NCEA-S-0455

TOXICOLOGICAL REVIEW

OF

BENZENE

(NONCANCER EFFECTS)

(CAS No. 71-43-2)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

September 1998

NOTICE

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U.S. Environmental Protection Agency
Washington DC

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FOREWORD

The National Center for Environmental Assessment-Washington Division, Office of Research and Development, has prepared this document on the Toxicological Review of Benzene to serve as a source document for updating the noncancer health effects summary on benzene in the Integrated Risk Information System (IRIS).

In the development of this document, the scientific literature has been reviewed, key studies have been evaluated, and summary/conclusions have been prepared so that the noncancer health effects from exposure to benzene are qualitatively and quantitatively characterized and the derivation of the reference dose (RfD) and reference concentration (RfC) are adequately described. The evaluation and review of the noncancer effects of exposure to benzene have been conducted under the standing guidance of several relevant risk assessment guidelines dealing with reproductive, developmental, neurotoxicity, and immunotoxicity effects. This draft has undergone internal peer review and will undergo an expert external peer panel review in October 1998.

The emphasis of this document is a detailed discussion of the noncancer adverse health effects of benzene, including the no-observed-adverse-effect levels (NOAELs), the lowest-observed-adverse-effect levels (LOAELs), uncertainty factors (UFs), and any other considerations used to develop the RfDs and RfCs for benzene.

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The National Center for Environmental Assessment-Washington Division (NCEA-W) of EPA's Office of Research and Development was responsible for the preparation of this document. It was prepared by TN & Associates, Inc. under EPA Contract No. 68-C6-0024, Work Assignment No. 1-09.1. David Bayliss of NCEA-W served as the Project Manager providing overall direction and technical assistance. Charalingayya Hiremath served as the Work Assignment Manager.

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LIST OF ACRONYMS AND ABBREVIATIONS

AChE	acetyl choline esterase
ANOVA	analysis of variance
ASA	acetylsalicylic acid
BFU-E	burst-forming unit-erythroid
CFU-C	colony-forming unit-culture
CFU-E	colony-forming unit-erythroid
CFU-HPP	high proliferative potential colony-forming unit
CFU-S	colony-forming unit-spleen
CI	confidence interval
Con A	concanavalin A
CYP2E1	cytochrome P450 2E1
DPP	differentiation and proliferation
ELISA	enzyme-linked immunosorbent assay
EMG	electromyographical
GD	gestation day
GM-CFU-C	granulocyte/macrophage colony-forming unit-culture
i.p.	intraperitoneally
IL-1	Interleukin-1
IL-1α	Interleuken-1alpha
IL-1β	Interleukin-1beta
Kd	kilodalton
LOAEL	lowest-observed-adverse-effect level
LPS	lipopolysaccharide
MCV	mean corpuscular volume
MF	modifying factor
NK	natural killer

LIST OF ACRONYMS AND ABBREVIATIONS (continued)

NOAEL	no-observed-adverse-effect level
NQO1 NAD(P)H	quinone oxidoreductase
OR	odds ratio
PBPK	physiologically based pharmacokinetic
PCE	polychromatic erythrocytes
PCV	packed cell volume
PWM	pokeweed mitogen
RBC	red blood cells
RfD	reference dose
RfC	reference concentration
TWA	time-weighted average
UF	uncertainty factor
WBC	white blood cells
YAC	yeast artificial chromosome

EXECUTIVE SUMMARY

In 1992, the U.S. Environmental Protection Agency's (EPA's) Office of Air Mobile Sources, Office of Air and Radiation, requested that the National Center for Environmental Assessment (NCEA) (then known as the Office of Health and Environmental Assessment) provide an updated characterization of human health risk from exposure to benzene. Recently, EPA published the Carcinogenic Effects of Benzene: An Update (EPA/600/P-97/001F, April 1998). The scope of this report is limited to the noncancer health risk assessment of exposure to benzene under the Agency's standing guidance of several relevant risk assessment guidelines dealing with reproductive, developmental, neurotoxic, and other noncancer effects, including derivation of an oral reference dose (RfD) and inhalation reference concentration (RfC). This toxicological review of benzene is to serve as a scientific source document for hazard identification and dose-response assessment in updating the noncancer health effects summary on benzene in the Integrated Risk Information System (IRIS).

Benzene, also known as benzol, is widely used as an industrial solvent, as an intermediate in chemical synthesis, and as a gasoline additive, hence, the potential for human exposure is great. Inhalation exposure is the major route of exposure to benzene, although oral and dermal exposure routes are also important. The toxicokinetics (absorption, distribution, metabolism, and elimination) of benzene have been studied in humans and experimental animal species. Benzene is readily absorbed by both test animals and humans and is distributed throughout several body compartments. The parent compound is preferentially stored in fat, and the relative uptake appears to be dependent on the blood perfusion rates of tissues. The metabolism of benzene is required for expression of benzene toxicity. Evidence indicates that following inhalation exposure to benzene, the major route of elimination of unmetabolized benzene is via exhalation in humans. Absorbed benzene is metabolized to phenol and muconic acid, followed by urinary excretion of conjugated sulfates and glucuronides. Limited data exist on excretion of benzene following dermal exposure in humans. Physiologically based pharmacokinetic (PBPK) models have been developed and are being improved to better define interactions of benzene metabolism, toxicity, and dosimetry. However, the current models are not sufficiently refined to allow them to predict with certainty the relationship between metabolism and toxicity.

Benzene exposure results in adverse noncancer health effects by all routes of administration to test animal species. Hematotoxicity and immunotoxicity have been consistently reported to be the most sensitive indicators of noncancer toxicity both in limited studies in humans and experimental animals. The bone marrow is the target organ for the expression of benzene hematoxicity and immunotoxicity. Chronic exposure to benzene results in progressive deterioration of hematopoietic function. Whether the hematotoxic/immunotoxic effects of benzene and its carcinogenic effects are due to a common mechanism has not been established. Although leukocytopenia has been consistently shown to be a more sensitive indicator of benzene toxicity than anemia in experimental animals, lymphocytopenia has been shown to be an even more sensitive indicator of benzene toxicity. A decrease in absolute lymphocyte count was observed to be the most sensitive indicator of benzene exposure in Chinese factory workers.

Benzene has also been shown to produce neurotoxic effects in test animals and humans after short-term exposure to relatively high concentrations; however; long-term neurotoxicity exposure studies are lacking. There is some evidence of reproductive and developmental effects due to benzene exposure from human epidemiological studies, but data are not conclusive to link low exposure concentrations to effects. No data from human studies were found to indicate that children are more sensitive to benzene toxicity than adults nor were there any gender differences found. The most frequently observed gender difference in test animals is a greater sensitivity of male mice with exposure to benzene, while in rats females appear to be more sensitive than males.

The human occupational inhalation study of Rothman et al. (1996) and the 28-day drinking water exposure study of Hsieh et al. (1988b) were considered to be the co-principal critical studies for derivation of RfC and RfD. Rothman et al. (1996) showed significant reductions in absolute lymphocyte counts in Chinese factory workers exposed to a median 8-hr TWA concentration of 7.6 ppm (24 mg/m^3). The findings from this study indicate that absolute lymphocyte count, WBC, RBC, hematocrit, and platelets were all significantly decreased, and mean corpuscular volume (MCV) was significantly increased in a group of 44 workers occupationally exposed to a median 8-hr TWA of 31 ppm (99 mg/m^3) in comparison to an age and sex matched control group. These effects are consistent with the well-known hematotoxic effects of benzene (Aksoy, 1989; Goldstein, 1988).

The co-principal study of Hsieh et al. (1988b) demonstrated significant (p <0.05) dose-dependent hematological effects (erythrocytopenia, leukocytopenia, lymphocytopenia, and increased MCV) at all exposure levels in (male Charles River) CD-1 mice. Hematological effects were accompanied by a biphasic response in several immunological tests, i.e., significantly increased responses at the low-exposure level (8 mg/kg-day) and significantly decreased responses at the mid- (40 mg/kg-day) and high-dose (180 mg/kg-day) levels. In one test, spleen cellularity was reported to be significantly decreased at all exposure levels, but in a separate test, only at the high-dose level.

Although the NTP (1986) study featured a 103-week chronic exposure regimen, the lowest dose tested (25 mg/kg-day) produced significant leukopenia and was higher than the lowest-observed-adverse-effect level (LOAEL) of 8 mg/kg-day identified in the Hsieh et al. (1988b) study.

To calculate an RfD at the oral equivalent dose of 1.2 mg/kg-day derived from the study of Rothman et al. (1996), the following uncertainty factors were selected. A factor of 10 was used for intraspecies differences in response (human variability) as a means of protecting potentially sensitive human subpopulations. A factor of 10 was applied for use of a LOAEL due to lack of a no-observed-adverse-effect level (NOAEL) in the Rothman et al. (1996) study. Two partial uncertainty factors of 3 were applied to account for extrapolation from subchronic exposure to chronic exposure and for database deficiencies to give a final uncertainty factor of 1000. Use of a partial uncertainty factor for exposure duration adjustment is necessary because the mean exposure duration in the study (6.4 years, range 0.7-16 years) is less than the Superfund interim guidance cutoff value of 7 years for designation as a chronic exposure (Means, 1989). Uncertainty regarding deficiencies in the database is small because of an adequate oral toxicity database and extensive supporting inhalation database. Although the database includes

developmental toxicity studies, a two-generation reproductive study was unavailable. No uncertainty factor was applied for route-to-route exposure because of benzene's well-documented target organ specificity irrespective of the route of administration. There is also little evidence of toxic effects in either the lungs or the gastrointestinal tract. No modifying factor is considered necessary. The calculation of the reference dose is as follows:

$$\text{RfD} = 1.2 \text{ mg/kg-day} \div 1000 = 1\text{E-}3 \text{ mg/kg-day}$$

To calculate an RfD using the LOAEL value of 8 mg/kg-day in male mice from the study of Hsieh et al. (1988b), the following uncertainty factors were selected. A factor of 10 was used for intraspecies differences in response (human variability) as a means of protecting potentially sensitive human subpopulations. A factor of 10 was applied for consideration of interspecies variation. A partial uncertainty factor of 3 was applied for extrapolation from subchronic (28 days) to chronic duration because the subchronic LOAEL from the study of Hsieh was lower than the LOAEL of 25 mg/kg-day determined in a chronic oral exposure study (NTP, 1986). A second partial uncertainty factor of 3 was applied for database deficiencies, due to the lack of a two-generation reproductive study. A factor of 10 was applied for use of a LOAEL due to lack of an appropriate NOAEL to give a final uncertainty factor of 10,000.

$$\text{RfD} = 8.0 \text{ mg/kg-day} \div 10{,}000 = 8\text{E-}4 \text{ mg/kg-day}$$

Although derivation of an RfD value based on the study of Rothman et al. (1996) requires route-to-route extrapolation, the level of uncertainty associated with this value is lower than the RfD calculated from the Hsieh et al. (1988b) study. Therefore, the RfD value of 1E-3 mg/kg-day is designated as the RfD.

The occupational study of Rothman et al. (1996) is also designated as the principal study for derivation of RfC. As described above, this cross-sectional study compared the hematological outcomes of 44 workers occupationally exposed to benzene and 44 unexposed workers that were age and gender matched from Shanghai, China. Absolute lymphocyte count, WBC, RBC, hematocrit, and platelets were all significantly decreased, and MCV was significantly increased in a group of 44 workers occupationally exposed to a median 8-hr TWA of 31 ppm (99 mg/m^3) in comparison with an age- and sex-matched control group. These effects are consistent with the known hematotoxic effects of benzene (Aksoy, 1989; Goldstein, 1988). Only the absolute lymphocyte count was reduced in the subgroup exposed to a median 8-hr TWA of 7.6 ppm (24 mg/m^3) benzene ($p<0.03$). The study of Rothman et al. (1996) is notable among epidemiology studies that were compared to matched controls. A dose-response relationship was established between the absolute lymphocyte count and benzene exposure as monitored by organic vapor passive dosimetry and renal benzene metabolites. The median 8-hr TWA of 7.6 ppm (24 mg/m^3) was the lowest exposure group examined, and it is therefore a LOAEL for benzene immunotoxicity in humans.

The principal study is also supported by a large database of inhalation and oral exposure experiments in a range of test animal species.

The co-principal study of Rothman et al. (1996) identified a median 8-hr TWA concentration of 7.6 ppm (24 mg/m^3) as a LOAEL for the immunological endpoint, decreased absolute lymphocyte counts. A LOAEL $_{(HEC)}$ was calculated by adjusting for the occupational ventilation rate and for the intermittent work week schedule for 24-hr exposure, 7 days per week. The calculation is as follows:

$$\text{LOAEL}_{(HEC)} = 24 \text{ mg/m}^3) \times (10 \text{ m}^3/20 \text{ m}^3) \times 5 \text{ days}/7 \text{ days} = 8.6 \text{ mg/m}^3$$

To calculate an RfC using the adjusted LOAEL $_{(HBC)}$ value of 8.6 mg/m^3, the following uncertainty factors were selected. A factor of 10 was used for intraspecies differences in response (human variability) as a means of protecting potentially sensitive human subpopulations. A factor of 10 was applied for use of a LOAEL due to lack of an appropriate NOAEL. Two partial uncertainty factors of 3 each were applied to account for extrapolation from subchronic exposure to chronic exposure and for database deficiencies, to give a final uncertainty factor of 1000. Use of a partial uncertainty factor for exposure duration adjustment is necessary because the mean exposure duration in the study (6.4 years, range 0.7-16) is less than the Superfund interim guidance cutoff value of 7 years for designation as a chronic exposure (Means, 1989). Uncertainty regarding deficiencies in the database is small because of an adequate oral toxicity database and an extensive supporting inhalation database. Although the database includes developmental toxicity studies, a two-generation reproductive study was unavailable. There is also little evidence of toxic effects in either the lungs or the gastrointestinal tract. No modifying factor is considered necessary.

$$\text{RfC} = 8.6 \text{ mg/m}^3 / (1000) = 9\text{E-}3 \text{ mg/m}^3$$

The human chronic dose of ingested benzene considered to be without any appreciable risk (the RfD) is 1E-3 mg/kg-day. This is 1/1000 of the oral equivalent dose extrapolated from the LOAEL $_{(HBC)}$, using decreased absolute lymphocyte count observed in the lowest exposure group investigated in a subchronic human occupational study as the indicator of adverse effects (Rothman et al., 1996). The RfD is supported by an RfD value of 8E-4 mg/kg-day, which is 1/10,000 of the LOAEL dose, using hematological effects of erythrocytopenia, leukocytopenia, lymphocytopenia, and increased MCV in a subchronic test animal drinking water study as the indicator of adverse effects (Hsieh et al., 1988b).

Quantitative estimates of human risk as a result of low-level chronic benzene inhalation exposure are based on subchronic human occupational inhalation exposure because no suitable chronic human data are available. Hematotoxicity and immunotoxicity in humans are the critical effects observed in humans.

The human chronic air concentration considered to be safe (the RfC) is 9E-3 mg/m^3. This is 1/1000 the LOAEL $_{(HEC)}$, using decreased absolute lymphocyte count observed in the lowest exposure group investigated in a subchronic human occupational study as the indicator of adverse effects (Rothman et al., 1996). The overall confidence in this RfC assessment is medium.

In summary, although a large number of human and experimental animal studies have been conducted, there have been few human studies with reliable estimates of exposure to benzene and

few long-term repeated dose experiments in test animals. The human studies are also complicated by exposure to other solvents. The long-term animal studies have employed exposure levels that were too high to establish reliable NOAEL values, as significant adverse effects were observed even in the lowest dose tested in all the long-term studies examined.

An area of scientific uncertainty in this assessment concerns the neurotoxic effects of benzene. In common with many other organic solvents, benzene has been shown to produce neurotoxic effects in experimental animals and humans after short-term exposures to relatively high concentrations. There is a lack of reliable information on dose-related neurotoxic effects under low-dose, chronic exposure conditions in either humans or experimental animal model systems.

1. INTRODUCTION

This document presents background and justification for the hazard and dose-response assessment summaries in EPA's Integrated Risk Information System (IRIS). IRIS summaries may include an oral reference dose (RfD), inhalation reference concentration (RfC) and a carcinogenicity assessment.

The RfD and RfC provide quantitative information for noncancer dose-response assessments. The RfD is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis but may not exist for other toxic effects such as some carcinogenic responses. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer effects during a lifetime. The inhalation RfC is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). It is generally expressed in units of mg/m^3.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral exposure and inhalation exposure. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates are presented in three ways. The *slope factor* is the result of application of a low-dose extrapolation procedure and is presented as the risk per mg/kg/day. The *unit risk* is the quantitative estimate in terms of either risk per µg/L drinking water or risk per $µg/m^3$ air breathed. Another form in which risk is presented is a drinking water or air concentration providing cancer risks of 1 in 10,000; 1 in 100,000; or 1 in 1,000,000.

Development of these hazard identification and dose-response assessments for benzene has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines that were used in the development of this assessment may include the following: the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA,1986a), *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986b), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986c), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Proposed Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1995a), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998b), *Proposed Guidelines for Carcinogen Risk Assessment* (1996a), and *Reproductive Toxicity Risk Assessment Guidelines* (U.S. EPA, 1996b); *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988); (proposed) *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a); *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b); *Peer Review and Peer Involvement at the U.S. Environmental Protection Agency* (U.S. EPA, 1994c); *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995b); *Science Policy Council Handbook: Peer Review* (U.S. EPA, 1998a); and memorandum

from EPA Administrator, Carol Browner, dated March 21, 1995, Subject: Guidance on Risk Characterization.

Literature search strategy employed for this compound were based on the CASRN and at least one common name. At a minimum, the following databases were searched: RTECS, HSDB, TSCATS, CCRIS, GENETOX, EMIC, EMICBACK, DART, ETICBACK, TOXLINE, CANCERLINE, MEDLINE, and MEDLINE backfiles. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

Benzene also is known as benzol. Some relevant physical and chemical properties of benzene are listed below (Merck Index, 1989; NFPA, 1994).

CASRN: 71-43-2
Empirical formula: C_6H_6
Molecular weight: 78.11 (Merck Index, 1989)
Vapor pressure: 75 mm Hg at 20°C (NFPA, 1994)
Water solubility: 1750 mg/L at 25°C (Banerjee et al., 1980)
Log K_{ow}: 2.13 (Hansch and Leo, 1985)
Henry's law constant: 5.5×10^{-3} atm-m^3/mol (Mackay and Leinonen, 1975)
Conversion factor: 1 ppm = 3.24 mg/m^3 at 20°C

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

The toxicokinetics of benzene have been extensively studied; inhalation exposure is probably the major route of human exposure to benzene, although oral and dermal exposure are also important. Absorption, distribution, metabolism, and elimination have been studied in both humans and test animals. Investigations of the metabolism of benzene have led to the identification of toxic metabolites and to hypotheses about the mechanism of toxicity.

3.1. ABSORPTION

Benzene is readily absorbed by both test animals and humans from inhalation, oral, and dermal exposure (ATSDR, 1997).

3.1.1. Gastrointestinal Absorption

Although definitive data are not available on oral absorption of benzene in humans, case studies of accidental or intentional poisoning indicate that benzene is absorbed by the oral route (Thienes and Haley, 1972).

