due to diverse occupations, with the largest contribution from engine mechanics and repairers (16%) and welders and flame cutters (6%).

Analysis of exposure to specific agents indicated that linkage level of fathers was unrelated to risk of preterm delivery in general and in the analysis of benzene exposure (OR = 0.9; 95% CI = 0.5-1.4). In contrast, the largest OR for paternal exposures and SGA was found for benzene (OR = 1.5; 95% CI = 1.1-2.3), adjusted for child's race, sex, maternal smoking, and restricted to mothers ≥20 years old. The analysis by linkage level for paternal benzene exposure and risk of SGA, according to the authors, "was highly suggestive", with ORs for low, medium and high linkages of 1.2, 1.5 (95% CI = 1.0-2.2), and 2.0 (95% CI = 1.1-3.7), respectively.

Because of spousal reporting of paternal occupation, accurate reporting is a concern in this study, as is potential movement into and out of jobs. According to the authors, the direction of the bias these imperfections of exposure assignment present is almost certainly toward the null. In addition, given the many comparisons made in the study, there is a need to distinguish etiologically meaningful results from the random spread of

odds ratios, relying on biologic plausibility, dose-response gradients, and the precision of risk estimates. The study's strengths include specific information on maternal and paternal occupations, and data on the most important potential confounders (e.g., maternal smoking, pregnancy history). Having a sufficient number of subjects to distinguish essentially independent groups defined by preterm delivery and SGA demonstrates the strength of the study. More intensive evaluation of paternal exposure as a possible determinant of pregnancy outcome is indicated by the results of this study.

E.1.2. Spontaneous abortion and perinatal mortality

Effects of paternal exposure to benzene on fetal loss, including perinatal mortality and spontaneous abortion, have been investigated in relation to paternal occupational benzene exposure in 2 studies (Savitz et al. 1989; Stucker et al. 1994). Paternal occupational exposure to benzene was associated with nonsignificant elevations of spontaneous abortion risk in 1 study (Stucker et al. 1994), and significant elevations of risk of stillbirth in the high exposure group in another (Savitz et al. 1989). These studies are discussed in detail below. More definitive studies with accurate assessment of benzene-specific exposure are needed to evaluate the associations suggested by these studies.

Savitz et al. (1989)

In a case-control analysis of stillbirth risk in the study of parental occupational exposures and risk of adverse pregnancy outcomes in the U.S. (described above, see Section C.1.1), Savitz et al. (1989) had data on 3,170 stillbirths and 5,669 live births for paternal occupation. Savitz et al. (1989) found that paternal benzene exposure at high linkage level was associated with stillbirth risk (OR = 1.4, 95% CI = 1.1-1.9), although no elevated risk was seen for all paternal benzene exposure combined (OR = 1.0; 95% CI = 0.8-1.3).

Stucker et al. (1994)

In a study in France, Stucker et al. (1994) evaluated the risk of spontaneous abortion in wives of men occupationally exposed to benzene. Information collected in a mailed questionnaire completed by the wives of 823 men employed in 2 chemical plants included descriptions of pregnancy outcomes, pregnancy order, previous spontaneous abortions, age and maternal tobacco use, among other things. Employees' occupational histories were supplied by the companies. Benzene exposure was categorized as none, <5 ppm and ≥ 5 ppm, as assigned by occupational physicians in relation to employees' functions in the plants, and was separately considered for those with exposure in the 3 months immediately prior to conception, and for any past exposure. Spontaneous abortion was defined as a pregnancy that ended before 28 weeks of gestation, excluding induced abortions. Analysis of pregnancies that ended in live birth or spontaneous abortion included 270 exposed (at any time) and 1277 unexposed.