Benzene appears to be efficiently absorbed following oral dosing in test animals. Oral absorption of benzene was first demonstrated by Parke and Williams (1953). After radiolabeled ^{14}C-benzene was administered orally to rabbits (340-500 mg/kg), the total radioactivity eliminated in exhaled air and urine accounted for approximately 90% of the administered dose, indicating that most of the dose was absorbed. Studies in rats and mice showed that gastrointestinal absorption was greater than 97% in both species when the test animals were administered benzene by gavage at doses of 0.5-150 mg/kg-day (Sabourin et al., 1987). In many test animal studies, benzene is administered in oil to ensure predictable doses; however, this is not the likely method of human exposure, which is likely to be in drinking water. There have been few studies conducted in which benzene was administered to test animals in drinking water, which more closely resembles the predicted human oral exposure (Hsieh et al., 1988a,b; Shell Chemical Co., 1980).

The bioavailability of pure as opposed to soil-adsorbed benzene was compared in adult male rats (Turkall et al., 1988). Animals were gavaged with an aqueous suspension of ^{14}C-benzene alone or adsorbed to clay or sandy soil. Plasma concentration, half-life, tissue distribution, respiratory excretion, and urinary excretion were monitored. Peak plasma concentration of radioactivity was increased in the presence of soil as opposed to benzene alone, and soil increased the area under the plasma radioactivity-time curve as opposed to benzene alone. The half-life in plasma was not affected by soil.

3.1.2. Respiratory Absorption

Several studies have examined the absorption of benzene from the respiratory tract in humans (Nomiyama and Nomiyama, 1974a,b; Pekari et al., 1992; Srbova et al., 1950). Results from a study of 23 subjects that inhaled 47-100 ppm (150-320 mg/m^3) benzene for 2-3 hours showed that absorption was 70%-80% in the first few minutes of exposure, but decreased to approximately 50% after 1 hour, although considerable individual variability (some as low as 20%) was noted (Srbova et al., 1950). Similar results were reported by Nomiyama and Nomiyama (1974a,b), who exposed three female and three male volunteers to 52 to 62 ppm (198 mg/m^3) benzene for 4 hours. Retention declined from 50%-60% initially to reach a stable level of 30%-40% after 3 hours. Average uptake of benzene was 47% after 3 hours with subsequent excretion through the lungs of 17%, resulting in a net retention of 30% of the benzene in the respired air. No differences were observed between males and females. In a similar study, three healthy nonsmoking volunteers were exposed to benzene at levels of 1.6 or 9.4 ppm (5-30 mg/m^3) for 4 hours (Pekari et al., 1992). The amount of benzene absorbed was estimated from the difference between the concentration inhaled and exhaled. Estimates were 48% for the high dose and 52% for the low dose.

A number of studies have also been conducted on the absorption of benzene from inhalation exposure in test animal studies. Schrenk et al. (1941) noted a linear relationship between benzene concentration (200-1300 ppm [639-4153 mg/m^3]) and the equilibrium concentration of benzene in the blood of dogs. A steady-state blood level was attained within 30 minutes for these exposure concentrations. Exposure concentration appears to affect the retention of inhaled benzene as demonstrated by Sabourin et al. (1987) in rats and mice. The retention of benzene by rats and mice during a 6-hr exposure decreased as exposure concentration increased; 33±6% to 15±9% for rats, and 50±1% to 10±2% for mice as exposure concentration increased from 26 to 2600 mg/m^3 (10 to 1000 ppm). This study also showed species variability in the uptake and retention of inhaled benzene. At all exposure concentrations, mice inhaled greater amounts of benzene. At exposure concentrations below 350 mg/m^3, the mice retained approximately 50% more radioactivity per kg body weight than did the rats, but there was no significant difference at the highest (2500 mg/m^3) concentration.

3.1.3. Dermal Absorption

Studies conducted both with humans and experimental animals indicate that benzene is rapidly absorbed through the skin from both liquid and vapor phases. The percutaneous absorption of benzene has been studied in humans (Conca and Maltagliati, 1955; Hanke et al., 1961; Franz, 1984) and laboratory animals (Maibach and Anjo, 1981; Franz, 1983; Susten et al., 1985). Although dermal absorption is minimal compared with inhalation or oral absorption, this is, in large part, a result of the experimental methods and calculations employed. Benzene volatilizes rapidly from the skin, and if the absorption is based on the amount applied to the skin without accounting for volatilization losses, then percentage absorption figures are low and usually less than 1%. Although this represents realistic exposure conditions for most situations, absorption will be underestimated in exposure situations where contact with the benzene source is maintained for a prolonged period, such as showering with contaminated water. A substantial portion of the absorbed benzene is excreted through the lungs. Thus, experiments that measure excretion only in urine and feces substantially underestimate true absorption.

In vivo experiments on four human volunteers, to whom 0.0026 mg/cm^2 or ^{14}C-benzene was applied to forearm skin, indicated that approximately 0.05% of the applied dose was absorbed (Franz, 1984). Absorption was rapid, with more than 80% of the total excretion of the absorbed dose occurring in the first 8 hours after application. Calculations were based on urinary excretion data, and no correction was made for the amount of benzene that evaporated from the applied site before absorption occurred. In another study, 35-43 cm^2 of the forearm was exposed to approximately 0.06 g/cm^2 of liquid benzene for 1.25-2 hours (Hanke et al., 1961). The absorption was estimated from the amount of phenol eliminated in the urine. The absorption rate of liquid benzene by the skin was calculated to be approximately 0.4 mg/cm^2/hour. The absorption due to vapors in the same experiment was negligible.

Laitinen et al. (1994) studied occupational exposure to benzene in car mechanics in Finland. Blood samples were taken 3-9 hours after exposure. The approximated benzene concentration in blood corresponding to the time point of 16 hours after the exposure showed much higher levels of exposure than expected based on corresponding air measurements in the

workplace. Comparison of measured blood concentrations to predictions based on air measurements suggested that dermal exposure could account for 68% of exposure. In a series of experiments conducted in a residence with a benzene-contaminated water supply, Lindstrom et al. (1993) calculated that a dose of 281 µg benzene was absorbed during a 20-min shower with 60% derived from dermal absorption.

In vitro experiments using human skin support the observation that benzene can be absorbed dorsally. An experiment on the permeability of excised human skin resulted in the absorption of 0.17 mg/cm^2 after 0.5 hours and 1.92 mg/cm^2 after 13.5 hours (Loden, 1986). When time to complete evaporation (actual exposure time) was determined, total absorption was found to increase linearly with exposure time (Franz, 1984).

Using results from an in vitro study, it was estimated that an adult working in ambient air containing 10 ppm (32 mg/m^3) benzene would absorb 7.5 µL/hour from inhalation and 1.5 µL/hour from whole-body (2 m^2) dermal exposure (Blank and McAuliffe, 1985). It was also estimated that 100 cm^2 of smooth and bare skin in contact with gasoline containing 5% benzene would absorb 7 µL/hr. Diffusion through the stratum corneum was considered the most likely rate-limiting step because of benzene's low water solubility (Blank and McAuliffe, 1985).

Several dermal absorption studies have also been conducted with experimental animals. In rhesus monkeys, minipigs, and hairless mice, dermal absorption was <1% following a single direct application of liquid benzene (Franz, 1984; Maibach and Anjo, 1981; Susten et al., 1985). Absorption was rapid with the highest urinary excretion observed in the first 8 hours following exposure (Franz, 1984; Susten et al., 1985). Multiple applications, as well as application to cellophane tape-stripped skin resulted in greater skin penetration (Maibach and Anjo, 1981).

Tsuruta (1989) reported that dermal absorption of benzene increased linearly with dose in hairless mice exposed to benzene vapors (the mice were attached to respirators to avoid inhalation exposure). The dermal absorption rates at exposure concentrations of 200, 1000, and 3000 ppm (639, 3195, and 9584 mg/m^3) were 4.11, 24.2, and 75.5 $mmol/cm^2$/hour, respectively. The skin absorption coefficient was 0.619 cm/hour. Tsuruta (1989), using the mouse dermal absorption data and human occupational exposure data, estimated that skin absorption of benzene by humans would be 3.7% that of inhalation exposure at the same concentration.

Permeability constants for dermal absorption of benzene vapors were also estimated by McDougal et al. (1990). In this study, rats that were supplied breathing air through latex masks were exposed to benzene vapor at a concentration of 40,000 ppm (127,787 mg/m^3) for 4 hours. Blood concentration was monitored at 0.5, 1, 2, and 4 hours. A physiologically based pharmacokinetic (PBPK) model was used to estimate the permeability of the vapor in rat skin as well as human skin; the rat and human permeability constants were estimated as 0.15 and 0.08 cm/hour, respectively. Based on these findings, dermal exposure studies in rats would likely provide a conservative estimate of the dermal absorption of benzene by humans. In an in vitro experiment using Fischer 344 rat skin, Mattie et al. (1994) determined a skin:air partition coefficient of 35 for benzene at 203 ppm (649 mg/m^3) with an equilibration time of 4 hours.

Skowronski et al. (1988) found that soil adsorption decreases the dermal bioavailability of benzene. Male rats were treated dermally with 0.004 mg/cm^2 ^{14}C-benzene, with or without 1 g of clay or sandy soil. Benzene absorption half-lives of 3.1, 3.6, and 4.4 hours were reported for pure benzene, sandy soil, and clay soil, respectively.

3.2. DISTRIBUTION

3.2.1. Oral Exposure

No studies were located regarding distribution of benzene in humans after oral exposure to benzene.

One test animal study examined the distribution of benzene and its metabolites following oral exposure. In Sprague-Dawley rats administered a single dose of 0.15, 1.5, 15, 150, or 500 mg/kg of ^{14}C-benzene by gavage, benzene was rapidly absorbed and distributed to various organs and tissues within 1 hour of administration (Low et al., 1989, 1995). One hour after rats were dosed with 0.15 or 1.5 mg/kg, tissue distribution of benzene was highest in liver and kidney, intermediate in blood, and lowest in the Zymbal gland, nasal cavity tissue, and mammary gland. At higher doses, beginning with 15 mg/kg, benzene disproportionately increased in the mammary glands and bone marrow. Bone marrow and adipose tissue proved to be depots of benzene at the higher doses. The highest tissue concentrations of the benzene metabolite hydroquinone 1 hour after administration of 15 mg/kg of benzene were in the liver, kidney, and blood, while the highest concentrations of the metabolite phenol were in the oral cavity, nasal cavity, and kidney. The major tissue sites of the conjugated benzene metabolites were blood, bone marrow, oral cavity, kidney, and liver for phenyl sulfate and hydroquinone glucuronide; muconic acid was also found in these sites. Additionally, the Zymbal gland and nasal cavity were depots for phenyl glucuronide, another conjugated metabolite of benzene. The Zymbal gland is a specialized sebaceous gland and a site for benzene-induced tumors. Therefore, it is reasonable to expect that a lipophilic chemical like benzene would partition readily into this gland. However, benzene did not accumulate in the Zymbal gland; within 24 hours after administration, a radiolabel derived from ^{14}C-benzene in the Zymbal gland constituted less than 0.0001% of the administered dose.

The bioavailability of pure as opposed to soil-absorbed benzene was conducted in adult male rats (Turkall et al., 1988). Animals were gavaged with an aqueous suspension of ^{14}C-benzene alone or adsorbed to clay or sandy soil. Two hours after exposure, stomach tissue contained the highest amount of radioactivity, followed by fat in all treatment groups. No differences in tissue distribution patterns were detected for the three treatments.

3.2.2. Inhalation Exposure

Human case studies provide some information on the distribution of benzene in the body following inhalation exposure (Tauber, 1970; Winek and Collom, 1971). Levels of 0.38 mg% in blood (mg% = mg per 100 mL of blood or mg/100 g of tissue), 1.38 mg% in the brain, and 0.26 mg% in the liver were reported in a worker who died from exposure to a very high benzene

concentration (Tauber, 1970). An autopsy of a youth who died from benzene inhalation showed concentrations of 2.0 mg% in blood, 3.9 mg% in brain, 1.6 mg% in liver, 1.9 mg% in kidney, 1 mg% in stomach, 1.1 mg% in bile, 2.23 mg% in abdominal fat, and 0.06% in urine (Winek and Collom, 1971). Benzene can cross the human placenta and has been found in umbilical cord blood in amounts equal to or greater than amounts in the mother's blood (Dowty et al., 1976).

Results from test animal studies indicate that absorbed benzene is distributed throughout several compartments. The parent compound is preferentially stored in the fat, although the relative uptake in tissues also appears to be dependent on the perfusion rate of tissues by blood. Steady-state benzene concentrations in rats exposed via inhalation to 1600 mg/m^3 (500 ppm) for 6 hr were: blood, 1.2 mg%; bone marrow, 3.8 mg%; and fat, 16.4 mg% (Rickert et al., 1979). Benzene was also found in the kidney, lung, liver, brain, and spleen. Levels of the benzene metabolites phenol, catechol, and hydroquinone were higher in the bone marrow than blood, with phenol being eliminated more rapidly after exposure than catechol or hydroquinone. Ghantous and Danielsson (1986) exposed pregnant mice to a benzene concentration of 6400 mg/m^3 (2000 ppm) for 10 min and found benzene and its metabolites in lipid-rich tissues such as brain and fat, as well as in perfused tissues such as liver and kidney. Benzene was also found in the placenta and fetuses immediately following exposure.

Benzene was rapidly distributed throughout the bodies of dogs exposed via inhalation to concentrations of 800 ppm (2556 mg/m^3) for up to 8 hours per day for 8-22 days (Schrenk et al., 1941). Fat, bone marrow, and urine contained about 20 times the concentration of benzene in blood: benzene levels in muscles and organs were 1-3 times that in blood; and erythrocytes contained about twice the amount of benzene found in plasma.

Studies in pregnant mice demonstrate that after inhalation exposure, ^{14}C-labeled benzene crosses the placenta. Volatile radioactivity (unmetabolized benzene) was observed in the placenta and fetuses immediately after and up to 1 hour after exposure (Ghantous and Danielsson, 1986). Nonvolatile metabolites were also detected in the fetus, but at lower levels than in maternal tissues. The label peaked in fetal tissues 30 minutes to 1 hour after inhalation, similar to but reaching lower concentrations than levels in maternal tissues; the label was not retained in the fetus. No firmly tissue-bound metabolites of benzene could be detected in the fetal tissues in late gestation, indicating that the mouse fetus does not have the ability to form the reactive metabolites.

3.2.3. Dermal Exposure

No studies were located regarding distribution in humans after dermal exposure to benzene.

A study of male rats treated dermally with 0.004 mg/cm^2 of ^{14}C-benzene, with and without 1 g of clay or sandy soil, revealed soil-related differences in tissue distribution following treatment. The ^{14}C activity (expressed as a percentage of initial dose per g of tissue) 48 hours after treatment with soil-absorbed benzene was greatest in the treated skin (0.059%-0.119%), followed by the kidney (0.024%) and liver (0.013%-0.015%), in both soil groups. In the pure

benzene group, the kidney contained the largest amount of radioactivity (0.026%), followed by the liver (0.013%) and treated skin (0.11%) (Skowronski et al., 1988). In all three groups, less than 0.01% of the radioactivity was found in the following tissues: duodenum, fat, bone marrow, esophagus, pancreas, lung, heart, spleen, blood, brain, thymus, thyroid, adrenal, testes, untreated skin, and carcass.

3.3. METABOLISM

The metabolism of benzene is required for expression of benzene toxicity, and the evidence has been summarized in several reviews (ATSDR, 1997; Snyder and Hedli, 1996; Ross, 1996). Although benzene metabolism is still under investigation, the current understanding of the pathways of benzene metabolism is shown in Figure 1. The first step in benzene metabolism is the formation of the epoxide benzene oxide via a cytochrome P450-dependent mixed-function oxidase (Jerina et al., 1968). The specific oxidase involved in the initial metabolism of benzene has been identified as cytochrome P450 2E1 (CYP2E1). After this occurs, two metabolic toxification pathways, one involving ring hydroxylation and the second involving ring opening, results in the formation of putative toxic benzene metabolites (Henderson et al., 1989). In the first pathway involving ring hydroxylation, acid-catalyzed opening of the epoxide ring is followed by aromatization resulting in the formation of phenol. Phenol is further converted into hydroquinone which is oxidized to benzoquinone. The conjugates formed from hydroquinone (hydroquinone glucuronide and hydroquinone sulfate) are markers for this toxification pathway leading to benzoquinone (Henderson et al., 1989). Phenol can also be metabolized to catechol and trihydroxy benzene. Metabolism of benzene oxide by epoxide hydrolase leads to the formation of benzene dihydrodiol. Catechol can also be formed from benzene dihydrodiol via metabolism by cytosolic dehydrogenases (Henderson et al., 1989).

The second pathway involving ring-opening leads to the formation of muconic acid via muconaldehyde (see Figure 1). Mouse liver microsomes and cytosol have been shown to catalyze ring-opening in the presence of nicotinamide adenine dinucleotide phosphate in vitro, producing *trans,trans*-muconaldehyde, a six-carbon dien dialdehyde also referred to as muconic dialdehyde (Goon et al., 1993; Latriano et al., 1986), a known hematotoxin (Witz et al., 1985) and toxic metabolite of benzene (Henderson et al., 1989). Metabolism of benzene and *trans,trans*-muconaldehyde in the isolated perfused rat liver indicated that benzene was metabolized to muconic acid, a ring-opened metabolite of benzene (Grotz et al., 1994). *Trans,trans*-muconaldehyde was metabolized to muconic acid and three other metabolites. These studies indicate that ring-opening of benzene occurs in the liver.

There are two detoxification pathways (see Figure 1). One pathway leads to the formation of mercapturic acid via glutathione conjugates of benzene oxide, which are subsequently metabolized to prephenyl mercapturic acid and phenyl mercapturic acid and eliminated via biliary excretion (Henderson et al., 1989; Sabourin et al., 1988; Schafer et al., 1993; Schlosser et al., 1993; Schrenk et al., 1992; van Sittert et al., 1993). The major portion of benzene oxide is nonenzymatically rearranged into phenol (Parke and Williams, 1953). The second detoxification pathway involves the formation of water-soluble urinary metabolites, which are glucuronide or sulfate conjugates of phenol (Henderson et al., 1989; Wells and

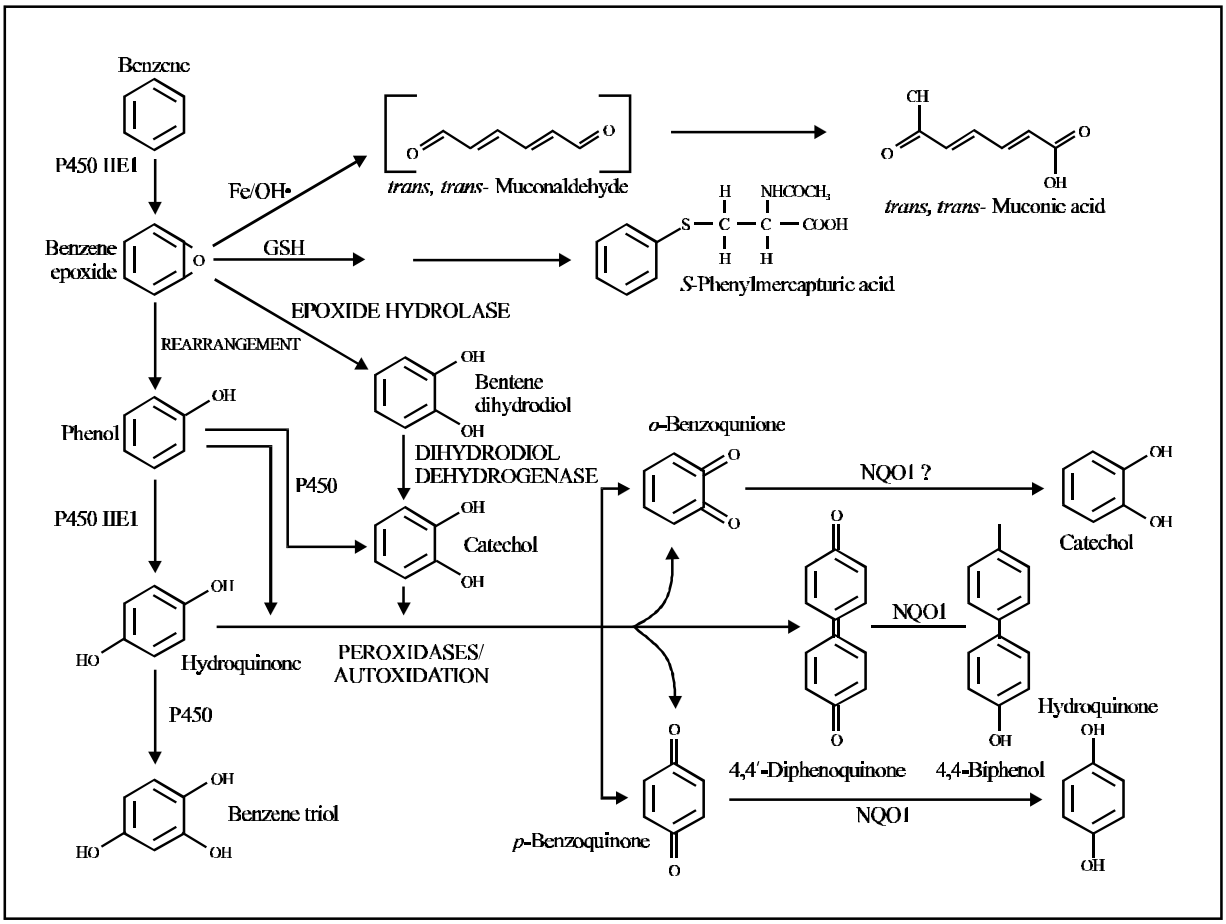

Figure 1. Metabolic pathways for benzene.

Nerland, 1991). Further metabolites of phenol and benzene dihydrodiol (e.g., catechol, hydroquinone, and trihydroxy benzene) are excreted as sulfate or glucuronide conjugates, which are considered detoxification products of benzene metabolism (Henderson et al., 1989; Schrenk and Bock, 1990). Electroanalytical methods have been used to further elucidate the metabolism of benzene and phenol by both microsomal and peroxidase enzyme (Lunte and Lunte, 1990).

Recent literature identifies the following metabolites after incubation of benzene with mouse liver microsomes: phenol, hydroquinone, *trans,trans*-muconaldehyde, 6-oxo-*trans,trans*-2,4-hexadienoic acid, 6-hydroxy-*trans,trans*-2,4-hexadienal, and 6-hydroxy-*trans,trans*-2,4-hexadienoic acid (Zhang et al., 1995a). β-hydroxymuconaldehyde, a new metabolite, was also identified. Additional work by Zhang et al. (1995b) suggests that *cis,cis*-muconaldehyde is formed first, followed by *cis,trans*-muconaldehyde, and finally converted to *trans,trans*-muconaldehyde. Muconic dialdehyde has been shown to be metabolized in vivo in mice to muconic acid (Witz et al., 1990a,b). These data suggest that muconic dialdehyde is the precursor of muconic acid in animals exposed to benzene. Small amounts of muconic acid were found in the urine of rabbits and mice that received oral doses of ^{14}C-benzene (Gad-El Karim et al., 1985; Parke and Williams, 1953). The percentage of this metabolite formed varied with the administered benzene dose and was quite high at low doses (17.6% of 0.5 mg/kg benzene

administered to C57BL/6 mice) (Witz et al., 1990a,b). Other studies in test animals support these results (Brondeau et al., 1992; Ducos et al., 1990; McMahon and Birnbaum, 1991; Schad et al., 1992). Because of its relative importance in benzene toxicity, additional modeling studies, including molecular orbital studies, have been conducted to further describe how *trans,trans*-muconaldehyde is transformed to muconic acid (Bock et al., 1994).

The above pathway also appears to be active in humans (Bechtold and Henderson, 1993; Ducos et al., 1990, 1992; Lee et al., 1993; Melikian et al., 1993, 1994; Yu and Weisel, 1996). Urine samples from male and female smokers and nonsmokers were obtained from subjects who applied for life insurance (Melikian et al., 1994). The levels of muconic acid and cotinine (a biomarker for cigarette smoking) in the urine of the groups of pregnant and nonpregnant smokers and nonsmokers were compared with previously reported data in male smokers. Results showed the mean levels of muconic acid in the groups of male, female nonpregnant, and female pregnant smokers were 3.6-, 4.8-, and 4.5-fold higher, respectively, than the mean concentration of this acid in the nonsmoking groups. Yu and Weisel (1996) measured *trans-trans* muconic excretion in human volunteers who were exposed to benzene in environmental tobacco smoke. Urinary muconic acid associated with the benzene exposure was excreted within 12 hours. The proportion of the sub-ppm benzene dose excreted as muconic acid averaged 25%, range 7.2%-58%. This is higher than the mean value of 17.6% muconic acid formed by mice (Witz et al., 1990a,b). The proportion of benzene excreted as muconic acid also varies with the dose in humans (Rothman et al., 1996). In this occupational study, the proportion of measured benzene metabolites excreted as muconic acid and hydroquinone decreased from 32% in workers exposed to less than 31 ppm (99 mg/m^3) to 24% in workers exposed to more than 31 ppm (99 mg/m^3).

Benzene metabolism in humans and test animals follows many similar pathways. Benzene is metabolized by cytochrome P450-dependent mixed-function oxidase enzymes. Cytochrome P450 enzymes are ubiquitous in all tissues; however, benzene is primarily metabolized in the liver. Sammett et al. (1979) provided evidence that the metabolism of benzene in the liver plays an important part in benzene toxicity by showing that partial hepatectomy of rats diminished both the rate of metabolism of benzene and its toxicity, suggesting that a metabolite and/or metabolites formed in the liver are necessary for toxicity.

Immunoinhibition studies in rat and rabbit hepatic microsomes also have implicated CYP2E1 as the major oxidative enzyme involved in benzene metabolism (Johansson and Ingelman-Sundberg, 1988; Koop and Laethem, 1992). Valentine et al. (1996) provide convincing evidence that benzene metabolism by CYP2E1 is in fact the critical step in benzene toxicity. Transgenic CYP2E1 knockout mice (cyp2e1$^{-/-}$) were used to investigate the involvement of CYP2E1 in benzene metabolism and toxicity. Absence of the CYP2E1 protein was confirmed by Western blot analysis of mouse liver. Male cyp2e1$^{-/-}$ and wild-type mice were exposed to 200 ppm (639 mg/m^3) ^{14}C-benzene by nose-only inhalation for 6 hr. Total radioactivity recovered in the urine over 48 hr for cyp2e1$^{-/-}$ mice was reduced to 13% of the wild-type mice, with large reductions in all oxidation products of benzene. The major urinary metabolite in cyp2e1$^{-/-}$ mice was phenylsulfate. For the toxicity studies, male cyp2e1$^{-/-}$, wild-type, and B6C3F1 mice were exposed to 0 or 200 ppm (640 mg/m^3) benzene 6 hr/day for 5 days. On day 5, blood, bone marrow, thymus, and spleen were removed for evaluation of micronuclei frequencies and tissue cellularities. No benzene-induced cytotoxicity or genotoxicity was observed in cyp2e1$^{-/-}$ mice. In

contrast, benzene exposure resulted in severe genotoxicity and cytotoxicity in both wild-type and B6C3F1 mice.

Benzene is also metabolized to hydroquinone in the lungs, although the rate of metabolism was slower (Chaney and Carlson, 1995). The CYP2E1 specific inhibitor, diethyldithiocarbamate, inhibited activity in both pulmonary and hepatic microsomes, thus indicating that CYP2E1 is also the predominant P450 isozyme in pulmonary microsomes.

Variation in CYP2E1 activity in human hepatic microsomes has been shown to vary by 13-fold (Seaton et al., 1994). Human hepatic microsomes were prepared from 10 trauma victims, and CYP2E1 activity was compared to rats and mice hepatic microsomes. Activity varied from 0.253-3.266 nmol/min/mg. Activities determined in mice (1.558 nmol/min/mg) and rats (0.625 nmol/min/mg) were within the range determined for humans. Phenol was the main metabolite formed in all but two high-activity human samples. In these two samples, the major metabolite was hydroquinone. Formation of hydroquinone and catechol was directly correlated with CYP2E1 activity. Seaton et al. (1995) measured the activity of the two major detoxification reactions, phenol sulfonation and hydroquinone glucuronidation in hepatic microsomes from the same 10 individuals. The initial rates of phenol sulfonation varied threefold (0.309-0.919 nmol/min/mg). The initial rates of hydroquinone glucuronidation also varied threefold (0.101-0.281 nmol/min/mg). Initial rates of phenol sulfonation were faster in rats than in mice (1.195 vs. 0.458 nmol/min/mg). In contrast, initial rates of hydroquinone glucuronidation were slower in rats than mice (0.077 vs. 0.218 nmol/min/mg). The values for rats and mice lie outside the observed ranges for humans.

The production of the initial benzene metabolites occurs primarily in the liver; however, there is little evidence of benzene hepatotoxicity, and the effects of benzene toxicity are primarily expressed as hematotoxicity and myelotoxicity in the bone marrow (ATSDR, 1997; Snyder and Hedli, 1996). The evidence suggests that secondary metabolism in the bone marrow is required for expression of the toxicity of benzene (Irons et al., 1980; Schlosser and Kalf, 1989; Subrahmanyam et al., 1990, 1991). Most research has focused on the possibility that catechol and hydroquinone, or *trans,trans*-muconaldehyde, generated by reactions in the liver are transported to the bone marrow and subsequently activated there by the action of myeloperoxidase (Smith et al., 1989; Rushmore et al., 1984). Benzene oxide was believed to be too reactive to escape the liver and to cause toxicity in the bone marrow. Lovern et al. (1997), however, recently showed that benzene oxide constituted 7% of the benzene metabolites after 18 minutes in vitro. Lindstrom et al. (1997) demonstrated the presence of benzene oxide in the blood and estimated its half-life to be about 8 min. Using a PBPK model, Lindstrom et al. (1997) predicted that the dose to the body from benzene oxide would be about 22 times greater than from 1,4 benzoquinone. Thus, circulating benzene oxide could contribute to observed DNA and protein adduct formation.

The ability of bone marrow to metabolize benzene independently of the liver has been tested in situ in male Fischer 344 rats. Benzene metabolism by bone marrow was complete and independent of metabolism by the liver, with concentrations of phenol greater than catechol and hydroquinone. Although the total metabolism by bone marrow was limited (total metabolites present were 25% of those in blood), the concentration of metabolites in the bone marrow exceeded that in the blood. The concentration of total metabolites in blood was 0.215 nmol/g as

compared with 81.3 nmol/g for bone marrow (Irons et al., 1980). Similar studies have been conducted in mice (Ganousis et al., 1992). Fibroblasts had elevated levels of glutathione-S-transferase activity relative to macrophages, whereas macrophages had higher levels of UDP-glucuronyltransferase and peroxidase activity. These data suggest that cell-specific metabolism of benzene in the marrow may contribute to the toxicity of benzene in this tissue compartment. In addition, comparison of the detoxifying activities of rat and mouse bone marrow stromal cells indicate that rats have higher levels of glutathione and quinone reductase, which are known to play critical roles in modulating hydroquinone-induced toxicity; this suggests a metabolic basis for the observed increased susceptibility of mice to benzene-induced hematotoxicity (Zhu et al., 1995).

Benzene has been found to stimulate its own metabolism, thereby increasing the rate of toxic metabolite formation. Pretreatment of mice, rats, and rabbits subcutaneously with benzene increased benzene metabolism in vitro without increasing cytochrome P450 concentrations (Arinc et al., 1991; Gonasun et al., 1973; Saito et al., 1973). In contrast, there was no significant effect on the metabolism of benzene when Fischer 344 rats and B6C3F1 mice, pretreated with repeated inhalation exposure to 600 ppm (316 mg/m^3) of benzene, were again exposed to 600 ppm (316 mg/m^3) benzene (Sabourin et al., 1990). The rate of benzene metabolism can be altered by pretreatment with various compounds. Benzene is a preferential substrate of one particular cytochrome P450 family, namely CYP2E1, which also metabolizes alcohol and aniline. CYP2E1 can be induced by these substrates and is associated with the generation of hydroxyl radicals, probably via futile cycling of the cytochrome (Chepiga et al., 1990; Parke, 1989; Snyder et al., 1993a). It is possible that hydroxy radical formation by CYP2E1 may play a role in the benzene ring-opening pathway, leading to the formation of *trans, trans*-muconaldehyde. Phenol, hydroquinone, benzoquinone, and catechol have also been shown to induce P-450 in human hematopoietic stem cells (Henschler and Glatt, 1995). Therefore, exposure to chemicals that stimulate the activity of this enzyme system prior to exposure to benzene could increase the rate of benzene metabolism.

Daiker et al. (1996) found that repeated oral benzene exposure of female B6C3F1 mice for 3 weeks at 50 mg/kg-day decreased CYP2E1 activity by 34% and activated the detoxification enzyme glutathione transferase by 30% without affecting aldehyde dehydrogenase, another detoxifying enzyme. The authors suggested that these changes in enzyme activity may serve a protective role against repeated benzene exposure.

In rats exposed to ethanol and benzene, it was found that benzene interfered with the disappearance of ethanol from the body (Nakajima et al., 1985). The results of further studies showed that ethanol treatment increased the production of hydroxylated benzene metabolites, phenol, and hydroquinone, suggesting induction of benzene metabolism (Nakajima et al., 1987). Ethanol is known to be an inducer of CYP2E1, the enzyme involved in the initial metabolism of benzene. The toxicity of alcohol, particularly necrosis of the gastrointestinal tract, is known to be associated with oxygen radical production. The possibility therefore exists of a synergism between alcohol and benzene, and of the role of reactive oxygen and the formation of circulating lipid peroxides in the hematopoietic toxicity and carcinogenicity of benzene (Parke, 1989).

The dose of benzene affects both the total metabolism and the concentrations of individual metabolites formed. In mice, the percentage of hydroquinone glucuronide decreased as the dose increased. In both rats and mice, the percentage of muconic acid decreased as the dose increased. The shift in metabolism may affect the dose-response relationship for toxicity, and has been observed in all animal species studies thus far (Sabourin et al., 1989a, 1992; Witz et al., 1990a,b).

Additional studies by Sabourin et al. (1987, 1988, 1989a, 1992) showed that differences in species, routes of exposure, and dosing regimens will affect the disposition and metabolic fate of benzene. The effect of species differences was evidenced by the fact that mice have a higher minute volume per kg body weight than rats (~1.5 times higher). This caused the blood concentration of benzene to reach equilibrium more quickly in mice than in rats, but the steady-state level in blood was not influenced (Sabourin et al., 1987). Steady-state blood levels are determined primarily by the blood:air partition coefficient rather than differences in metabolism. Rats and mice were administered benzene by gavage at doses of 0.5-150 mg/kg-day (Sabourin et al., 1987). At doses below 15 mg/kg, >90% of the benzene was metabolized, while at doses above 15 mg/kg, an increasing percentage of orally administered benzene was exhaled unmetabolized. Total metabolites per unit body weight were equal in rats and mice at doses up to 50 mg/kg-day. However, total metabolites in mice did not increase at higher doses, suggesting saturation of metabolic pathways (Sabourin et al., 1987).

The integrated dose to a tissue over a 14-hr period (6-hr exposure, 8 hr following exposure) was calculated for benzene metabolites in Fischer 344 rats and B6C3F1 mice that were exposed to 50 ppm (160 mg/m^3) of radiolabeled (^{3}H) benzene (Sabourin et al., 1988). The major metabolic products in rats were detoxification products that were marked by phenyl conjugates. In contrast, mice had substantial quantities of the markers for toxification pathways (muconic acid, hydroquinone glucuronide, and hydroquinone sulfate) in their tissues. Muconic acid and hydroquinone glucuronide were also detected in mouse bone marrow. These results may explain why mice are more susceptible to benzene-induced toxicity than rats.

In a study by Orzechowski et al. (1995), hepatocytes from adult male Wistar rats and NMRI mice were incubated for 1 hr with 0.5 mM ^{14}C-benzene, and the supernatant analyzed for metabolites. Formation of sulfate conjugates of benzene, hydroquinone, and 1,2,4-benzenetriol was also studied in a separate experiment. Mouse hepatocytes produced two metabolites (1,2,4-trihydroxybenzene sulfate and hydroquinone sulfate) that were not found in rat hepatocyte incubations. These sulfate metabolites were found in incubations including benzene, or the metabolites themselves, hydroquinone and 1,2,4-benzenetriol. Mouse hepatocytes were almost three times more effective in metabolizing benzene compared with rat hepatocytes. This difference was accounted for in the formation of hydroquinone, hydroquinone sulfate, and 1,2,4-trihydroxybenzene sulfate. These in vitro experiments indicate that there are both quantitative and qualitative differences in rodent metabolism of benzene.

Data produced in vitro by mouse and rat liver microsomes also indicate species differences in benzene metabolism (Schlosser et al., 1993). Quantitation of metabolites from the microsomal metabolism of benzene indicated that after 45 min, mouse liver microsomes from male B6C3F1 mice had converted 20% of the benzene to phenol, 31% to hydroquinone, and 2% to catechol. In contrast, rat liver microsomes from male Fischer 344 rats converted 23% to phenol, 8% to

hydroquinone, and 0.5% to catechol. Mouse liver microsomes continued to produce hydroquinone and catechol for 90 min, whereas rat liver microsomes had ceased production of these metabolites by 90 min. Muconic acid production by mouse liver microsomes was <0.04 and <0.2% from phenol and benzene, respectively, after 90 min.

There are quantitative differences in the benzene metabolites produced by different species (Sabourin et al., 1988). Fischer 344 rats exposed to 50 ppm (160 mg/m^3) benzene had undetectable amounts of phenol, catechol, and hydroquinone in the liver, lungs, and blood. The major water-soluble metabolites were muconic acid, phenyl sulfate, prephenyl mercapturic acid, and an unknown metabolite. The unknown metabolite was present in amounts equal to the amounts of phenyl sulfate in the liver; phenyl sulfate and the unknown metabolite were the major metabolites in the liver. B6C3F1 mice exposed to 50 ppm (160 mg/m^3) benzene had detectable levels of phenol and hydroquinone in the liver, lungs, and blood; catechol was detectable only in the liver and not in the lungs or blood. As in the rat, the unknown metabolite was present in amounts equal to the amounts of phenyl sulfate in the liver. Mice had more muconic acid in the liver, which indicates a greater risk for them from *trans,trans*-muconaldehyde (Sabourin et al., 1988).

A PBPK model based on the ventilation rate, cardiac output, tissue blood flow rates, and volumes as well as measured tissue:air and blood:air partition coefficients has been developed (Medinsky et al., 1989a; Travis et al., 1990a). Experimentally determined data and model simulations indicated that during and after 6 hr of inhalation exposure to benzene, mice metabolized benzene more efficiently than rats (Medinsky et al., 1989a). After oral exposure, mice and rats appeared to metabolize benzene similarly up to oral doses of 50 mg/kg, above which rats metabolized more benzene than did mice on a per kg body weight basis (Medinsky et al., 1989b). This model may be able to predict the human response based on test animal data. Benzene metabolism followed Michaelis-Menton kinetics in vivo primarily in the liver and to a lesser extent in the bone marrow. Additional information on PBPK modeling is presented in Section 3.5.