The frequency of spontaneous abortion was slightly elevated for those exposed at any time before conception (11.3%) compared to those unexposed (8.8.%), but was not statistically significant (OR = 1.3; 95% CI = 0.9-2.0). Although the authors report that the association between benzene exposure and spontaneous abortion was not modified when the results were adjusted separately for mother's age at delivery (≥30 years), pregnancy order, and maternal smoking, the adjustment for these 3 risk factors simultaneously in a logistic regression decreased the OR to 1.1 (95% CI = 0.7-1.8) for any past exposure. The number of pregnancies in the various subgroup analyses were less than that noted by the authors as the minimum required to detect a 2-fold increase. In the analyses of those exposed during the 3 months prior to conception, none of the odds ratios were elevated. In analyses based on all past exposures and separated by level of exposure, the frequency of spontaneous abortions was 9.6% in those exposed to < 5 ppm and 12.5% in those exposed to ≥ 5 ppm (for the higher exposure group, we calculated the crude OR = 1.49, 95% CI = 0.89-2.46); however, the authors report that neither the test for linear trend nor the adjusted odds ratios were statistically significant. In a subcategory of subjects (30 pregnancies) with higher level and duration of exposure (≥5 ppm benzene for >5 years), Stucker et al. (1994) reported a slight excess of spontaneous abortions (13.3%; no crude OR reported nor could it be calculated from the information provided); following adjustment, the risk was not elevated (adjusted OR = 0.93; 95% CI = 0.3-3.01). Although information was collected on subjects' previous history of spontaneous abortions, usually considered an important risk factor, the authors did not indicate adjustment for this factor.

E.1.3. Childhood leukemia

Two well-designed epidemiologic studies (McKinney et al. 1991; Shaw et al. 1984) have investigated the association between paternal benzene exposure and childhood leukemia. A statistically significant association with childhood leukemia and non-Hodgkin's lymphoma was found (OR = 5.81; 95% CI = 1.67-26.44) by McKinney *et al.* (1991), but not in the study by Shaw *et al.* (1984); greater precision in the exposure assessment in the more recent study may at least partially explain the discrepancy. These studies are discussed in detail below. More definitive studies with accurate assessment of benzene-specific exposure are needed to evaluate the association between pre-conceptional paternal exposure to benzene and childhood leukemia found in the McKinney et al. (1991) study.

Other studies of paternal occupational exposure to solvents or petrochemicals may provide additional information (Van Steensel-Moll et al. 1985; Lowengart et al. 1987; Knox 1994; Buckley et al. 1989; Shu et al. 1988). Van Steensel-Moll et al. (1985) examined all hydrocarbon-related occupations together and found no association between paternal exposure and childhood leukemia. Lowengart et al. (1987) found no association with pre-conceptional paternal exposure to "petroleum-chemicals". A population registry-based matched case-control study of childhood leukemia in China (Shu et al., 1988) failed to detect an association between childhood leukemia and paternal occupation in the chemical industry (chemical processors and related workers, rubber and plastic

products makers, leather workers, painters, and chemical analysts), although an association was found with mothers employed in similar occupations (see C.1.1.4); paternal exposure during pregnancy rather than pre-conceptional exposures to chemicals were discussed in this report.

Buckley et al. (1989) found an association between acute nonlymphocytic leukemia and paternal occupational exposure to solvents (OR = 2.1 for prolonged exposure; p for trend = 0.003) and petroleum products (OR = 2.4 for prolonged exposure, 95% CI = 1.3-4.1; p for trend = 0.002). When timing of exposure was examined in the Buckley et al. (1989) study, the association between exposure to petroleum products and leukemia was found to be greater when exposure occurred during pregnancy (OR = 2.8, $p<0.05$) than when it occurred before (OR = 2.0, $p<0.05$) or after (OR = 1.6, $p<0.10$) pregnancy; for solvent exposure, the association was similar for exposures before (OR = 2.2, $p<0.05$) and during pregnancy (OR = 2.1, $p<0.05$), and lower after (OR = 1.5, $p<0.05$). A recent study by Knox (1994) demonstrates clustering of British childhood leukemia cases in geographic proximity to areas of exposure to leakage, processing, and use of petrochemicals. Although questions have been raised about methodology of the Knox study (Bithell and Draper 1995), the study author suggests the possibility that benzene exposure may explain the clusters.