The effect of dosing regimen on benzene metabolism was studied in Fischer 344 rats and B6C3F1 mice that had either long inhalation exposures to low concentrations or short exposures to high concentrations of benzene (Sabourin et al., 1989a). Inhalation occurred at 1 of 3 exposure regimens, all having the same integral amount of benzene: 600 ppm (1917 mg/m^3) benzene for 0.5 hr, 150 ppm (479 mg/m^3) for 2 hr, or 50 ppm (160 mg/m^3) for 6 hr. Results indicated no dose-rate effect in rats. In mice, however, the fast exposure rate (600 ppm [1917 mg/m^3] for 0.5 hr) produced less muconic acid in the blood, liver, and lungs. In the blood and lungs, less hydroquinone glucuronide and more prephenyl mercapturic acid were produced at the higher exposure rates. At the highest benzene exposure concentrations or fastest benzene exposure rate in mice, there was a reduction in the ratios of muconic acid and hydroquinone glucuronide to the metabolite phenyl sulfate. Furthermore, with increased dose rate or increased exposure concentration, mice tended to shift a greater portion of their benzene metabolism toward detoxification pathways. Likewise, the detoxification pathways for benzene appear to be low-affinity, high-capacity pathways, whereas pathways leading to the putative toxic metabolites appear to be high-affinity, low-capacity systems (Henderson et al., 1989). Accordingly, if the exposure dose regimen, via inhalation, extends beyond the range of linear metabolism rates of

benzene (~200 ppm [639 mg/m^3] by inhalation) (Sabourin et al., 1989b), the fraction of toxic metabolites formed relative to the amount administered will be reduced.

Kenyon et al. (1995) compared the urinary profile of metabolites in B6C3F1 mice after oral dosing with phenol with the result of Sabourin et al. (1989a), who administered a comparable oral dose of benzene to B6C3F1 mice. Their analysis indicated that phenol administration resulted in lower urinary levels of hydroquinone glucuronide and higher levels of phenol sulfate and phenol glucuronide compared with benzene administration. They hypothesized that the differences in the urinary metabolite profiles between phenol and benzene after oral dosing were due to zonal differences in the distribution of metabolizing enzymes within the liver. Conjugating enzymes are more concentrated in the periportal area of the liver, the first region to absorb the compound, whereas oxidizing enzymes are more concentrated in the pericentral region of the liver. Based on this hypothesis, during an initial pass through the liver after oral administration, phenol would have a greater opportunity to be conjugated as it was absorbed from the gastrointestinal tract into the periportal region of the liver, thus resulting in less free phenol being delivered into the pericentral region of the liver to be oxidized. With less free phenol available for oxidation, less hydroquinone would be produced, relative to conjugated phenol metabolites. In contrast, benzene must be oxidized before it can be conjugated. Therefore, metabolism of benzene would be minimal in the periportal region of the liver, with most of the benzene reaching the pericentral region to be oxidized to hydroquinone. Based on this scheme, the authors suggest that benzene administration would result in more free phenol being delivered to oxidizing enzymes in the pericentral region of the liver than administration of phenol itself (Kenyon et al., 1995).

A number of investigators have suggested that covalent binding of benzene metabolites to cellular macromolecules is related to benzene's mechanism of toxicity, although the relationship between adduct formation and toxicity is not clear. Benzene metabolites have been found to form covalent adducts with proteins from blood in humans (Bechtold et al., 1992b). Benzene metabolites have been found to form covalent adducts with nucleic acids and proteins in rats (Norpoth et al., 1988); to covalently bind to proteins in mouse or rat liver, bone marrow, kidney, spleen, blood, and muscle in vivo (Bechtold and Henderson, 1993; Bechtold et al., 1992a,b; Longacre et al., 1981a,b; Sun et al., 1990); to bind to proteins in perfused bone marrow preparations (Irons et al., 1980) and in rat liver DNA in vivo (Lutz and Schlatter, 1977); and to bind to DNA in rabbit and rat bone marrow mitochondria in vitro (Rushmore et al., 1984). The inhibition of RNA synthesis in liver and bone marrow mitochondria has been correlated with covalent binding of benzene metabolites to DNA in vitro (Kalf et al., 1982). In vitro studies showed that benzene metabolites inhibited RNA synthesis and formed adducts similar to benzene; p-benzoquinone was the most effective inhibitor of RNA synthesis. When ^{14}C-hydroquinone was added to ^{3}H-deoxyguanosine, two adducts were formed. Two adducts were also formed when ^{14}C-hydroquinone was reacted with DNA from calf thymus and *M. lysodeikticus* (Snyder et al., 1987).

Fischer 344 rats and B6C3F1 mice were administered a single dose of 50, 100, 200, and 400 mg/kg ^{13}C/^{14}C-radiolabeled benzene in corn oil via gastric intubation (McDonald et al., 1994). Animals were sacrificed 24 hr after administration of labeled benzene. Radiolabeled (^{14}C/^{13}C) adducts in hemoglobin (Hgb) and bone marrow were monitored. Cysteine adducts of benzene oxide and 1,2- and 1,4-benzoquinone in the rats were assayed, and the proportions of cysteine-

bound adducts to total protein binding were estimated. Increasing production of Hgb adducts was observed over all dose levels tested. Benzene oxide adducts with rat Hgb represented 27% of the total Hgb binding and 73% of the cysteinyl binding, whereas quinone adducts represented relatively small proportions. In the bone marrow, benzoquinone adducts were more abundant than those of benzene oxide. 1,2-benzoquinone adducts predominated in rat marrow with 9% of binding. The relationship between marrow adducts and dosage was linear. Protein adduct levels in the bone barrow were about 20% of the levels in rat Hgb. Comparisons of adduct production in the bone marrow indicated that 1,2-benzoquinone adducts were approximately 2.3-fold greater than those of 1,4-benzoquinone in the rat. In the mouse, increasing production on Hgb adducts was observed over all dose levels tested. With mouse Hgb, the 1,4-benzoquinone adducts accounted for 5.5% of the total Hgb binding and 12.2% of the cysteinyl binding, while 1,2-benzoquinone and benzene oxide each accounted for less than 3% of the total. In the bone marrow, benzoquinone adducts were more abundant than those of benzene oxide. 1,4-benzoquinone adducts were more abundant in the mouse with 21% of binding. Protein adduct levels in the bone marrow were about 60% of the levels in mouse Hgb. Comparisons of adduct production in the bone marrow indicated that 1,4-benzoquinone adducts were approximately 17-fold greater than those of 1,2-benzoquinone in the mouse.

The use of $^{13}C/^{14}C$-radiolabeled benzene enabled McDonald et al. (1994) to show that some phenol and hydroquinone adducts (those containing nonradiolabeled ^{12}C) were present prior to experimental exposure of the test animals to benzene, most likely from nonbenzene sources, i.e., background levels. Background levels of adducts (nonradiolabeled) of 1,2-benzoquinone and 1,4-benzoquinone with Hgb and bone marrow proteins of both rats and mice were much greater than those of the radiolabeled benzene-specific adducts. In rat Hgb, background levels of 1,2-benzoquinone and 1,4-benzoquinone were 68-fold and 473-fold higher, respectively, than the adduct formed from administered radiolabeled benzene of 50 mg/kg, and 11- and 25-fold higher at the 400 mg/kg dose. Similarly, mouse Hgb showed background levels of adducts that were 6- to 18-fold higher than the radiolabeled adducts at the low dose, and 2.7- to 5.2-fold higher at the high dose. When levels of benzoquinone adducts were expressed as ratios of the background levels, the levels of adducts from benzene exposure approached background levels at the two highest doses (200 and 400 mg/kg), but not at $\leq$100 mg/kg-day. The authors suggest that the background levels of quinone adducts accumulated over the life span of the protein, and that a chronic steady-state exposure to benzene itself would result in levels of quinone adducts that would equal or exceed background levels. The existence of background levels of quinone adducts is important in assessing risk of exposure to benzene, especially at low doses.

Hedli et al. (1996) investigated the covalent binding to DNA (adduct formation) of the benzene metabolites hydroquinone and 1,2,4-benzenetriol in combination with investigations of the effects of these two metabolites on cell differentiation in a hematopoiesis model system. Hydroquinone formed DNA adducts in human promyelocytic leukemia cells, but 1,2,4-benzenetriol did not. Both metabolites, however, inhibited retinoic acid-induced maturation of human promyelocytic leukemia cells to granulocytes. Thus, DNA adduct formation may be important in hydroquinone but not in 1,2,4-benzenetriol toxicity.

Creek et al. (1997) showed that DNA and protein adduct formation following intraperitoneal (i.p.) administration of ^{14}C-benzene was linear over a dose range spanning eight

orders of magnitude in B6C3F1 mice. At doses greater than 16 mg/kg body weight, however, adduct formation was nonlinear. This corresponds very closely to the metabolic saturation level of 15 mg/kg body weight observed by Sabourin et al. (1987). Benzene was administered to male B6C3F1 mice over a dose range of 700 pg/kg to 500 mg/kg body weight. Liver DNA adduct levels peaked at 0.5 hours after exposure, but bone marrow DNA adduct levels peaked between 12 and 24 hours. The dose-response assessment of protein adduct formation in the liver after 1 hr was linear up to 16 mg/kg body weight and leveled off at higher concentrations. These results indicate that adduct formation is linear in the range of benzene concentrations where benzene toxicity is first detected, and becomes saturated at higher doses where most toxicity experiments have been conducted.

The mechanisms involved in benzene toxicity and carcinogenicity may include synergism between metabolites (Eastmond et al., 1987; Snyder et al., 1989) or synergism between glutathione-depleting metabolites of benzene and oxygen radicals (generated by futile cycling of cytochrome P450 or cycling of quinone metabolites) (Parke, 1989). The benzene metabolites hydroquinone and muconic dialdehyde can produce hematotoxic effects (Eastmond et al., 1987; Gad-El Karim et al., 1985; Latriano et al., 1986). The co-administration of phenol (75 mg/kg-day) and hydroquinone (25-75 mg/kg-day) twice daily for 12 days to B6C3F1 mice produced myelotoxicity similar to that induced by benzene (Eastmond et al., 1987). The proposed mechanism suggested that selective accumulation of hydroquinone occurred in the bone marrow after the initial hepatic conversion of benzene to phenol and hydroquinone. Additionally, phenol is thought to stimulate the enzymatic activity of myeloperoxidase, which uses phenol as an electron donor, thus producing phenoxy radicals. These radicals further react with hydroquinone to form 1,4-benzoquinone, a toxic intermediate that inhibits critical cellular processes (Eastmond et al., 1987).

Legathe et al. (1994) investigated the pharmacokinetics of hydroquinone and phenol in blood and recovery in urine after i.p. administration of 75 mg/kg alone or in combination to $B6C3F_1$ mice. Combined administration resulted in a 2.6-fold increase in the area under the curve for blood concentration of hydroquinone and increased the half-life of hydroquinone from 9 to 15 min. The area under the curve of phenol was increased by a factor of 1.4, and clearance of phenol was decreased from 89 mL/min/kg when injected alone, to 62 mL/min/kg after co-administration. Recovery of conjugated metabolites in the urine indicated that both conjugation pathways were diminished. The authors suggest that enhanced myelotoxicity, observed after co-administration, is the result of reduced elimination of hydroquinone and phenol, leaving more phenol available for conversion to hydroquinone.

In studies on the erythroid cell line, Longacre et al. (1981b) demonstrated a 60%-80% decrease in the incorporation of iron (as ^{59}Fe) into bone marrow precursors of mice injected subcutaneously with 12-20 doses of 440 or 880 mg/kg of benzene. When given 48 hours before measuring ^{59}Fe uptake, benzene produced a dose-dependent decrease in erythroid cells (Snyder et al., 1989). Following i.p. administration of benzene, hydroquinone, *p*-benzoquinone, and *trans,trans*-muconaldehyde, ^{59}Fe uptake was reduced. The combination of metabolites most effective in reducing ^{59}Fe uptake was hydroquinone plus *trans,trans*-muconaldehyde (Snyder et al., 1989).

The stromal microenvironment of the bone marrow that normally modulates stem cell proliferation and differentiation is a potential target for the hematotoxicity of benzene (Cox, 1991; Snyder et al., 1989; Kalf, 1987). The interaction of the stroma with the stem cells is necessary for hematopoiesis. Furthermore, the stromal macrophage produces interleukin-1 (IL-1), a cytokine also essential for hematopoiesis. Patients with aplastic anemia usually exhibit monocyte dysfunction and decreased IL-1 production (Renz and Kalf, 1991).

Renz and Kalf (1991) demonstrated the disruption of IL-1 production by the stromal macrophages of mice exposed to benzene. The mice were injected i.p. with 600 or 800 mg/kg-day of benzene for 2 days. The stromal macrophages removed from these mice and cultured with lipopolysaccharide (LPS) produced the IL-1 precursor, 34-kilodalton (Kd) pre-IL-1α, but could not convert the precursor to the 17-Kd mature cytokine. Hydroquinone added in vitro also inhibited the conversion of the pre-IL-1α to the mature cytokine in mouse macrophages. However, administration of recombinant mouse IL-1α to mice before a bone-marrow-suppressing dose of benzene ameliorated the bone marrow depression, probably by circumventing the inability of the stromal macrophage in benzene-treated animals to process pre-ILα to the mature cytokine. Thus Renz and Kalf (1991) suggested that benzene-induced depression of bone marrow cellularity may result from the failure of the stromal macrophages to process pre-IL-1α to mature IL-1α, which activates the stromal fibroblast production of the colony-stimulating factor required for the differentiation of stem cells.

Niculescu et al. (1995, 1996) demonstrated that *p*-benzoquinone, the oxidation product of hydroquinone in the cell, causes a concentration-dependent inhibition of highly purified human platelet calpain with an IC_{50} of 3 μM. Calpain is a calcium-activated, cysteine-dependent protease that catalyzes the processing of pre-ILα to the mature cytokine in vivo. The investigators also demonstrated that *p*-benzoquinone inhibits the processing of interleukin-1β (IL-1β), the product of a distinct second gene, to IL-1 by the sulfhydryl-dependent protease referred to as IL-1β convertase.

3.4. ELIMINATION AND EXCRETION

3.4.1. Oral Exposure

No studies were located regarding excretion in humans after oral exposure to benzene. Data on excretion of benzene or its metabolites in human breast milk after oral exposure were not found.

Radiolabeled benzene (~340 mg/kg) was administered by oral intubation to rabbits; 43% of the label was recovered as exhaled unmetabolized benzene and 1.5% was recovered as carbon dioxide (Parke and Williams, 1953). Urinary excretion accounted for about 33% of the dose. The isolated urinary metabolites were mainly in the form of conjugated phenols. Phenol was the major metabolite, accounting for about 23% of the dose or about 70% of the benzene metabolized and excreted in the urine. The other phenols excreted (percentage of dose) were hydroquinone (4.8%), catechol (2.2%), and trihydroxybenzene (0.3%). L-phenyl-N-acetyl cysteine accounted

for 0.5% of the dose. Muconic acid accounted for 1.3%; the rest of the radioactivity (5%-10%) remained in the tissues or was excreted in the feces (Parke and Williams, 1953).

In a study using male C57BL/6N mice given single oral doses of ^{14}C-benzene (10 or 200 mg/kg), McMahon and Birnbaum (1991) reported the effects of age on benzene metabolism. Radioactivity was monitored in urine, feces, and breath. Consistent with previous reports, the following urinary metabolites were detected: hydroquinone glucuronide, muconic acid, phenyl glucuronide, phenyl sulfate, catechol glucuronide, hydroquinone sulfate, and prephenylmercapturic acid. At various time points up to 48 hours after dosing with 10 mg/kg, a significant decrease in the urinary excretion of benzene-derived ^{14}C was observed for 18- versus 3-month-old mice, while fecal elimination was significantly increased at 72 hours; this was not observed for the 200 mg/kg dose group. When expressed as the percent of total benzene administered, the relative amounts of some urinary metabolites varied between the 10 and 200 mg/kg dose groups, thereby indicating dose-related quantitative changes in the urinary excretion of benzene metabolites. At the low dose, urinary excretion was the major route of elimination. Hydroquinone glucuronide, phenyl sulfate, and muconic acid were the major metabolites at this dose, accounting for 40%, 28%, and 15% of the dose, respectively. At 200 mg/kg, urinary excretion decreased to account for 42%-47% of the administered dose, while respiratory excretion of volatile components increased to 46%-56% of the administered dose. Fecal elimination was minor and relatively constant over both doses, accounting for 0.5%-3% of the dose. Although age-related differences in benzene disposition were observed, they could be attributed to alterations in physiologic function occurring with age; the significance relative to toxicity versus aging could not be ascertained.

The effect of dose on the excretion of radioactivity, including benzene and metabolites, following oral administration of ^{14}C-benzene (0.5-300 mg/kg) has been studied in rats and mice (Sabourin et al., 1987). At doses of <15 mg/kg for 1 day, $\geq$90% of the administered dose was excreted in the urine of both species. There was a linear relationship for the excretion of urinary metabolites up to 15 mg/kg; above that level, there was an increased amount of ^{14}C eliminated in the expired air. Mice and rats excreted equal amounts up to 50 mg/kg; above this level, metabolism apparently became saturated in mice. In rats, 50% of the 150 mg/kg dose of ^{14}C was eliminated in the expired air; in mice, 69% of the 150 mg/kg dose of ^{14}C was eliminated in expired air (Sabourin et al., 1987). The label recovered during exhalation was largely in the form of unmetabolized benzene, suggesting that saturation of the metabolic pathways had occurred. Dose also affected the metabolite profile in the urine. At low doses, a greater fraction of the benzene was converted to putative toxic metabolites than at high doses, as reflected in urinary metabolites.

The bioavailability of pure as opposed to soil-adsorbed benzene was conducted in adult male rats (Turkall et al., 1988). Animals were gavaged with an aqueous suspension of benzene alone, or benzene adsorbed to clay or sandy soil. Plasma concentration, half-life, tissue distribution, respiratory excretion, and urinary excretion were monitored. Excretion patterns were altered by the presence of the soils. Expired air was the primary excretion route for benzene alone, with lesser amounts eliminated in the urine during the 48 hours after exposure. Urine and expired air were equal routes of excretion with sandy soil, while clay soil produced results opposite to those seen with pure benzene. Urine and expired air were equal routes of excretion with sandy soil, while clay soil produced results opposite to those seen with pure benzene. The

increase in urinary excretion in the presence of soil suggests that the soil allows greater absorption, and thus greater opportunity for metabolism of benzene, compared to benzene alone. For clay soil, particularly, the increased potential for metabolism is supported by the significantly lower amount of unmetabolized benzene in expired air. Less than 2% of the dose was eliminated in the feces for all treatments, although sandy soil treatment resulted in the highest amount of benzene associated with fecal excretion. These results further confirm that clay soil binds benzene.

3.4.2. Inhalation Exposure

Evidence indicates that in humans following inhalation exposure to benzene, the major route for elimination of unmetabolized benzene is via exhalation. Absorbed benzene is also excreted in humans via metabolism to phenol and muconic acid followed by urinary excretion of conjugated derivatives (sulfates and glucuronides). Respiratory uptake (the amount of benzene absorbed from the lungs following inhalation of the vapors) in six male and female volunteers exposed to 52-62 ppm (166-198 mg/m^3) benzene for 4 hours was determined to be approximately 47% (Nomiyama and Nomiyama, 1974a). Respiratory excretion (the amount of absorbed benzene excreted via the lungs) in a total of six male and female volunteers exposed to 52-62 ppm (166-198 mg/m^3) benzene for 4 hours was determined to be approximately 17%. No differences in respiratory excretion were observed between men and women (Nomiyama and Nomiyama, 1974a). Results from a study of 23 subjects that inhaled 47-110 ppm (150-351 mg/m^3) benzene for 2-3 hours showed that 16.4%-41.6% of the retained benzene was excreted by the lungs within 5-7 hours (Srbova et al., 1950). The rate of excretion of benzene was greatest during the first hour. The study also showed that only 0.07%-0.2% of the retained benzene was excreted in the urine.

Other studies suggest that benzene in the urine may be a useful biomarker of occupational exposure (Ghittori et al., 1993). Results of a study involving a single human experimental subject exposed to concentrations of benzene of 6.4 and 99 ppm (20 and 316 mg/m^3) for 8 hours and 1 hour, respectively, suggested that excretion of benzene in breath has three phases and could possibly have four phases. The initial phase is rapid and is followed by two (or three) slower phases (Sherwood, 1988). The initial phase, with a high-exposure concentration (99 ppm [198 mg/m^3]) and a short-term exposure duration (1 hr), has a more rapid excretion rate (half-life = 1.2 hr, percentage of total dose excreted = 9.3%). Subsequent phases showed an increase in the half-lives. These results also showed that urinary excretion of phenol conjugate was biphasic, with an initial rapid excretion phase, followed by a slower excretion phase. A greater proportion of the total dose was excreted in urine than in breath (Sherwood, 1988). The urinary excretion of phenol in workers was measured following a 7-hr work shift exposure to 1-200 ppm (3.2-640 mg/m^3) benzene. A correlation of 0.881 between exposure level and urinary phenol excretion was found (Inoue et al., 1986). Urine samples were collected from randomly chosen subjects not exposed to known sources of benzene, from subjects exposed to sidestream cigarette smoke, or from supermarket workers presumed exposed to benzene from polyvinyl chloride meat packing wrap (Bartczak et al., 1994). Samples were analyzed for identification of muconic acid. Muconic acid concentrations of 8-550 ng/mL were found in all urines. Kok and Ong (1994) report blood and urine levels of benzene in nonsmokers as 110.9 and 116.4 ng/L, respectively, and in smokers,

328.8 and 405.4 ng/L, respectively. A significant correlation was found between benzene levels in blood and benzene levels in urine. Similar results were found for filling station attendants in Italy (Lagorio et al., 1994b).

Popp et al. (1994) reported a mean blood benzene level in car mechanics of 3.3 µg/L. Urinary muconic acid and S-phenyl-N-acetyl cysteine levels increased during the work shift, and were well correlated with the blood levels and the benzene air levels, which reached a maximum of 13 mg/m^3.

As discussed in Section 3.3, the mean urinary levels of muconic acid in groups of male, female nonpregnant, and female pregnant smokers were 3.6-, 4.8-, and 4.5-fold higher, respectively, than the mean concentration of this acid in the nonsmoking groups (Melikian et al., 1994). The differences in the mean muconic acid concentrations between smoking and nonsmoking groups were significant in male (p<0.001), nonpregnant (p<0.001), and pregnant female smokers (p<0.002). Mean concentrations of muconic acid levels in nonpregnant female smokers were similar to that of male smokers. Mean concentrations of muconic acid in groups of 42 male smokers and 53 female smokers were 0.22 $\pm$ 0.03 and 0.24 $\pm$ 0.02 mg/g creatinine, or 0.24 $\pm$ 0.06 and 0.13 $\pm$ 0.07 mg/mg cotinine, respectively. Mean concentrations of urinary cotinine in pregnant smokers were significantly lower than in the group of nonpregnant female smokers (1.13 $\pm$ 0.12 mg/g creatinine compared to 1.82 $\pm$ 0.14 mg/g creatinine). Data on excretion of benzene or its metabolites in human breast milk after inhalation exposure were not found.

Test animal data show that exhalation is the main route for excretion of unmetabolized benzene and that metabolized benzene is excreted primarily in urine. Only a small amount of absorbed dose is eliminated in feces. A biphasic pattern of excretion of unmetabolized benzene in expired air was observed in rats exposed to 500 ppm (1597 mg/m^3) for 6 hours, with half-times for expiration of 0.7 hours for the rapid phase and 13.1 hours for the slow phase (Rickert et al., 1979). The half-life for the slow phase of benzene elimination suggests the accumulation of benzene. The major route of excretion following a 6-hr nose-only inhalation exposure of rats and mice to various concentrations of ^{14}C-benzene appeared to be dependent on the inhaled concentration (Sabourin et al., 1987). At similar exposures to vapor concentrations of 10-1000 ppm (32-3195 mg/m^3), the mice received 150%-200% of the equivalent dose in rats on a per kg body weight basis. At all concentrations, fecal excretion accounted for <3.5% of the radioactivity for rats and <9% for mice. At lower exposure concentrations (i.e., 13-130 ppm [42-415 mg/m^3] in rats and 11-130 ppm [35-415 mg/m^3] in mice), less than 6% of the radioactivity was excreted in expired air. At the highest exposure concentrations (rats, 870 ppm [2779 mg/m^3]; mice, 990 ppm [3162 mg/m^3]), both rats and mice exhaled a significant amount of unmetabolized benzene (48% and 14%, respectively) following termination of the exposure. The majority of the benzene-associated radioactivity that was not exhaled was found in the urine and in the carcass 56 hours after the end of exposure to these high concentrations. The radioactivity in the carcass was associated with the pelt of the animals. The authors assumed that this was due to contamination of the pelt with urine, since the inhalation exposure had been nose-only. Further investigation confirmed that the radioactivity was associated with the fur of the animals. Accordingly, the percentage of the total radioactivity excreted by these animals (urine and urine-contaminated pelt) that was not exhaled or associated with feces was 47%-92% for rats and 80%-94% for mice. At

exposures of ≤260 ppm (831 mg/m^3) in rats, 85%-92% of the radioactivity was excreted as urinary metabolites. The total urinary metabolite formation was 5%-37% higher in mice than in rats at all doses. This may be explained by the greater amount of benzene inhaled by mice per kg of body weight (Sabourin et al., 1987). Purebred Duroc-Jersey pigs were exposed to 0, 20, 100, and 500 ppm (0, 64, 319, and 1597 mg/m^3) benzene vapors 6 hours per day, 5 days per week for 3 weeks (Dow, 1992a). The average concentration of phenol in the urine increased linearly with dose.

3.4.3. Dermal Exposure

Limited data on excretion of benzene after dermal exposure in humans were found. Four human male subjects were given a dermal application of 0.0024 mg/cm^2 ^{14}C-benzene (Franz, 1984). A mean of 0.023% (range, 0.006%-0.054%) of the applied radiolabel was recovered in the urine over a 36-hr period. Urinary excretion of the radiolabel was greatest in the first 2 hours following skin application. More than 80% of the total excretion occurred in the first 8 hours. In another study, 35-43 cm^2 of the forearm was exposed to approximately 0.06 g/cm^2 of liquid benzene for 1.25-2 hours (Hanke et al., 1961). The absorption was estimated from the amount of phenol eliminated in the urine. The absorption rate of liquid benzene by the skin (under the conditions of complete saturation) was calculated to be low, approximately 0.4 mg/cm^2/hour. The absorption due to vapors in the same experiment was negligible. Although there was a large variability in the physiological values, the amount of excreted phenol was 8.0-14.7 mg during the 24-hr period after exposure. It is estimated that approximately 30% of dermally absorbed benzene is eliminated in the form of phenol in the urine.

Data on excretion of benzene or its metabolites in human breast milk after dermal exposure were not found.

Monkeys and minipigs were exposed dermally to 0.0026-0.0036 mg/cm^2 of ^{14}C-benzene (Franz, 1984). After application, the urine samples were collected over the next 2-4 days at 5-hr internals. The rate of excretion was highest in the first two collection periods. The total urinary excretion of radioactivity was found to be higher in monkeys than in minipigs with the same exposure. Mean excretion in monkeys was 0.065% (range 0.033%-0.135%) of the applied dose compared with 0.042% (range 0.030%-0.054%) in minipigs.

Results of a study in which male rats were dermally treated with 0.004 mg/cm^2 of ^{14}C-benzene, with or without 1 g of clay or sandy soil, showed that for all treatment groups the major routes of excretion were the urine and, to a lesser extent, the expired air (Skowronski et al., 1988). The highest amount of radioactivity in urine appeared in the first 12-24 hours after treatment (58.8%, 31.3%, and 25.1% of the absorbed dose, respectively, for pure benzene, sandy soil-adsorbed benzene, and clay soil-adsorbed benzene). In the group treated with pure benzene, 86.2% of the absorbed dose was excreted in the urine. Sandy soil and clay soil significantly decreased urinary excretion to 64.0% and 45.4%, respectively, of the absorbed dose during the same time period. Rats receiving pure benzene excreted 12.8% of the absorbed dose in expired air within 48 hours. Only 5.9% of the radioactivity was collected in expired air 48 hours after treatment with sandy soil-adsorbed benzene, while experiments with clay soil-adsorbed benzene

revealed that 10.1% of the radioactivity was located in expired air. Less than 1% of the absorbed dose was expired as $^{14}CO_2$ in all groups. The ^{14}C activity in the feces was small (<0.5% of the applied radioactivity) in all groups 48 hours after treatment. Phenol was the major urinary metabolite detected in the 0-12 hour urine samples of all treatment groups. The percentage of total urinary radioactivity associated with phenol was 27.7% for benzene alone, 44.2% for benzene adsorbed to sandy soil, and 45.5% for benzene adsorbed to clay soil. Smaller quantities of hydroquinone, catechol, and benzenetriol were also detected (Skowronski et al., 1988).

3.4.4. Other Routes of Exposure

The metabolic fate of benzene can be altered in fasted animals. In nonfasted rats that received an intraperitoneal injection of 88 mg of benzene, the major metabolites present in urine were total conjugated phenols (14%-19% of dose), glucuronides (3%-4% of dose), and free phenol (2%-3% of dose). However, in rats fasted for 24 hours preceding the same exposure, glucuronide conjugation increased markedly (18%-21% of dose) (Cornish and Ryan, 1965). Free phenol excretion (8%-10% of dose) was also increased in fasted, benzene-treated rats. There was no apparent increase in total conjugated phenol excretion in fasted rats given benzene.

When ^{14}C-benzene (0.5 and 150 mg/kg) was injected intraperitoneally into rats and mice, most of the ^{14}C-benzene and ^{14}C-metabolites were excreted in the urine and in the expired air. A smaller amount of ^{14}C-benzene was found in the feces due to biliary excretion (Sabourin et al., 1987). Monkeys were dosed intraperitoneally with 5-500 mg/kg radiolabeled benzene, and urinary metabolites were examined (Sabourin et al., 1992). The proportion of radioactivity excreted in the urine decreased with increasing dose, whereas as the dose increased, more benzene was exhaled unchanged. This indicated saturation of benzene metabolism at higher doses. Phenyl sulfate was the major urinary metabolite. Hydroquinone conjugates and muconic acid in the urine decreased as the dose increased. When C57BL/6 mice and DBA/2 mice were given benzene subcutaneously in single doses (440, 880, or 2200 mg/kg) for 1 day, or multiple doses (880 mg/kg) 2 times daily for 3 days, no strain differences were observed in the total amount of urinary ring-hydroxylated metabolites (Longacre et al., 1981a).

Although each strain excreted phenol, catechol, and hydroquinone, differences in the relative amounts of these metabolites were noted. The more sensitive DBA/2 mice excreted more phenol but less hydroquinone than the more resistant C57BL/6 mice, while both strains excreted similar amounts of catechol. DBA/2 mice excreted more phenyl glucuronide but less sulfate conjugate. Both strains excreted similar amounts of phenyl mercapturic acid (Longacre et al., 1981a).

3.5. PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELING

A PBPK model is a mathematical description of the uptake, distribution, metabolism, and excretion of a compound. There have been several attempts to develop PBPK models to refine the understanding of the interactions of benzene metabolism and toxicity. The first PBPK model for benzene was developed by Sato (Sato and Nakajima, 1979; Sato, 1988), who exposed three

men to either 25 or 100 ppm (80 or 319 mg/m^3) benzene vapor for 2 hours and then observed a triexponential decay of benzene from their blood. They constructed a three-compartment model consisting of richly perfused tissues, poorly perfused tissues, and fat, which acted as a major sink for benzene. Subsequently, more complex models have been developed, to take differences in benzene metabolism between species and individuals into account, using both experimental data and simulations, by Medinsky et al. (1989a), Travis et al. (1990a,b), Bois et al. (1991b), and Cox (1991, 1996).

The Medinsky model (Medinsky et al., 1989a,b,c) was one of the first PBPK models to be developed. The model was developed to describe and predict the fate of benzene and to determine if differences in the metabolic pathways between rats and mice could explain the differences in toxicity between these species. The model indicates that for inhalation concentrations up to 1000 ppm (3195 mg/m^3) mice metabolize at least 2-3 times as much benzene as rats. However, following oral exposure rats metabolized more benzene on a body weight basis than did mice at doses greater than 50 mg/kg. Patterns of metabolites also differed between rats and mice. Mice produced primarily hydroquinone glucuronide and muconic acid, metabolites linked to toxic effects, whereas rats produced primarily phenyl sulfate, a detoxification product. These simulated results agree with experimental data and provide a framework for understanding the greater sensitivity of the mouse to benzene toxicity.

The Medinsky model was based on an earlier PBPK model developed by Ramsey and Anderson (1984). The tissue compartments initially included in the model were: (a) the liver, presumed to be the only organ where benzene metabolism takes place; (b) a group of poorly perfused tissues including muscle and skin; (c) a group of richly perfused tissues including bone marrow, kidney, and intestines; and (d) a fat compartment. All metabolism of benzene, consisting of initial metabolism to benzene oxide which is then further metabolized by one of four pathways, was modeled by Michaelis-Menten kinetic parameters. Metabolic rate constants were determined by fitting the results of model simulations to experimental data obtained by exposing mice and rats to benzene orally and by inhalation (Medinsky et al., 1989b; Sabourin et al., 1987). Human metabolic rate constants were derived by using metabolic parameters obtained for mice, the more sensitive rodent species. Bois et al. (1991a) applied the data of Rickert et al. (1979) to the Medinsky model and found that the Medinsky model tended to overestimate data to which it was not specifically fitted. This indicated that the model needed to be further refined.

Medinsky and co-workers have used the results of their modeling efforts to focus their research on further defining values for parameters that generate the greatest uncertainties in explaining the toxicity of benzene. Since the original model was published, additional compartments have been added to reflect the advancing understanding of benzene metabolism, and specific biochemical and toxicokinetic parameters have been refined to reflect age, sex, and species specific differences (Schlosser et al., 1993; Seaton et al., 1994; McMahon et al., 1994; Kenyon et al., 1995). Seaton et al. (1994) measured 13-fold variability in CYP2E1 (cytochrome P-450 2E1) activity in human hepatic microsomes and compared this with the activity in mouse and rat liver microsomes. The model predicted the dependence of benzene metabolism on the measured CYP2E1 activity, and the proportion of hydroquinone (the suspected toxic metabolite) produced in vitro was correlated with the level of CYP2E1 activity. Seaton et al. (1995) measured the initial rates of the two major detoxification reactions, phenol sulfonation and

hydroquinone glucuronidation, in the same human hepatic microsome preparations, and in rats and mice. This information was used in a physiological compartment model to predict steady-state concentrations of phenol and hydroquinone in blood. Among humans, predicted steady-state concentration of phenol varied sixfold (0.38-2.17 nM) and predicted hydroquinone concentrations varied fivefold (6.66-31.44 nM). Predicted steady-state concentrations of phenol were higher in mice than rats (2.28 vs. 0.83 nM), and predicted hydroquinone concentrations were also higher (42.44 vs. 17.99 nM). The predicted concentrations for mice were higher than the range for humans, but the rat values were within the predicted concentrations for humans. On this basis the authors suggested that the rat may be a good model for humans with respect to tissue dosimetry for these benzene metabolites.

McMahon et al. (1994) investigated age-related changes in benzene disposition in male mice. They found that the rate of urinary benzene elimination was decreased in 18-month-old mice in comparison to 3-month old mice. Kenyon et al. (1995) investigated the metabolism of phenol. They sought to explain the reason that although phenol is thought to be a key intermediate in benzene metabolism leading to toxicity, orally administered phenol is neither carcinogenic or genotoxic (NCI, 1980). They found marked higher excretion of hydroquinone glucuronide after oral benzene exposure as compared to phenol. Also, phenol sulfate and phenol glucuronide excretion was much lower following benzene exposure than following phenol exposure. This could be explained by differences in the zonal distribution of CYP2E1 and detoxification enzymes in the liver. Phenol initially entering the liver had a relatively greater chance for conjugation (sulfonation or glucuronidation) in periportal hepatocytes of zone 1 than of oxidation by CYP2E1 located in pericentral hepatocytes in zone 3. Benzene, on the other hand, was more likely to pass through to zone 3 and be oxidized to phenol. The Medinsky PBPK model has served to organize the available information into a coherent model, that has helped to refine the specific experimental approaches used to fill the gaps in the understanding of the mechanism of benzene toxicity. The knowledge derived from this PBPK model has helped to reduce the scientific uncertainty in predicting the risk of adverse health effects at low exposure concentrations relevant for humans (Medinsky et al., 1995, 1996).

Travis et al. (1990a, 1990b) developed a model to describe the pharmacokinetics of benzene in rats, mice, and humans. The model contained five compartments, consisting of liver, fat, bone marrow, muscle, and organs such as brain, heart, kidney, and viscera. The different compartments were connected by the arterial and venous blood pathways. Metabolism of benzene was assumed to follow Michaelis-Menten kinetics in all species and was assumed to occur primarily in the liver, and to a lesser extent in the bone marrow. Model simulations were compared to experimental data from Sabourin et al. (1987, 1988), Andrews et al. (1977, 1979), Nomiyama and Nomiyama (1974a,b), Snyder et al. (1981), Sato et al. (1975), and Rickert et al. (1979). The Travis model successfully simulated uptake, metabolism, and excretion for mice, rats, and humans using experimental data from the studies that were used to develop the model. However, the model is limited by the fact that it does not predict other data very accurately (Bois et al., 1991a). For instance, the concentration of benzene in fat is poorly predicted.

The model developed by Bois and Paxman (1992) provided strong evidence that the exposure rate had a strong influence on the rate of formation of several important metabolites of benzene. Their model had three components, which described the pharmacokinetics of benzene

and the formation of metabolites in the rat. Distribution and elimination of benzene from a five-compartment system comprised of liver, bone marrow, fat, poorly perfused tissues, and well-perfused tissues made up the first component of the model. The bone marrow compartment was included for its relevance to human leukemia. Parameter values for this component were derived from the literature and from the previously published work of Rickert et al. (1979) and Sabourin et al. (1987). The second component described the metabolic transformations of benzene and its by-products in the liver and bone marrow. The reactions were assumed to follow Michaelis-Menten kinetics, with the exception of transformation of benzene oxide into phenol, which occurs spontaneously and may be described by a first-order reaction. The third component was the distribution of phenol. In addition to the compartments described for benzene, phenol was also assumed to distribute to the lung and gastrointestinal tract.

The Bois and Paxman (1992) model was validated against the data of Cassidy and Houston (1984), Sabourin et al. (1987, 1988, 1989a), and Sawahata and Neal (1983). The model was also used to predict metabolite production for male rats exposed to benzene by nose-only procedures to three different concentrations for three different lengths of exposure, and these results were compared to the experimental data of Sabourin et al. (1989a). The three exposure regimens were established to maintain a constant product of exposure length times concentration. Simulation results indicated that the model may over- or underestimate the level of urinary metabolites.