The biologic plausibility of the possible association of paternal benzene exposure and childhood leukemia needs to be considered. Studies have shown associations between preconceptional x-ray exposure of the father and risk of childhood leukemia; for example, Shu et al. (1988) found statistically significant elevated odds ratios associated with paternal preconceptional x-rays (1-5 x-rays: OR = 1.4; 6-10 x-rays: OR = 2.4, 95% CI = 1.5-5.0; >11 x-rays: OR = 3.9, 95% CI = 1.7-8.6). Associations have also been found with exposure to chlorinated solvents (Lowengart et al., 1987), “plastic or rubber” (Van Steensel-Moll et al., 1985), and plastics and pesticides (Buckley et al., 1989), though not all of these were statistically significant. Other studies have failed to detect associations of paternal occupational exposure and childhood leukemia (e.g., Shu et al., 1988). Difficulties in exposure assessment may contribute to the variability in study findings. Most studies were not able to separately assess effects of exposure before, during, and after pregnancy.

Shaw et al. (1984)

In a matched case-control study evaluating 255 cases of childhood leukemia reported to the California Tumor Registry, Shaw et al. (1984) examined the association between disease and risk factors including maternal age, birth order, socio-economic status, and paternal occupation. Occupational classifications determined by the National Occupational Hazard Survey were used to classify fathers in the study as “potentially exposed” or “not exposed” based on the paternal occupation at the time of birth as stated on the birth certificate. This study found no association between paternal benzene exposure and childhood leukemia. Using this method of assessing exposure, 76% of the cases and 75% of the controls were classified as having potential paternal exposure. As

noted by the authors, it is possible that the failure to detect an association in this study is due to misclassification of exposure status.

McKinney *et al.* (1991)

In another matched case-control study, McKinney *et al.* (1991) evaluated the associations between self-reported parental exposures to specific agents and childhood leukemia and non-Hodgkins lymphoma in 3 areas of England with previously documented high rates of these diseases. Data in this study were collected through face-to-face home interviews which asked questions specifically about parental exposure at work or through a hobby to a variety of suspected toxicants. Children diagnosed with leukemia or non-Hodgkin's lymphoma in the study area between 1974 and 1988 were included in the study. Cases occurring during this period included 113 cases of acute lymphoblastic leukemia (75%), 21 other cases of leukemia (14%), and 17 cases of non-Hodgkin's lymphoma (11%). Each case was matched to 2 controls by sex, date and health district of birth. Cases were included in the analysis if data were available for the case and at least 1 control. Twelve (12%) of 101 cases compared to 6 (3%) of 178 controls had fathers who reported pre-conceptional exposure to benzene (OR = 5.81; 95% CI = 1.67-26.44).

E.2. Animal male reproductive toxicity studies

The majority of the information relevant to male reproductive toxicity consists of data on sperm genotoxicity, sperm count sperm morphology and testicular histopathology. Mice appear to demonstrate benzene testicular toxicity in these studies. There are also 4 dominant lethal studies, none of which reported effects, but no available multigeneration studies; thus, very limited information on fertility is available. Animal male reproductive toxicity studies are outlined in Tables E.2.1-E.2.3 at the end of Section E.

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E.2.1. Effects on sperm

A screening test was used in mice to determine induction of sperm head abnormalities, (which are thought to arise from small deletions or point mutations) by 54 agents in mice (Topham 1980). Benzene was 1 of 5 agents found to induce a significant increase in sperm head abnormalities out of 54 agents tested. (The other 4 agents were: benzo(a)pyrene; estradiol; ICI 42464, an alkylating agent; and hexamethylphosphoramide, a carcinogenic agent.) Benzene was administered i.p. on 5 successive days at 7 doses (0.1- 1.0 mL/kg/day). The dose response curve showed statistically significant effects above 0.4 mL/kg with a peak effect at 0.6 mL/kg. No general toxicity data was reported, although all doses were stated to be below 0.5 LD50.