More recent efforts on development of the original PBPK model of Bois and Paxman (1992) have focused on defining the physiological pharmacokinetic parameter distributions needed to develop models useful in risk assessment (Spear and Bois et al., 1991, 1994; Watanabe and Bois, 1996; Bois et al., 1996). Spear and Bois (1994) described the outcome of their modeling efforts to explain the basis for the paradoxical observation that although phenol is a major initial metabolite of benzene, a known carcinogen, the NCI (1980) chronic study did not demonstrate carcinogenic activity for phenol. The approach selected was to apply Monte Carlo methods using parameter distributions coupled with a pass-fail fit criterion. The advantage of this approach is that it acknowledges that in most biological applications there is no clear way to select a “best” set of fixed parameters. Based on their modeling effort-hydroquinone was rejected as the ultimate toxic agent, and the pathway through benzene glycol to catechol and muconaldehyde appeared to provide a better fit to the data.

Watanabe and Bois (1996) examined three methods (multiplicative, additive, allometric) to extrapolate physiological parameter distributions across species, specifically from rats to humans. The results indicate that the multiplicative and allometric techniques were able to extrapolate the model parameter distributions. The results also indicated that rats do not provide a good kinetic model of benzene disposition in humans.

Bois et al. (1996) applied techniques from population pharmacokinetics, Bayesian statistical inference, and physiological modeling to model the distribution and metabolism in humans. Statistical distributions for the parameters of a physiological model of benzene were derived based on existing data. The relationship between the fraction of benzene metabolized in the bone marrow and benzene exposure is linear up to 10 ppm (32 mg/m^3). The median population estimate of the fraction metabolized in bone marrow was 52% (90% confidence

interval 47%-67%). At levels approaching occupational inhalation exposure (continuous 1 ppm [3.2 mg/m^3]) the estimated quantity metabolized in bone marrow ranges from 2 to 40 mg/day.

PBPK models continue to improve as additional data become available and are incorporated, and additional techniques from other scientific fields are applied to modeling benzene dosimetry. However, the models are not sufficiently refined to allow them to predict human metabolism accurately.compartment system comprised of liver, bone marrow, fat, poorly perfused tissues, and well-perfused tissues made up the first component of the model. The bone marrow compartment was included for its relevance to human leukemia. Parameter values for this component were derived from the literature and from the previously published work of Rickert et al. (1979) and Sabourin et al. (1987). The second component described the metabolic transformations of benzene and its by-products in the liver and bone marrow. The reactions were assumed to follow Michaelis-Menten kinetics, with the exception of transformation of benzene oxide into phenol, which occurs spontaneously and may be described by a first-order reaction. The third component was the distribution of phenol. In addition to the compartments described for benzene, phenol was also assumed to distribute to the lung and gastrointestinal tract.

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PBPK models continue to improve as additional data become available and are incorporated, and additional techniques from other scientific fields are applied to modeling benzene dosimetry. However, the models are not sufficiently refined to allow them to predict human metabolism accurately.

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS

4.1.1. Oral Exposure

Individual case reports of death from acute oral exposure to benzene have appeared in the literature since the early 1900's. The benzene concentrations ingested by the victims often were not known. However, lethal oral doses for humans have been estimated at approximately 125 mg/kg (Thienes and Haley, 1972). Lethality in humans has been attributed to respiratory arrest, central nervous system depression, or cardiac collapse (Greenburg, 1926). Accidental ingestion and attempted suicide with lethal oral doses of benzene have produced the following signs and symptoms: staggering gait, vomiting, shallow and rapid pulse, somnolence, and loss of consciousness, followed by delirium, pneumonitis, collapse, and then central nervous system depression, coma, and death (Thienes and Haley, 1972). Ingestion of lethal doses may also result in visual disturbances or feelings of excitement and euphoria, which may suddenly change to weariness, fatigue, sleepiness, convulsion, coma, and death (Von Oettingen, 1940).

4.1.2. Inhalation Exposure

4.1.2.1. *Hematotoxicity*

In the 19th century, benzene was first identified as the etiologic agent in the development of aplastic anemia, a potentially life-threatening suppression of bone marrow activities, and pancytopenia, a reduction of the cellular elements of the peripheral blood (Goldstein, 1988). Exposure to benzene has been associated with acute myelogenous leukemia and lymphoid malignancies in humans (Goldstein, 1988; Snyder, 1987). The relationship between benzene-induced malignancies and frank pancytopenia is unclear (Snyder, 1987). The hematopoietic abnormalities observed in humans exposed to benzene in industry have been reproduced in numerous test animal experiments.

Human exposure to benzene occurs primarily via inhalation in the workplace and from gasoline vapors and automotive emissions (ATSDR, 1997). Individuals exposed to benzene exhibit bone marrow depression, as evidenced by anemia (decreased red blood cell [RBC] count), leukopenia (decreased white blood cell [WBC] count), and/or thrombocytopenia (decreased platelet count) (U.S. EPA, 1985). A depression of all three elements is called pancytopenia, and the simultaneous depression of RBC, WBC, and platelets, accompanied by necrosis of the bone marrow, is diagnostic of aplastic anemia (U.S. EPA, 1985). Patients with aplastic anemia also have exhibited mild bilirubinemia, changes in osmotic fragility of erythrocytes, shortened erythrocyte survival time, increased fecal urobilinogen, and mild reticulocytosis (Aksoy, 1991). The bone marrow picture may vary from aplasia to hyperplasia, and these symptoms may vary in frequency and severity in different patients.

Other hematological changes observed in humans exposed chronically to benzene include decreased leukocyte osmotic resistance, decreased phagocytic function of neutrophils, reduced glycogen content and decreased activity of peroxidase of neutrophils, increased delta-aminolevulinic acid activity in erythrocytes, and increased coproporphyrins in the urine (Aksoy, 1991).

Epidemiologic studies on persons exposed to various levels of benzene in the workplace for intermediate and chronic periods of time have indicated potentially serious health effects (Table 4-1). These studies, however, often have limitations, such as concomitant exposure to other chemicals and lack of appropriate control groups. In addition, lack of adequate exposure data precludes quantitative determination of the relationship between severity of effects and exposure levels. The hematological effects in humans have been extensively reviewed (U.S. EPA, 1985; ATSDR, 1997). Summaries of the studies that provide the best evidence for deriving an RfC are briefly summarized below.

Aksoy et al. (1971) assessed hematological abnormalities in 217 apparently healthy men exposed in the shoe manufacturing industry. The test battery included RBC, WBC, platelet, and differential cell counts; packed cell volume (PCV); bone marrow examination; and Hgb analysis. The subjects, 12–58 years old, were compared with 100 unexposed, age-matched hospital workers. Benzene levels ranged from 15 ppm (48 mg/m^3) during nonworking hours to 210 ppm (95–671 mg/m^3) during the use of adhesives containing benzene; exposure (reported as

Table 4-1. Hematotoxicity of benzene—occupational exposure

Population/Industry	No.	Exposure	Effects	Reference
Rotogravure printers	6	77–3386 mg/m^3 of benzene	Pancytopenia	Erf and Rhoads, 1939[a]
Rotogravure printers, New York	332	35–3386 mg/m^3 of benzene	23 cases of significant cytopenias	Goldwater, 1941; Goldwater and Tewksbury, 1941; Greenburg et al., 1939
Shoe factory, Finland	147	≤1278 mg/m^3 of benzene for ~10 yrs	107 cases of hematological abnormalities	Savilahti, 1956[b]
Shoe factory, Finland	147	Followup of Savilahti (1956) study, 9 yr later	Persistent cytopenias; one death from acute leukemia	Hernberg et al., 1966
Rubber factory, Ohio	1104	≤1597 mg/m^3 (mean, ~319 mg/m^3) of benzene	83 cases of mild hematological abnormalities, 25 cases of more severe pancytopenia (9 required hospitalization, 3 of whom died)	Wilson, 1942
Watch industry	216	No exposure data; workers followed for 10 yr after cessation of exposure to benzene	4 cases of persistently decreased blood counts; one death from aplastic anemia 9 years after cessation of exposure	Guberan and Kocher, 1971
Rubber-coating industry		<80–399 mg/m^3 of benzene (prior to installation of control measures)	Followup suggested mild persistent anemia	Pagnotto et al., 1961
Shoe manufacturing, Turkey	217	96–671 mg/m^3 of benzene for 3 mo–17 yr	51 cases of hematological abnormalities, including 6 cases of pancytopenia	Aksoy et al., 1971
Controls (healthy hospital workers)	100			
Various industries using benzene-containing adhesives, Turkey	32 diagnosed with blood dyscrasias	479–1597 mg/m^3 of benzene for 4 mo–15 yr	32 cases of significant aplastic anemia; 8 deaths from thrombocytopenic hemorrhage and infection	Aksoy et al., 1972
Various industries using benzene-containing adhesives, Turkey	44 diagnosed with blood dyscrasias	Followup study over 2–17 yr after last exposure to 479–2077 mg/m^3 of benzene for 4 mo–15 yr	Complete remission in 23 (52%), death due to complications of pancytopenia in 14 (32%), leukemia in 6 (14%), myeloid metaplasia in 1 (2%)	Aksoy and Erdem, 1978

Table 4-1. Hematotoxicity of benzene—occupational exposure (continued)

Population/Industry	No.	Exposure	Effects	Reference
Turkish, various	231	Exposure to solvents, thinners, and similar materials in 40 small industries; in one facility, benzene concentrations averaged 351 mg/m^3, in the remainder, benzene levels <1 ppm	351 mg/m^3: 2 cases of acute myeloblastic leukemia in facility where levels measured 351 mg/m^3 <1 ppm: mild abnormalities in 14 workers (6.1%); differential diagnosis of 186 workers, monocytosis in 8, eosinophilia in 5, and basophilia in 3; 1 case each of acute myeloblastic leukemia, Hodgkin's disease, and lymphoma	Aksoy et al., 1987
Chemical production facility	282 men	Jobs assigned to exposure categories: (1) <6 mg/m^3 TWA (2) 6–29 mg/m^3 TWA (3) 32–77 mg/m^3 TWA (4) 80– ~96 ppm TWA exposures ranged from <1 year to >20 years	Slight statistically significant (p<0.05) (but not biologically significant) decreases in bilirubin and RBC counts; no correlations between peripheral blood counts and latency, duration, or intensity of benzene exposure	Townsend et al., 1978
Refinery	303 men	Exposure to benzene levels of 1.70 mg/m^3 (mean) for an average of 8 years for white employees and 4.5 years for nonwhite employees); average length of followup, 13 years	No significant changes in hematology of workers as a group; one case of multiple myeloma; one death from multiple myeloma and one from malignant melanoma	Tsai et al., 1983
Chinese shoemaking and printing	300	Mean time-weighted average exposures: Benzene—105 and 188 mg/m^3 (55 and 65 mo) for men and women, respectively; Toluene—173 and 154 mg/m^3 of toluene (95 and 73 mo); Mixture—45 mg/m^3 benzene + 68 mg/m^3 toluene and 45 mg/m^3 benzene + 79 mg/m^3 toluene (159 and 120 mo)	Changes in absolute and/or relative peripheral cell counts (statistically significant at p<0.01 or p<0.05): Benzene—decreased lymphocyte counts (women; men and women, combined); eosinophilia (women; men and women combined); decreased monocytes (women) Toluene—decreased lymphocyte counts (women; men and women, combined); eosinophilia (all groups) Mixture—decreased lymphocyte counts (men; women; men and women combined); significant eosinophilia (men and women combined)	Yin et al., 1987

Table 4-1. Hematotoxicity of benzene—occupational exposure (continued)

Population/Industry	No.	Exposure	Effects	Reference
Rubber industry	459	Mean estimated exposure from 1948 to 1975, 48–64 mg/m^3	Significant increases in WBC, RBC, and Hgb during the 1940s (Pearson correlations: r=0.50 for WBC, r=0.44 for RBC, and r=0.71 for Hgb), but not during the ensuing 25 years; rapid decline in exposure for the workers consistent with increases in blood count values	Kipen et al., 1989a,b
Workers Unexposed controls	200 268	0.03–4.5 mg/m^3 8-hr TWA over a 10-yr period	No abnormal hematology	Collins et al., 1991
Workers in various Chinese industries Low-exposure group Below median Above median Unexposed controls	 11 22 22 44	Workers exposed to benzene, but not to other solvents, at TWA of 24 (low-exposure), 44 (below-median), or 294 (above-median) mg/m^3 for an average of 6.3 yr, doses were measured by personal dosimetry for 2 wk	Significant increase in MCV and significant decreases in absolute lymphocyte count (ALC), WBC, RBC, hematocrit, and platelets in above-median group; significant reductions in ALC, RBC, and platelet count in below-median group; but only reduction in ALC was significant in low-exposure group	Rothman et al., 1996
Rubber industry	657	Estimates of benzene exposure ($\leq$109 mg/m^3) for last 30, 90, or 180 days before blood tests were correlated with hematological data	Weak correlation between RBC and benzene exposure, but strong correlation between low WBC counts and benzene exposure with no evidence of a threshold	Ward et al., 1996

[a]Reviewed in ATSDR (1989).
[b]Reviewed in U.S. EPA (1985).

30–210 ppm [95–671 mg/m^3]) duration ranged from 3 months to 17 years. Of the 217 exposed workers involved in the study, 51 (23.5%) had hematological abnormalities. Leukopenia was present in 9.7%, thrombocytopenia in 1.8%, leukopenia associated with thrombocytopenia in 4.6%, pancytopenia in 2.8%, acquired pseudo-Pelger Huet anomaly in 0.5%, eosinophilia in 2.4%, and basophilia in 0.5%.

Bone marrow examination of 11 of the 51 workers with abnormal peripheral blood cell counts revealed (a) normocellularity with no abnormal histology (2 patients), (b) slight hypocellularity (3 patients), (c) hypercellularity (1 patient), or (d) normocellularity with abnormal histology. Abnormal findings present in various combinations included maturation arrest in the erythroid and myeloid series, maturation arrest in the granulocytic series, and marked vacuolization in the myeloid series. One worker with normal hematology developed acute erythroleukemia 4 years later. The investigators concluded, based on the incidences of the various blood abnormalities, that benzene had a greater effect on leukocytes (with basophilia and eosinophilia as inconsistent findings) than on platelets. In a followup study, Aksoy et al. (1972) reported that 8 of 32 workers diagnosed with pancytopenia died, mainly from infection and bleeding.

About 18,000 peripheral blood counts from hematological surveillance records on 459 workers employed in the rubber industry between 1940 and 1975 were examined (Kipen et al., 1989a,b). Mean concentrations of benzene decreased from 137 to 66 ppm (437–210 mg/m^3) between 1940 and 1948, with a mean 8-hr time-weighted average (TWA) of 75 ppm (239.6 mg/m^3). During the period 1940–1948, significant depressions in erythrocyte, Hgb, and leukocyte levels were observed in the workers. As the benzene levels steadily decreased, the erythrocytes and leukocyte levels steadily increased. Between 1948 and 1975, when the workers were exposed to mean 8-hr TWA concentrations of 15–20 ppm (48–64 mg/m^3), no significant relationships between benzene levels and erythrocyte, Hgb, or leukocyte levels were established.

Hematological parameters (peripheral blood RBC, WBC, Hgb, platelets, and mean corpuscular volume [MCV]) were examined in workers (n<200) exposed to benzene over a 10-year period (Collins et al., 1991). Within this 10-year period, the mean length of exposure was 7.3 years. The workers were exposed to an 8-hr TWA of 0.01–1.4 ppm (0.03–4.5 mg/m^3) benzene. The mean TWA exposure was 0.045 ppm (0.14 mg/m^3). A group (n=268) of workers in the same plant not exposed to benzene were used as controls. There were statistically significant differences on demographic (age, race, sex) and personal habit (current smokers, regular exercise) variables between the benzene-exposed workers and the control group. Multiple regression analyses were applied using the confounding factors and current exposure as independent variables. No significant correlations between cumulative exposure and hematological parameters were identified. Thus, this study identifies a free-standing no-observed-adverse-effect level (NOAEL) of 0.045 ppm (0.14 mg/m^3) for hematological effects in humans.

Hematological parameters (RBC, WBC, hematocrit, Hgb, MCV, platelets, differential blood counts, clot retention determinations, sedimentation rate, and blood indices) were examined in 10 employees exposed to high benzene concentrations (8-hr TWA of >25 ppm [>80 mg/m^3]) for 2.5–22.9 years, with an average of 9.6 years of exposure (Fishbeck et al., 1978). Concentrations of benzene in the work area were especially high in 1963, with the 8-hr TWA

ranging from 37 to 132 ppm (1118–422 mg/m^3). After 1963, conditions were altered to ensure that concentrations of benzene remained below 25 ppm (80 mg/m^3, the acceptable limit at that time). Examination of the 10 employees in 1963 revealed enlarged RBC, high MCV (10/10), slightly low Hgb levels (9/10), and transient anemia; bone marrow was examined at this time and no abnormalities were found. After 1963, hematological values for these employees improved (in 1977, 5/10 workers had increased MCV values), and by 1978 none of the employees had developed serious health problems. The authors concluded that the workers' exposure to high benzene produced transient hematological effects that did not influence their long-term overall health.

Rothman et al. (1996) provided evidence that exposure to a median 8-hr TWA concentration of 7.6 ppm (24 mg/m^3) causes a significant reduction in absolute lymphocyte count in humans. They also provided evidence of a dose-response relationship between various measures of current benzene exposure (i.e., personal air monitoring, benzene metabolites in urine) and absolute lymphocyte count, total WBC count, and the MCV.

Rothman et al. (1996) conducted a cross-sectional study of 44 workers exposed to a wide range of benzene concentrations and 44 age- and gender-matched unexposed controls from Shanghai, China. From a large cohort study of 75,000 workers exposed to benzene, workers from three workplaces where benzene was used as a solvent, and unexposed workers from two workplaces in the same geographic area that did not use benzene, were selected as controls. The three workplaces using benzene included a factory manufacturing rubber padding for printing presses, a factory manufacturing adhesive tape, and a factory using benzene-based paint. The control workplaces included a factory manufacturing sewing machines and an administrative facility. Subjects with a prior history of cancer, therapeutic radiation, chemotherapy, or current pregnancy were excluded. Requirements for inclusion in the study were current employment for at least 6 months in a factory that used benzene, minimal exposure to other aromatic solvents, and no exposure to other known marrow-toxic chemicals or ionizing radiation. Controls with no history of occupational exposure to benzene or other marrow-toxic agents were frequency-matched to the exposed subjects on age (5-year intervals) and gender.

Benzene exposure was monitored by organic vapor passive dosimetry badges worn by each worker for a full workshift on 5 days within a 1–2 week period prior to collection of blood samples. Benzene exposure of controls in the sewing machine factory were monitored for 1 day, but no exposure monitoring was performed in the administrative facility. Benzene exposure was also evaluated by analyzing for benzene metabolites in urine samples collected at the end of the benzene-exposure period for the exposed subjects. Historical benzene exposure of the subjects was evaluated by examining employment history. Data on age, gender, current and lifelong tobacco use, alcohol consumption, medical history, and occupational history were collected by interview.

Five hematological measurements were evaluated: total WBC count, absolute lymphocyte count, hematocrit, RBC count, platelet count, and MCV. Total WBC counts and absolute lymphocyte counts were performed using a Coulter T540 blood counter. Abnormal counts were confirmed by hand. Benzene metabolites in urine were measured by an isotope dilution GC/MS assay. Correlation analyses were performed with Spearman rank order correlation. The

Wilcoxon rank sum test was used to test for hematological differences. Twenty-one of 44 subjects in the exposed and control groups were female.