Benzene-induced chromosomal damage in sperm has also been studied (Ciranni et al. 1991) . Single oral doses were administered to mice. Chromosome damage was scored in bone marrow cells as well as differentiating spermatogonia. In a dose response study, a higher percentage of both bone marrow cells and spermatogonia demonstrated

chromosome aberrations (breaks, fragment, exchanges) at 0.25, 0.5 and 1 mL/kg than in controls; a dose-response relationship was seen. The clastogenic effect was considered more severe in the bone marrow cells. In a time-response study, the highest number of spermatagonia with chromosome damage was found at 24 h after dosing with 1 mL/kg.

A more complete evaluation of sperm cytotoxicity by cell type was conducted using a higher dose range (5 doses between 1 and 7 mL/kg), and gavage administration in mice (Spano et al. 1989). Evaluations were conducted 7, 14, 21, 28 and 70 days after treatment to detect sperm cycle effects, and various cell types (elongated spermatids, round spermatids, diploid elongated spermatids, diploid cells, S-phase cells, tetraploid cells) were separated by DNA content flow cytometry. No effects on body or testes weight were reported at these doses, which were below an estimated LD50 of 7.5 mL/kg. Dose dependent declines in various populations with subsequent recovery was noted in this experiment. Differentiating spermatogonia were identified as the main target of benzene cytotoxicity. This is consistent with the profile produced by a known spermatotoxic treatment, radiation. As was the case for the sperm head abnormality test (Topham 1980), a peak effect was found below the highest dose.

E.2.2. Fertility/dominant lethal

Although benzene-induced genotoxicity and spermatotoxicity have been described, clear demonstrations of fertility or dominant lethal effects were not reported in available dominant lethal studies.

A large collaborative project in the 1970s used benzene as a test agent to examine the inter-laboratory reliability of the dominant lethal assay (Ehling 1977; Ehling et al. 1978). Male mice given a single dose of 1, 2 or 4 mL/kg benzene by gavage were followed over a 12 week mating period. A complete report of the data from a total of 7 studies was published in German. All 7 studies were consistent in failing to identify any dominant lethal effects (dead implants/total implants). Increased paternal mortality and decreased fertility at the 2 highest doses (2 or 4 mL/kg by gavage) may have interfered with evaluation of dominant lethal effects during the first 8 days after treatment. Three of the 7 studies reported a statistically significant decrease in implantation efficiency (implants/corpora lutea) at the high dose (4 mL/kg) during days 13-16 after mating. However, 3 of the remaining 4 studies failed to indicate any trend in this direction. A separate brief report (Feldt and Zhurkov 1985) stated that no dominant lethal mutations were induced by benzene gavage in mice (4 doses ranging from 0.001 to 0.2 LD50, 5 week dosing period). Induction of PCE micronuclei and bone marrow chromosomal aberrations was found at these doses.

Two other studies used rats, which are generally less sensitive than mice to benzene toxicity. The first rat dominant lethal study administered benzene by the inhalation route (1, 10, 30 and 300 ppm) for 10 weeks and then mated the rats for a 2 week period (2 females per week) (Bio/dynamics Inc. 1980b). No effect of benzene was demonstrated by statistical analysis for mortality, body weight or pregnancy rate. Analysis of variance

across dose groups was significant for corpora lutea, implantation sites and viable fetuses in matings from week 1. No individual dose group was identified as significantly different from controls, and a clear pattern of dose dependence was seen only for the viable fetuses measure. Analysis of variance across dose groups was not reported as significant for implantation efficiency (implants/corporea lutea) or mutagenic ratio (dead implants/total implants) in either week. This study reported testicular damage (histopathology) in 2 of 20 males exposed to 300 ppm benzene. Females mated to 1 of these males demonstrated high preimplantation loss and mutagenic ratios. A high incidence of postimplantation loss was also noted in another male in the 300 ppm group who was not reported for testicular histopathology.

The second dominant lethal study in rats used a single i.p. injection of 0.5 mL/kg body weight benzene and a subsequent 10 week mating period. No statistically significant effects were found on pregnancy rate, live, dead or total implants or preimplantation loss (Lyon 1975). This same dose produced clastogenic effects as reflected in the micronucleus test but sperm genotoxicity was not evaluated.

E.2.3. Reproductive organ pathology

Indications of testicular pathology in the absence of general toxicity were reported in a subchronic inhalation study of mice (Ward et al. 1985). At inhalation exposures of 300 ppm, 6 h/day, 5 d/wk a decrease in testes weight was recorded in mice killed after 28, 56, and 91 days of exposure; no effects were seen at the first time point (14 days). The size of the reduction in testes weight was not reported. After 91 days of exposure, histopathologic evidence of atrophy and degeneration (7 of 20 mice), decreases in epididymal sperm (6 of 20 mice), and increases in the number of abnormal sperm forms (9 of 20 mice) were reported. Although hematologic effects were also noted at this inhalation exposure, no effects on mortality or body weight were reported. In contrast to mice, male rats (n = 10/group) showed no testicular damage at the same exposures. Hematotoxicity was also less pronounced in rats than mice.

In chronic toxicity studies that administered benzene by gavage (Huff et al. 1989; NTP 1986), tumors were noted in a number of organ systems of male rats and mice; mice demonstrated preputial gland tumors. No histopathological observations of reproductive tract abnormalities independent of carcinogenesis were apparent; neither reproductive organ weight data or sperm counts were given in the reports.

A briefly reported older study (Wolf et al. 1956) described effects on testes weight in rats, rabbits and guinea pigs using benzene inhalation doses that also produced increased mortality, general growth retardation and/or bone marrow pathology. Benzene was administered 7 h/day, 5 days/week. The effective benzene concentration and study durations were: rat, 6,600 ppm, 70 days; guinea pig, 88 ppm, 193 days; rabbit, 80 ppm, 243 days. A subjective rating of the extent of the problem was provided in a table but no incidence or quantitative data were included.

E.3. Male reproductive toxicity: Other relevant data

E.3.1. Distribution and metabolism in males

No data was located regarding the distribution or metabolism of benzene in testes or other male reproductive organs. For a discussion of general metabolism, see Section B.4. In general, the observations that benzene and its metabolites are widely distributed suggest that they would reach the male reproductive organs.

E.3.2. Chromosomal aberrations and related effects of benzene metabolites

Several metabolites of benzene have been found to produce chromosomal aberrations and related effects *in vivo* or *in vitro* in a variety of cell types. This data has been discussed in Sections B.5 and D.3.2. It may be that the same effect would occur with germ line cells. This could adversely affect reproduction. However, no evidence has been located which indicates that benzene metabolites would selectively target (or selectively avoid targeting) male reproductive organs.

A link between chromosomal aberrations in sperm in animals (Ciranni et al. 1991) and an increased risk of childhood leukemia from paternal benzene exposure (McKinney et al. 1991) is difficult to establish at this time. Chromosome damage to sperm such as aneuploidy and reciprocal translocation would be likely to have widespread consequences for early development and later function of somatic cells, and would thus be less likely as an initiating event in childhood cancer. However, chromosome deletion in sperm could lead to a situation where normal development and somatic function are attained through the other allele, but cancer risk is increased when the other allele is genetically damaged by environmental factors. Such a mechanism would not necessarily be confined to childhood cancers.

E.4. Integrative evaluation

Animal studies have reported decreased testicular weight, testicular atrophy and degeneration, reduced sperm count, and abnormal sperm morphology due to benzene exposure. These effects are considered adverse for the purposes of hazard identification under current USEPA guidelines (USEPA 1996). Many studies used single or short term gavage or injection administrations, but a subchronic inhalation study is also included. On the other hand, no fertility or dominant lethal effects were detected in available rat and mouse studies using similar routes and doses. No multigeneration breeding study is available. However, some, but not all, human epidemiological studies have reported statistically significant associations between paternal occupational exposure to benzene and effects on fetal growth and fetal loss. More definitive human studies with accurate assessment of benzene-specific exposure are needed to evaluate these paternally mediated effects.