Mean (sd) years of occupational exposure to benzene were 6.3 (4.4) with a range of 0.7–16 years. The median 8-hr TWA benzene exposure concentration for all exposed workers was 31 ppm (99 mg/m^3). Exposure to toluene and xylene was ≤0.2 ppm (0.6 mg/m^3) in all groups. The exposed group was subdivided into 2 equal groups of 22 of those exposed to greater than the median concentration and those exposed to less than the median concentration. The median (range) 8-hr TWA exposure concentration was 13.6 (1.6–30.6) ppm (43.4 [5.1–97.8] mg/m^3) for the low-exposure group and 91.9 (31.5–328.5) ppm (294 [101–1049] mg/m^3) for the high-exposure group. A subgroup of the low-exposure group composed of 11 individuals who were not exposed to >31 ppm (100 mg/m^3) at any time during the monitoring period was also examined in some comparisons. The median (range) 8-hr TWA exposure of these individuals was 7.6 (1–20) ppm (24 [3.2–64] mg/m^3). The urinary concentrations of phenol, muconic acid, hydroquinone, and catechol were all significantly correlated with measured benzene exposure.

All five blood elements measured were significantly different in the high benzene-exposure group compared with controls. Absolute lymphocyte count (ALC), WBC, RBC, hematocrit, and platelets were all significantly decreased, and MCV was significantly increased. These effects are consistent with the hematotoxic effects of benzene (Aksoy, 1989; Goldstein, 1988). The ALC was reduced from 1.9×10^3/μL blood in controls to 1.6×10^3/μL (p<0.001) in the <31 ppm (99 mg/m^3) group and to 1.3×10^3/μL (p<0.01) in the group exposed to >31 ppm (99 mg/m^3) benzene. In the subgroup exposed to a median 8-hr TWA of 7.6 ppm (24 mg/m^3) benzene, the ALC (1.6×10^3/μL) was also significantly reduced (p<0.03). The RBC and platelet counts were also significantly reduced in the <31 ppm (99 mg/m^3) exposure group, but only ALC was significantly different in the low-exposure subgroup. Thus, ALC was the most sensitive measure of benzene hematotoxicity.

The study of Rothman et al. (1996) is notable among occupational studies because benzene exposure concentrations were monitored, exposure to toluene was minimal, and subjects were compared with matched controls. A dose-response relationship was established between the ALC and benzene air level and benzene urine metabolites. The median 8-hr TWA of 7.6 ppm (24 mg/m^3) is a lowest-observed-adverse-effect level (LOAEL) for benzene hematotoxicity in humans.

Hematologic screening data collected over a 35-year period at a rubber hydrochloride manufacturing plant were analyzed: an increased risk of leukemia had been demonstrated previously among workers at the plant (Ward et al., 1996). Hematologic screening data were available for 657 of 1037 individuals employed at the plant from 1939 through 1976. There was a total of 21,710 blood test records. The study utilized a case-control design and estimated benzene exposures using the job exposure matrix developed by Rinsky et al. (1987). The effects of benzene exposure in the 30, 90, and 180 days before the blood test date, as well as cumulative exposure up until the blood test date, were examined using conditional logistic regression. For WBC there was a strong exposure response, and all of the exposure metrics selected showed a significant relationship with low blood count. For RBC there was a weak positive exposure response, which was significant (p<0.03) for one of the dose metrics. The maximum daily

benzene exposure estimate in this study was 34 ppm (109 mg/m^3). There was no evidence for a threshold for the hematological effects of benzene exposure, suggesting that even exposure to relatively low levels of benzene (e.g., <5 ppm [16 mg/m^3]) could result in hematological suppression. The results of this study are consistent with test animal studies that have demonstrated a decrease in peripheral lymphocyte counts at benzene exposures as low as 10 ppm (32 mg/m^3), and a stronger effect of benzene exposure on lymphocytes than on red cells.

The analysis of Ward et al. (1996) differs from the earlier investigations of the same rubber worker cohort by Kipen et al. (1989 a,b) and Cody et al. (1993), in that a nested case-control design was used. Incident cases were defined as the first occurrence of a low WBC or RBC count, and matched controls were chosen from those tested within 6 months of the case's blood test date. In contrast to the earlier analyses of hematologic screening data from the same plant, the Ward et al. (1996) study used the entire dataset, evaluated the exposure-response relationship based on individual dose metrics, and controlled for the temporal trends in preemployment blood count screening.

4.1.2.2. *Neurotoxicity*

Humans have displayed symptoms of neurotoxicity following acute inhalation of relatively high concentrations of benzene (Snyder, 1987; ATSDR, 1997). Benzene produces generalized symptoms such as dizziness, headache, and vertigo at levels of 250–3000 ppm (799–9584 mg/m^3) (Brief et al., 1980; ATSDR, 1997), leading to drowsiness, tremor, delirium, and loss of consciousness at 700–3000 ppm (2236–9584 mg/m^3) (ATSDR, 1997). Death may result from exposure to 20,000 ppm (63,894 mg/m^3) of benzene for 5–10 min (Sandmeyer, 1981). Neurological signs and symptoms are similar for both lethal and nonlethal exposures to benzene and for exposure to multiple solvents including benzene. These neurological symptoms are reversible upon removal of the subject from exposure (Kraut et al., 1988).

Autopsies of two victims of acute benzene poisoning revealed elevated levels of benzene in the brain and other tissues (Tauber, 1970; Winek and Collom, 1971). One victim died immediately after inhaling high atmospheric levels of benzene. The other victim was anesthetized while sniffing benzene in a plastic bag and accidentally suffocated (Winek and Collom, 1971).

Chronic exposure to benzene and toluene was studied in 121 workers exposed to benzene for 2–9 years (Kahn and Muzyka, 1973). The air concentration of benzene between 1962 and 1965 was 6–15.6 ppm (20–50 mg/m^3), whereas the toluene vapors did not exceed the 5 mg/m^3 level. Subsequently, the air levels of both benzene and toluene did not exceed the 5 mg/m^3 level. Seventy-four of the examined workers complained of frequent headaches (usually at the end of the workday), became tired easily, had difficulty sleeping, and complained of memory loss. The limitations of this study are that workers were exposed to both benzene and toluene and that the dose and duration of exposure were unknown.

Few studies have examined the neurological effects of chronic exposure to benzene. Herregods et al. (1984) diagnosed transverse myelitis in a young man exposed daily to benzene while working in the warehouse of a wholesale supplier of drugs and chemicals. The duration of

exposure was not given by the authors. The diagnosis of benzene poisoning was based on differential diagnosis, ruling out other possible causes of poisoning. The clinical diagnosis was based on the patient's occupation, high urinary phenol levels (28 mg/L in contrast to the normal level, <10 mg/L), and the coincidental decrease in urinary phenol and improvement of clinical symptoms over about 6 months after cessation of exposure. The diagnosis of "transverse myelitis" is consistent with an acute transection of the spinal cord affecting both the gray and white matter.

Baslo and Aksoy (1982) performed neurological examinations on eight patients with history of chronic exposure to benzene, who were diagnosed with aplastic anemia (cases 1–6) or preleukemia (cases 7 and 8). The seven males and one female ranged in age from 19 to 51 years (average age, 35.3 years). Cases 1, 2, 4, 5, 7, and 8 worked in the shoemaking industry, case 3 was a leather worker, and case 6 was a whistle maker who dipped plastic material into an open vessel of solution known to contain 88.42% benzene and 9.25% toluene. At times, the concentrations of benzene in the working environments reached 210 ppm (671 mg/m^3) (ATSDR, 1997). Exposure duration for the six patients with aplastic anemia ranged from 6 months to 8 years (mean, 6 years), and the period between the cessation of exposure and the neurological examinations ranged from 1 to 96 months. The two patients with preleukemia (7 and 8) were exposed for approximately 15 and 10 years, respectively, and were not exposed during the 8 and 6 months preceding neurological examination.

Neurological, electromyographical (EMG), and motor conduction velocity examinations were performed on all patients (with the exception of case 8, who did not have the EMG test). At least three different muscles were tested in the EMG and motor conduction velocity examinations. Sensory conduction velocities were measured in the upper and lower extremities of patients 1, 5, 6, 7, and 8.

Four patients (1, 2, 3, and 8) had other neurological symptoms that included decreased sensory vibration, atrophy of the leg, and exaggerated deep tendon reflexes; the other four (patients 4, 5, 6, and 7) had normal neurological test results. EMG tests revealed neurogenic involvement in patients 1 and 2 but were normal in patients 3–7. Five of the patients (1, 2, 3, 7, and 8) had abnormal motor conduction velocity tests, involving at least one of the nerves tested. These effects were characterized by decreased motor conduction velocity and lengthening of latency in distal nerves. Conduction velocity was decreased in the sensory nerves of patients 1, 6, and 7. In case 6, the amplitudes of nerve action potentials were low. These effects reflected benzene-induced effects on the axons of the peripheral nerves.

Baslo and Aksoy (1982) concluded that the neurological abnormalities in the four pancytopenic individuals resulted from a direct effect of benzene or toluene (case 6) on peripheral nerves and/or the spinal cord. They also concluded that the effects were related to the period of nonexposure. For example, patients 3 and 4 had moderate to severe hematological findings and aplastic anemia, but no significant neurological findings. For these two patients, long periods of nonexposure (53 and 96 months, respectively) preceded neurological testing.

The study is based on a small number of patients, lacks exposure and control data, and does not rule out the possibility that the workers were exposed to chemicals other than benzene

and toluene (case 6); therefore, a reliable LOAEL or NOAEL could not be determined. The clinical impression indicated a predominant involvement of the white matter in the peripheral nervous system.

The reports summarized in this section have obvious deficiencies, such as small numbers of patients under study and a lack of information regarding the intensity or duration of benzene exposure. Thus, a reliable quantitative neurotoxic risk assessment is impossible at present. Nevertheless, the occurrence of symptoms following exposure and the amelioration of effects upon removal from exposure leave little doubt that benzene affects the central as well as the peripheral nervous system. It appears likely from the experience of benzene workers that neurotoxicity may be less of a public health concern than either hematotoxicity (leukopenia, anemia, or aplastic anemia) or carcinogenicity (preleukemia or acute nonlymphocytic leukemia).

4.1.2.3. *Reproductive Toxicity*

Vara and Kinnunen (1946) examined 12 female workers with gynecological disorders attributed to benzene exposure: three were 25–28 years old, seven were 37–40 years old, and two were 43–44 years old, and their exposure to benzene ranged from 1 to 10 years. The investigators identified the third group as "nearing menopause," but found no evidence of menopause in the women; however, the symptoms of toxicity, consisting of hypermenorrhea and hypomenorrhea, ovarian hypoplasia, sterility, degeneration of the ovary, and/or dysfunction of the ovary, were more severe in the two subjects nearing menopause. Peripheral blood counts revealed distinct leukopenia in four subjects and decreased neutrophils and platelets in most subjects. The investigators tentatively attributed the sparseness in menstruation to the ovarian hypoplasia, rather than to the benzene. The available studies on the reproductive toxicity of benzene in humans are summarized in Table 4-2.

One study did not specify if exposure was to benzene only. Pushkina et al. (1968) evaluated a group of workers with ovarian hypofunction related to exposure to benzene. Compared with clinically healthy female workers in the same factory, the levels of ascorbic acid (changes in ascorbic acid metabolism were used as an index of toxicity) were reduced in the blood of the workers with ovarian dysfunction (subjects, 0.36 ± 0.02 mg%; controls, 0.49 ± 0.03 mg%; $p<0.001$). The details of this study were sparse, but the results were supported by a study in rats that showed a similar effect (Pushkina et al., 1968).

A Russian study evaluated 360 women exposed to gasoline (a major source of benzene) and chlorinated hydrocarbons via inhalation and skin contact (Mukhametova and Vozovaya, 1972). Benzene levels were <1.6 ppm (5 mg/m^3); chlorinated hydrocarbon levels were 1.2–2.4 times higher than the permissible limits. Compared with workers with no exposure to benzene, the subjects had increased incidences of menstrual disturbances. As exposure duration increased, so did the number of premature interruptions of pregnancy, the percentage of cases where the placental membrane rupture during parturition was impeded, and the number of cases of intrauterine asphyxia of the fetus. This study, although demonstrating exposure-related

Table 4-2. Reproductive toxicity of inhaled benzene in humans

Population	No.	Exposure	Effects	Reference
Female workers with symptoms of benzene poisoning	30	Overt symptoms suggested exposure to levels greater than 3.2 mg/m^3	12 subjects had menstrual disorders (e.g., hypermenorrhea) and/or ovarian hypoplasia; women nearing menopause most severely affected; 4 had leukopenia and the majority had reduced neutrophil and platelet counts	Vara and Kinnunen, 1946
Female Polish factory workers	ND[a]	100 mg/m^3 benzene (no other details available)	Heavy menstrual bleeding	Michon, 1965
Female factory workers with benzene-induced ovarian hypofunction	ND	ND	Workers exhibiting ovarian hypofunction had decreased ascorbic acid concentrations in the blood, as compared with clinically healthy female workers ($p<0.001$)	Pushkina et al., 1968
Female gluing operators	360	Exposure to gasoline (major source of benzene) and chlorinated hydrocarbons (hc's) via skin and inhalation; benzene levels, <5 mg/m^3; 40% of chlorinated hc measurements exceeded permissible limits by 1.2- to 2.4-fold; controls had no chemical exposure	Functional disturbances of the menstrual cycle	Mukhametova and Vozovaya, 1972
Female solvent workers	174	(1) <131 mg/m^3 benzene (2) ≥131 mg/m^3 benzene (3) control	(1) hypermenorrhea in 4/40 (10%) ($p<0.05$) (2) hypermenorrhea in 4/47 (8.5%) ($p<0.05$) (3) hypermenorrhea in 1/87 (1.2%)	Yin et al., 1987
Female workers in shoemaking industry	223	Subjects exposed to benzene and toluene (compared with 327 controls)	Increased menstrual disorders (exposed, 48.9%; control, 16.2%); abortion (exposed, 5.7%; control, 2.4%); gestosis (exposed 22.6%; control, 10.5%); all differences were statistically significant	Huang et al., 1991

[a]ND = no data

reproductive effects and fetal toxicity, fails to distinguish between the effects of benzene and chlorinated hydrocarbons.

Yin et al. (1987) conducted a comprehensive study of the toxicity of benzene, in which 300 workers exposed to benzene, toluene, or a mixture of the two, were examined for subjective symptoms and hematological and biochemical effects. For 174 women, the mean TWA exposure to benzene was 59 ppm (188 mg/m^3) (exposure duration, 65 months). The women were divided into low-exposure (1–40 ppm [3.2–128 mg/m^3]) and high-exposure (41–210 ppm [131–671 mg/m^3]) groups and were examined separately for subjective and pancytopenia-related subjective symptoms. The benzene-exposed subjects reported an increase in the incidence of hypermenorrhea, but the effect was not concentration-related (prevalence was 10% for lower exposures, 8.5% for higher exposures; $p<0.05$ for both doses). Although the only hematologic abnormality noted was a mild, but statistically significant, decrease in lymphocyte count ($p<0.05$), this hypermenorrhea was tentatively considered to be related to pancytopenia. These findings are strengthened by the large number of subjects, the measured exposure and statistical evaluation, and by similar observations in other studies; however, the significance of subjective symptoms is debatable.

A Chinese report described reproductive dysfunction in female workers exposed to benzene and toluene in the leather shoe-making industry (Huang et al., 1991). The exposed group (223 women) exhibited increased incidence in the rate of menstrual disorders compared with the data for 327 controls. The incidence rates of "mense-blood anomaly" (author's words) and dysmenorrhea had a tendency to increase with duration of employment. The incidences of spontaneous abortion and gestosis (toxemia) were also increased. All increases were statistically significant. The investigators concluded that both benzene and toluene had a deleterious effect on the reproductive function of female workers.

4.1.2.4. *Developmental Toxicity*

There is no convincing evidence that benzene produces malformations in humans or test animals; however, a few studies suggest that the chemical induces adverse reproductive effects in humans (ATSDR, 1997) and causes retarded fetal growth in test animals, manifested mainly as decreased fetal weight and delayed ossification in the presence or absence of maternal toxicity (Chatburn et al., 1981; ATSDR, 1997). The following discussion reviews the developmental toxicity of benzene in both humans and test animals, focusing on more recent reports. Available studies on the developmental toxicity of benzene in humans is summarized in Table 4-3.

Benzene crosses the human placenta and is present in cord blood in quantities equal to or greater than levels in the maternal blood (Dowty et al., 1976). Summaries of developmental toxicity of benzene in humans have been described in individual case reports (Forni et al., 1971; Holmberg, 1979; Bordarier et al., 1991), and occupational studies (Vara and Kinnunen, 1946; Michon, 1965; Pushkina et al., 1968; Mukhametova and Vozovaya, 1972; Funs-Cravat et al., 1977; Axelsson et al., 1984; Yin et al., 1987; Selves et al., 1989; Huang et al., 1991). Exposures were either to benzene alone or, as is characteristic of occupational exposure, to multiple

Table 4-3. Developmental toxicity of benzene—humans

Population	No.	Exposure	Effects	Reference
Pregnant worker	1	Exposure to benzene during entire pregnancy	Maternal effects included severe pancytopenia and increased chromosomal aberrations; no fetal effects	Forni et al., 1971
Employees in university laboratory (~745 subjects, 1160 pregnancies), divided into those with and without exposure to organic solvents	745	Responders to questionnaire reported exposure to at least 14 solvents; 41 workers remembered using benzene during the first trimester of pregnancy and 5 used phenol	All women exposed to solvents had slight, but not statistically significant difference in miscarriage rate over those not exposed (RR^a=1.31, 95% CI^b=0.89–1.91); 35 of 41 workers exposed to benzene delivered, 1 had induced abortion, and 5 miscarried (miscarriage rate for benzene-exposed subjects, 12.2%; miscarriage rate for all responders to questionnaire, 11.1%; miscarriage rate for unexposed responders, 10.1%); all 5 workers exposed to phenol delivered; exposure to solvents did not affect perinatal death rates or the incidence of malformations	Axelsson et al., 1984
Female gluing operators	360	Exposure to gasoline (major source of benzene) and chlorinated hydrocarbons (hc's) via skin and inhalation; benzene levels, <5 mg/m^3; 40% of chlorinated hc measurements exceeded permissible limits by 1.2- to 2.4-fold; controls had no chemical exposure	Spontaneous abortions and premature births (17.2% vs. 4.9% in controls), incidence of late membrane rupture, and intrauterine asphyxia of the fetus increased with exposure duration	Mukhametova and Vozovaya, 1972
Adult female workers and 14 of their children	29	Adults exposed to benzene and other organic solvents during pregnancy (compared with 42 control adults and 7 of their children)	Lymphocytes from adults exhibited approximately twofold increase over controls in incidence of chromosomal aberrations and breaks; their children exhibited increased frequency of chromatid breaks, isochromatid breaks (p<0.01, 14 children), and sister chromatid exchanges (p<0.001, 4 children) in lymphocytes	Funes-Cravioto et al., 1977
32-year-old pregnant worker	1	Personal interview revealed exposure to benzene and other solvents (dichloromethane, methanol, and ether) in laboratory during first trimester of pregnancy; compared with matched control	Stillborn anencephalic fetus	Holmberg, 1979

Table 4-3. Developmental toxicity of benzene—humans (continued)

Population	No.	Exposure	Effects	Reference
23-year-old female	1	21 intramuscular injections of benzene to induce abortion during first trimester of pregnancy	Following normal delivery, infant exhibited slight dysmorphy (hypotelorism and deep nasal bridge), moderate axial hypotonia and abnormal ocular movements; at 1.5 months of age, child was microcephalic, had severe axial hypotonia, severe peripheral hypertonia, and bilateral optic atrophy, and CT[c] scanning revealed bilateral porencephalic cavities that created communication between lateral ventricles and subarachnoid space; interventricular septum lacking; child died from aspiration pneumonia at 2 months of age	Bordarier et al., 1991

[a]RR = relative risk
[b]CI = confidence interval
[c]CT = computerized transverse tomography

chemicals including benzene. With only two exceptions (Bordarier et al., 1991; Mukhametova and Vozovaya, 1972), all adult exposures in the studies summarized in these sections were via inhalation.