An association between paternal occupational exposure to benzene and childhood leukemia was also reported in 1 of 2 studies. There are no parallel studies in animals with

benzene, but paternally mediated cancer in offspring has been demonstrated in a few instances in animal models (Tomatis 1994), and has received considerable attention in epidemiology (Gold and Sever 1994). Results have been controversial and no mechanisms have been identified. As regards biological plausibility, a chromosome deletion transferred in one allele from sperm could lie dormant during early development, but increase the risk of cancer if the maternal allele was damaged later in life.

No information is available concerning benzene transport to testes and transformation to reactive metabolites in male reproductive organs; however, chromosomal abnormalities detected in sperm after *in vivo* treatment support the possibility of a direct action of benzene on testes. Thus, although a coherent picture of benzene male reproductive toxicity is not available, there are a number of studies of good quality reporting effects on relevant parameters. Human studies are suggestive and indicate a need for further research.

Table E.2.1. Animal studies of the male reproductive effects of benzene by inhalation

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Reference	Study Design	Reported effects[(1)]
(Bio/dynamics Inc. 1980b)	rat 10 wks, 6 hr/d, 5 d/wk 0, 1, 10, 30, 300 ppm [Treated males mated with 2 untreated females, for 1 week, each week for 2 weeks.]	Male death not increased, weight gain not reduced at any concentration. Male fertility not reduced, pre- and post-implant losses not increased (i.e. dominant lethal effects not found) at any concentration. Testicular histopathological effects found in 2/20 animals at 300 ppm (statistical significance for this endpoint not addressed.)
(Ward et al. 1985)	mouse 13 wks, 5 d/wk, 6 hr/d 0, 1, 10, 30, 300 ppm	"No exposure related mortality." Male weight gain not reduced at any concentration. Hematological effects: RBCs, hemoglobin, hematocrit, WBCs reduced (SS) at 300 ppm. Testicular histopathological effects found, testes weight reduced (SS), epididymal sperm count reduced, sperm abnormalities increased at 300 ppm.
	rat 13 wks, 5d/wk, 6 hr/d 0, 1, 10, 30, 300 ppm	"No exposure related mortality." Male weight gain not reduced at any concentration. Hematological effects: WBCs reduced (SS) at 300 ppm; RBCs, hemoglobin, hematocrit not reduced at any concentration. Testicular histopathological effects not found, testes weight not reduced at any concentration.
(Wolf et al. 1956)	rat 7-8 hr/d number of days not stated: 0 ppm, 136 out of 204 d: 88 ppm, 133 out of 212 d: 2200 ppm, 28 out of 38 d: 4400 ppm, 70 out of 93 d: 6600 ppm, 1 to 10 out of 1 to 19 d: 9400 ppm	Male mortality increased at 4400, 6600, 9400 ppm. Male weight gain reduced (SS) at 2200, 4400, 6600, 9400 ppm. Hematology: blood and/or bone "histopathology" effects at 88, 2200, 4400, 6600, 9400 ppm. Narcosis found at 2200, 4400, 6600, 9400 ppm. Testes weight increased (SS) at 6600 ppm [not 9400 ppm]. (Statistical significance of body and organ weights, but not other endpoints, addressed)
	rabbit 7-8 hr/d number of days not stated: 0 ppm, 175 out of 243 d: 80 ppm	[Male mortality and weight gain results not reported; only adverse effects were reported in table.] Hematology: blood "histopathology" effects at 80 ppm. Testicular histopathological effects at 80 ppm.

Table E.2.1. Animal studies of the male reproductive effects of benzene by inhalation

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Reference	Study Design	Reported effects[1]
(Wolf et al. 1956) (continued)	guinea pig 7-8 hr/d number of days not stated: 0 ppm, 23 out of 32, and 193 out of 269 days: 88 ppm	[Male mortality results not reported; only adverse effects were reported in table.] Male weight gain reduced (SS) at 88 ppm (193 out of 269 days). Hematology: blood "histopathology" effects at 88 ppm (23 out of 32 or 193 out of 269 days), bone "histopathology" effects at 88 ppm (193 out of 269 days). Testicular weight increased (SS) at 88 ppm (193 out of 269 days). (Statistical significance of body and organ weights, but not other endpoints, addressed)

[1] Effects reported by authors to be statistically significant (SS) at $p \leq 0.05$ or biologically noteworthy.

Table E.2.2. Animal studies of the male reproductive effects of benzene by gavage

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Reference	Study Design	Reported effects[1]
(Ciranni et al. 1991)	mouse 1x Spermatogonia study: 0, 0.25, 0.5, 1.0 mL/kg (0, 0.22, 0.44, 0.88 mg/kg)[2] Bone marrow study: 0, 0.1, 0.5, 1.0 mL/kg (0, 0.088, 0.44, 0.88 mg/kg)[2]	Chromatid aberrations in differentiating spermatagonia increased (SS) at 0.25, 0.5, 1.0 mL/kg. Chromatid aberrations in bone marrow cells increased (SS) at 0.1, 0.5, 1.0 mL/kg
(Ehling 1977; Ehling et al. 1978)	mouse 1x 0, 1, 2, 4 mL/kg Note: 7 studies had full data and statistical analysis.	Male death increased at 1 mL/kg (2/7 studies, not SS), 2 mL/kg (3/7 studies, not SS), 4 mL/kg (5/7 studies, SS). Male fertility reduced at 1 mL/kg (2/7 studies, SS), 2 mL/kg (2/7 studies, SS), 4 mL/kg (5/7 studies, SS). Pre-implant loss increased at 1 mL/kg (1/7 studies, SS), 2 mL/kg (1/7 studies, SS), 4 mL/kg (4/7 studies, SS). Post-implant loss not increased at any dose in any study.
(Feldt and Zhurkov 1985) (short article)	mouse Dominant lethal study: 5 wks Micronuclei study: 2 times Bone marrow study: 10 times "4 doses ranging from 0.001 to 0.2 LD_{50}"	No dominant lethal effects at any dose tested. Micronuclei increased (SS) in polychromatic erythrocytes at 40 mg/kg and up. Chromosome aberrations increased (SS) in bone marrow cells at 20 mg/kg and up.

Table E.2.2. Animal studies of the male reproductive effects of benzene by gavage
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Reference	Study Design	Reported effects[1]
(Huff et al. 1989; NTP 1986)	rat 103 wks, 5 d/wk 0, 50, 100, 200 mg/kg/d	Male death increased: 12/50 at 0 mg/kg/d, 18/50 at 50 mg/kg/d, 19/50 at 100 mg/kg/d, 30/50 at 200 mg/kg/d (SS). Male weight gain reduced at 100, 200 mg/kg/d. Hematological effects: WBCs reduced (SS) at 50, 100, 200 mg/kg/d. RBCs not reduced at any dose. Testicular histopathological effects not found, aspermatogenesis not increased at any dose.
	mouse 103 wks, 5 d/wk 0, 25, 50, 100 mg/kg/d	Male death increased: 20/50 at 0 mg/kg/d, 24/50 at 25 mg/kg/d, 31/50 at 50 mg/kg/d, 41/50 at 100 mg/kg/d (SS). Male weight gain reduced at 100 mg/kg/d. Hematological effects: WBCs reduced (SS) at 25, 50, 100 mg/kg/d. [No data on RBCs.] Testicular histopathological effects not found at any dose.
(Spano et al. 1989)	mouse 1x 0, 1, 2, 4, 6, 7 mL/kg (0, 0.88, 1.8, 3.5, 5.3, 6.2 g/kg)[2]	Alteration of sperm maturation, as determined by DNA flow cytometry, at 1, 2, 4, 6, 7 mL/kg. Male weight gain not reduced, testes weight not altered at any dose. LD_{50} = 7.5 mL/kg.

[1] Effects reported by authors to be statistically significant (SS) at $p \leq 0.05$ or biologically noteworthy.
[2] Benzene doses reported in mL/kg were converted to g/kg using density = 0.87865 g/mL (CRC 1976).

Table E.2.3. Animal studies of the male reproductive effects of benzene by injection

Reference	Study Design	Reported effects[1]
(Lyon 1975)	i.p. rat 1x 0, 0.5 mL/kg (0, 0.44 mg/kg)[2] Treated males mated to 2 untreated females, for 1 week, each week for 10 weeks.	LD_{50} calculated approximately 2 mL/kg. Male deaths not increased at dose tested. Male weight gain reduced (SS). Male fertility not reduced, pre- and post-implant losses not increased (i.e. dominant lethal effects not found) at dose tested. Chromosomal aberrations in bone marrow and peripheral blood cells increased (SS) at 0.5 mL/kg.
(Topham 1980)	i.p. mouse 5 d 0, 0.1, 0.25, 0.4, 0.5, 0.6, 0.8, 1.0 mL/kg/d (0, 0.088, 0.22, 0.35, 0.44, 0.53, 0.70, 0.88 g/kg)[2]	Sperm abnormalities increased (SS) at 0.5, 0.6, 0.8, 1.0 mL/kg

[1] Effects reported by authors to be statistically significant (SS) at $p \leq 0.05$ or biologically noteworthy.
[2] Benzene doses reported in mL/kg were converted to g/kg using density = 0.87865 g/mL (CRC 1976).

F. Summary

F.1. *Developmental Toxicity*

Delayed intrauterine development has been a consistent finding of studies in rats, rabbits and mice which used benzene exposure by inhalation to pregnant dams during organogenesis. The relevant endpoints are fetal weight reduction (in the range of 7-10%) and delayed ossification. In some of these studies, but not all, maternal toxicity was reported to occur concurrent with exposures that produced adverse fetal effects. There is little indication that benzene causes structural malformations. There are no studies with postnatal endpoints. Dose dependence is seen and some benzene concentrations produce these effects in the absence of reported maternal toxicity. Human studies of pregnancy outcome from maternal exposure are characterized by limited exposure ascertainment, simultaneous exposure to multiple chemicals and low power. They neither support nor contradict the animal data.

Two benzene effects seen in adults, chromosome damage and changes in populations of hematopoietic precursors, are also seen in mouse fetuses whose dams are treated with benzene. Some effects on hematopoietic cell populations persist in the postnatal period. Hematopoietic effects occur at distinctly lower benzene inhalation exposure concentrations than delayed development. Functional consequences at the organismic level have not been explored. These endpoints have not been examined in human studies.

F.2. *Female Reproductive Toxicity*

There are relatively few studies of female reproductive toxicity in animals and they vary in quality and completeness of presentation. One large, well-conducted single generation study in rats by the inhalation route failed to find effects on female reproductive toxicity measures. There is no multigeneration study or continuous breeding study. In human studies, consistent reports of abnormal menstruation and excessive blood loss during childbirth in women occupationally exposed to benzene were identified in 3 cross-sectional studies and in case series and case reports. All 3 of the cross-sectional studies are limited: the comparison groups were not exposed to the factory environment under study but were not otherwise described; the studies had no apparent matching and poorly described methods. These cross-sectional studies and most of the case series and case reports involve women working in leather and/or rubber factories, and many of the women had benzene-associated toxicity; concurrent exposure to other solvents is likely to have occurred in most cases. More definitive studies with accurate assessment of benzene-specific exposure are needed to further evaluate the associations suggested by these studies.

F.3. *Male Reproductive Toxicity*

Studies in animals have demonstrated testicular damage and effects on sperm count, morphology and chromosome damage after benzene exposure. Associated general

toxicity was not reported in many of these studies. Separate studies of the potential consequences of sperm effects in terms of fertility or dominant lethal measures failed to find such effects in rats or mice. Of potential interest in this regard are human studies reporting associations between paternal exposure to benzene and increased risk of stillbirth, small-for-gestational age infants, and childhood leukemia. These studies, while suggestive, are not definitive and further research is needed. No human studies of benzene effects on sperm are available.

G. References

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