Only one study evaluated fetal effects following inhalation of benzene alone. A worker who was exposed to benzene during her entire pregnancy suffered from severe pancytopenia and exhibited increased chromosomal aberrations, but there were no effects on the fetus (Forni et al., 1971).

One study evaluated by questionnaire the outcome of pregnancy among personnel employed in laboratory work at the University of Gothenburg between 1968 and 1979 (Axelsson et al., 1984). Of 745 women who responded to the questionnaire, 556 had been pregnant (a total of 1160 pregnancies). The pregnant women were divided into two groups: those with exposure to organic solvents during laboratory work and those without exposure. Responders to the questionnaire reported exposure to at least 14 solvents; 41 workers remembered using benzene during the first trimester of pregnancy and 5 used phenol. A slightly increased, but not significant, difference in the miscarriage rate was found (relative risk=1.31, 95% confidence interval [CI]=0.89–1.91). There were no differences in perinatal death rates or prevalence of malformations between infants whose mothers were exposed to solvents and those who were not exposed. The investigators suggested that shift work, number of pregnancies, and age may have contributed to an increase in miscarriage rate in the group of pregnant women not exposed to solvents, resulting in an underestimation of the miscarriage rate of the exposed women. An additional concern was that in spite of the fact that confounders were reduced by using as controls laboratory workers not exposed to solvents, a mutagenic effect occurring during laboratory work (laboratory work implies exposure to a variety of agents) could theoretically cause a miscarriage even after the woman had stopped working at the laboratory, thereby altering the miscarriage rate in the control group. Another weakness in the study was that the subjects had worked in the laboratory during 1968–1979, and some had to remember several years back.

Four other studies evaluated the developmental toxicity of multiple solvents. These effects consisted of spontaneous abortions, premature births, and effects on "condition of the fetus" (Mukhametova and Vozovaya, 1972), chromosomal abnormalities (Funs-Cravat et al., 1977), and stillbirth (Holmberg, 1979).

The data presented provide inconclusive results regarding developmental toxicity of benzene in humans. Most studies consisted of small numbers of subjects, lacked important experimental details, involved (in almost all cases) concomitant exposure to other chemicals, and did not provide monitoring data or quantitative dose-response information.

In a recent epidemiology study, Selves et al. (1989) examined the effects of parental (paternal as well as maternal) occupational exposures on fetal development. The subjects, employed in various industries, were exposed to a number of chemicals. Summarized here are the data pertaining only to benzene exposures. The investigators accessed the National Natality and Fetal Mortality surveys to obtain data on probability samples of live births and fetal deaths that occurred in the United States in 1980 among married women. These data were merged with data provided by mothers from questionnaires and from information from medical care providers for

the public use data set. Omitted from the analysis were unmarried mothers, mothers who had not worked within 12 months of delivery, plural births, and births of unknown plurality. The analysis, based on three case control studies of pregnancy outcome, included case groups of stillbirths, preterm deliveries (birth before 37 weeks of gestation), and small-for-gestational-age infants. The investigators used an exposure linkage system to designate exposures. The exposure to a specific agent was assigned based on the occupation and industry of employment and on past studies and review of industrial processes. Linkages of none, low, medium, high, or unknown were assigned to each agent, representing probability (rather than intensity) of exposure. Unexposed working women served as controls. The odds ratios (OR) relating exposure to effect were adjusted for selected potential confounders. Elevated OR were found for maternal exposure to benzene and stillbirth (OR=1.3, CI=1.0–1.8]. Increased risk across low-, medium-, and high-linkage levels (crude odds ratios of 0.9, 1.2, and 1.4, respectively) were observed. Exposure to benzene was also associated with risk elevation for the fathers (OR=1.5, 95%, CI=1.1–2.3). The OR for low, medium, and high linkages were 1.2, 1.5, and 2.0, respectively, with reasonably good precision for the two highest levels (95% CI=1.0–2.2 and 1.1–3.7, respectively). Benzene exposure linkage level was unrelated to risk of preterm delivery for both parents. The investigators recognized that the study had limitations. These included small population sizes, poor quality of exposure information, and absence of statistical testing and the high rate of nonresponse to questionnaires among women who were under 20 years old, 40 or more years old, black, and having parity four or greater, little or no prenatal care, and low education. However, in spite of these limitations, they concluded that the results of the study encourage further evaluation of the developmental effects of paternal exposure to benzene.

4.2. ACUTE AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS

4.2.1. Hematotoxicity—Oral Exposure

4.2.1.1. *Subchronic Studies*

Subchronic oral studies have been conducted in F344 rats and B6C3F1 mice of both sexes (NTP, 1986; Huff et al., 1989) and female Wistar rats (Wolf et al., 1956). Other subchronic oral studies have been conducted to examine immunotoxicity, and these are described in Section 4.4.2.1.

NTP (1986) treated F344 rats and B6C3F1 mice (10/species/group/sex; 6–8 weeks of age) with 0, 25, 50, 100, 200, 400, or 600 mg/kg benzene, by gavage in corn oil, 5 days/week for 17 weeks. The adjusted doses were 0, 18, 36, 71, 143, 286, or 429 mg/kg-day. An additional five animals/species/group/sex were tested at the 0, 200, or 600 mg/kg dose levels and killed at 60 days of treatment. Hematological analyses were performed on all the animals killed at 60 days and on five animals/species/group/sex at the end of the study. In addition, necropsies were performed on all animals, and the spleens of all animals were examined histopathologically. Results of this study were also reported by Huff et al. (1989).

No compound-related deaths were observed for rats. Final body weight depression of ≥10% relative to controls was observed in male and female rats at dose levels of 200 mg/kg and greater. Significant (p<0.05) leukopenia and lymphocytopenia were observed in male and female rats after 60 days of treatment with 200 or 600 mg/kg (the only treatment groups tested on day 60). On day 120 of treatment, significant leukopenia and lymphocytopenia were observed in female rats at 25 mg/kg and higher, and significant lymphocytopenia was observed in male rats at 400 mg/kg. Lymphoid depletion of B-cells in the spleen was observed in 100% of male and female rats exposed to 600 mg/kg for 60 or 120 days. Increased extramedullary hematopoiesis in the spleen was observed in 4/5 male and 3/5 female rats treated with 600 mg/kg for 120 days. This study identified a LOAEL of 25 mg/kg (18 mg/kg-day) in female rats and a LOAEL of 200 mg/kg (143 mg/kg-day) in male rats for hematological effects following treatment by gavage for 17 weeks. The LOAEL for female rats was at the lowest dose tested; thus no NOAEL was established.

There were no compound-related deaths in the mice. A final body weight depression of 7% was seen in the 100 mg/kg dose group. Tremors were observed intermittently in male and female mice treated with 400 or 600 mg/kg. No leukopenia or lymphocytopenia was observed in male or female mice after 60 days of treatment with 200 or 600 mg/kg. At 120 days, significant (p<0.05) leukopenia and lymphocytopenia were observed in male mice at dose levels of 50 mg/kg and greater, and in female mice at 400 (only lymphocytopenia) and 600 mg/kg. A NOAEL of 25 mg/kg (18 mg/kg-day) and a LOAEL of 50 mg/kg (36 mg/kg-day) for hematological effects were identified in male mice treated by gavage for 17 weeks. A NOAEL of 200 mg/kg (143 mg/kg-day) and a LOAEL of 400 mg/kg (286 mg/kg-day) for hematological effects were identified in female mice treated by gavage for 17 weeks.

Female Wistar rats (10/group) were treated by gavage with benzene in olive oil, 5 days/week for 6 months (Wolf et al., 1956). The reported doses were 0, 1, 10, 50, or 100 mg/kg-day (0, 0.7, 7.1, 35.7, and 71.4 mg/kg-day). Parameters measured included mortality, clinical signs, body and organ weights, hematology, blood biochemistry, bone marrow counts, and gross and microscopic pathology of lungs, heart, liver, kidneys, spleen, testes, adrenals, and pancreas. Leucopenia was reported for 10 mg/kg; at higher dose levels erythrocytopenia and leucopenia were also observed. No quantitative data or statistical analysis was reported. The authors reported that rats fed 1 mg/kg had "no evidence of ill effects." This study identified a NOAEL of 0.7 mg/kg-day and a LOAEL of 7.1 mg/kg-day for hematological effects in female rats treated by gavage for 6 months.

4.2.1.2. *Chronic Studies*

In the NTP (1986) study, F344 rats and B6C3F1 mice of both sexes were treated by gavage with benzene, 5 days/week for 103 weeks. Results of this study have also been reported by Huff et al. (1989). For rats, males (60/group) were administered doses of 0, 50, 100, or 200 mg/kg (0, 36, 71, or 143 mg/kg-day), and females (60/group) were administered doses of 0, 25, 50, or 100 mg/kg (0, 18, 36, or 71 mg/kg-day). Survival decreased with increasing dose in rats of both sexes, and was significantly decreased (p<0.05) at 200 mg/kg in male and at 50 and 100 mg/kg in females. Body weight depression of ≥10% relative to controls was observed in male

rats treated with 100 mg/kg. Dose-related leucopenia was significant in female rats treated with ≥25 mg/kg for 3, 6, 9, and 12 months; leukocyte levels were comparable to controls after 15, 18, 21, and 24 months of treatment. In male rats, dose-related leucopenia was significant at ≥50 mg/kg. Lymphoid depletion was observed in the thymus of 0/44, 4/42, 8/41, and 10/34 male rats treated with 0, 50, 100, and 200 mg/kg benzene, respectively. In the spleen, lymphoid depletion was observed in 0/49, 19/58, 8/47, and 23/47 male rats treated with 0, 50, 100, and 200 mg/kg, respectively. This study identified a LOAEL of 25 mg/kg (18 mg/kg-day) for leukopenia and lymphocytopenia in female F344 rats and 50 mg/kg(36 mg/kg-day) in male F344 rats. These were the lowest doses tested, and thus no NOAEL was identified.

B6C3F1 mice (60/sex/group) were administered doses of 0, 25, 50, or 100 mg/kg (0, 18, 36, or 71 mg/kg-day). Survival decreased with increasing dose in mice of both sexes and was significantly decreased ($p<0.05$) at 100 mg/kg. Body weight depression of ≥10% relative to controls was observed in mice of both sexes treated with 100 mg/kg. Significantly decreased leukocyte counts were observed in males after 3, 6, 9, 12, 15, 18, and 21 months of treatment with 50 or 100 mg/kg, but males treated with 25 mg/kg had significantly decreased leukocyte counts only after 6 and 21 months. In female mice, leucopenia was observed only at 12 and 18 months, in both cases significant at all treatment levels. Significantly decreased lymphocyte counts were observed in males after 3, 6, 9, 12, 15, 18, and 21 months with 50 or 100 mg/kg, but males treated with 25 mg/kg had significantly decreased lymphocyte counts only after 12 months. In female mice, significant lymphocytopenia was observed at ≥25 mg/kg at 12 and 18 months and at 100 mg/kg at 3 months. Hematopoietic hyperplasia of the bone marrow was observed in 0/49, 11/48, 10/50, and 25/49 male mice, and in 3/49, 14/45, 8/50, and 13/49 female mice treated with 0, 25, 50, or 100 mg/kg, respectively. Increased splenic hematopoiesis was observed in 5/49, 9/48, 19/49, and 24/47 male mice and in 9/49, 10/45, 6/50, and 14/49 female mice treated with 0, 25, 50, or 100 mg/kg, respectively. In the female mice, increased incidences of epithelial hyperplasia of the ovary occurred at all three doses, and increased incidence of senile atrophy of the ovary occurred at the lower two doses compared with controls. This study identified a LOAEL of 25 mg/kg (18 mg/kg-day) for leukopenia and lymphocytopenia in male and female B6C3F1 mice. The observed LOAEL was at the lowest dose tested; thus a NOAEL was not identified.

Beginning in 1976, a series of carcinogenicity studies on oral treatment of rodents with benzene were performed at the Bologna Institute of Oncology, including 52- and 104-week studies on Sprague-Dawley and Wistar rats and Swiss and Rf/J mice (Maltoni et al., 1983, 1985). The results of the studies from this laboratory were reported in numerous publications. Limited information regarding noncarcinogenic effects were reported for Sprague-Dawley rats, but only carcinogenicity data were published for Wistar rats and mice. No statistical information was included, making interpretation of the data difficult.

Maltoni et al. (1985) treated Sprague-Dawley rats (13 weeks of age, 30–35/sex/group) by gavage with 0, 50, or 250 mg/kg benzene in oil, 4–5 days/week for 52 weeks, then observed until death. Expanded doses were 0, 32, and 161 mg/kg-day. In addition, Sprague-Dawley rats (7 weeks of age, 40–50/sex/group) were treated by gavage with 0 or 500 mg/kg benzene in oil, 4–5 days/week for 104 weeks, then observed until death. The expanded doses were 0 and 321 mg/kg-day. Mortality was higher in benzene-treated groups and appeared to be dose related; body

weights were not affected. Maltoni et al. (1983) stated that mortality in the first portion of the study was due to direct toxic effects of treatment, and in the later portion was partially due to tumors. Mortality was similar to that of controls during treatments with 500 mg/kg for 92 weeks; body weight appeared to be somewhat depressed relative to controls. In Sprague-Dawley rats exposed to 500 mg/kg for 84 or 92 weeks, decreased total RBC (only at 92 weeks), WBC, and lymphocytes were observed. Insufficient information was provided to establish LOAEL or NOAEL levels from these studies.

4.2.2. Hematotoxicity—Inhalation Exposure

4.2.2.1. *Subchronic Studies*

Benzene-induced hematotoxicity results from short-term as well as long-term exposure to the chemical. Early general toxicity studies reported leukopenia in dogs and fatal anemia in mice exposed to 600 ppm (1917 mg/m^3) of benzene for 12–15 days (Hough and Freeman, 1944); changes in bone marrow histopathology or leukopenia in rats, guinea pigs, and rabbits exposed to 80–85 ppm (256–272 mg/m^3) of benzene for 23–187 exposures (Wolf et al., 1956); and leukopenia in rats exposed to 61 ppm (195 mg/m^3) of benzene for 2–4 weeks or to 44 ppm (141 mg/m^3) for 5–8 weeks (Deichmann et al., 1963). The following discussion summarizes more recent studies of the effects of inhaled benzene on the peripheral blood of experimental animals. The available studies on the hematoxicity of inhaled benzene to test animals are summarized in Table 4-4.

Male CD-1 mice (11–12/group) were exposed for 6 hr/day, 5 days/week to concentrations of 0 or 10 ppm (0 or 32 mg/m^3) benzene for 10 weeks or to 0 or 300 ppm (0 or 958 mg/m^3) for 26 weeks (Green et al., 1981a,b). On the day of the last exposure, samples (pooled from groups of 3–4 mice) were obtained from the peripheral blood, bone marrow, and spleen to evaluate hematological and hematopoietic cells. In mice exposed to 10 ppm (32 mg/m^3), no adverse effects were observed with respect to mortality, body weight, or cells in the peripheral blood or bone marrow. Spleen weight, total nucleated cells per spleen, and nucleated RBC per spleen were significantly increased (p<0.05) in mice exposed to 10 ppm (32 mg/m^3). Mice exposed to 300 ppm (958 mg/m^3) had the following significant (p<0.05) changes: increased mortality rate; decreased numbers of lymphocytes and RBC in peripheral blood; decreased granulocyte/macrophage progenitor cells in bone marrow; decreased spleen weight and numbers of lymphocytes; multipotential hematopoietic stem cells and committed granulocyte/macrophage progenitor cells in the spleen; and increased incidence of atypical cell morphology in the peripheral blood, bone marrow, and spleen. These studies identify a LOAEL of 10 ppm (32 mg/m^3) for slight hematopoietic effects in mice exposed to benzene for 10 weeks.

In a subchronic inhalation toxicity study, Ward et al. (1985) evaluated the peripheral blood and bone marrow of benzene-exposed CD-1 mice and Sprague-Dawley rats. Groups of 50 male and 50 female rats and 150 male and 150 female mice inhaled benzene concentrations of 0, 1, 10, 30, or 300 ppm (0, 3.2, 32, 96, or 958 mg/m^3) 6 hr/day, 5 days/week for up to 13 weeks. Serial sacrifice of 30 mice and 10 rats/group took place after 7, 14, 28, 56, and 91 days of

Table 4-4. Peripheral blood and hematopoietic effects of benzene in animals—inhalation exposure

Strain/Species	Endpoints	Exposure	Effects	LOAEL/NOAEL (mg/m^3)	Reference
CD-1 mice (11–12 males/ group)	Body weight, cells in peripheral blood and bone marrow, spleen weight, total nucleated cells per spleen, lymphocytes	0, 32 mg/m^3, 6 hr/day, 5 days/wk for 10 wk	Increased spleen weight, total nucleated cells per spleen, and nucleated cells per spleen	LOAEL: 32	Green et al., 1981 a,b
CD-1 mice (30 males and 30 females/group)	HCT; Hgb; WBC, RBC, platelet, reticulocyte, and differential counts; MCV; MCH; myeloid/erythroid ratio from bone marrow; leukocyte alkaline phosphatase; RBC glycerol lysis time; and other more general parameters of subchronic toxicity	0, 3.2, 32, 319, or 958 mg/m^3 6 hr/day, 5 days/wk for up to 91 days; serial sacrifices after 7, 14, 28, 56, and 91 days of exposure	At 958 mg/m^3, decreased HCT, total Hgb, RBC, leukocyte, and platelet counts; decreased myeloid/erythroid ratios and percentage of lymphocytes; increased mean cell volume, mean cell Hgb, glycerol lysis time, and incidence and severity of RBC morphologic changes (statistically significant [$p<0.05$] for males and/or females); many effects occurred and were statistically significant by 14 days, persisting throughout exposure; microscopic changes in the thymus, bone marrow, lymph nodes, spleen, ovaries, and testes. Changes in males occurred more often and with greater severity than those in the females	LOAEL: 958 NOAEL: 319	Ward et al., 1985
AKR/J mice (60 males, test; 60 males, control)	WBC and RBC counts (Coulter), reticulocyte counts, differential counts, Hgb, HCT, lactic acid dehydrogenase (LDH), RBC acetylcholinesterase, reduced glutathione, cytogenetic analyses of bone marrow, and microscopic examination of lung, liver, spleen, and kidney	958 mg/m^3 benzene, 6 hr/day, 5 days/wk for life	Early mortality (mean survival time, 11 wk for test animals, 39 wk for controls), severe lymphocytopenia, bone marrow hypoplasia, granulocytosis, and reticulocytosis	LOAEL: ND[a] NOAEL: ND	Snyder et al., 1978

Table 4-4. Peripheral blood and hematopoietic effects of benzene in animals—inhalation exposure (continued)

Strain/Species	Endpoints	Exposure	Effects	LOAEL/NOAEL (mg/m^3)	Reference
AKR/J mice (50 males, test; 50 males, control)	RBC and WBC counts (Coulter), and differential count; microscopic examination of lung, liver, spleen, kidney, and bone marrow	319 mg/m^3 benzene, 6 hr/day, 5 days/wk for life	No statistically significant differences between test and control mice in median survival time, rate of weight gain or lymphoma type or occurrence; statistically significant lymphocytopenia (cell counts were depressed to ~65% of normal throughout exposure); depressed RBC counts (±2 SEM at 9/19 monitoring periods); increased neutrophils (±2 SEM at 3/19 monitoring periods); bone marrow hypoplasia in 20% of the treated mice ($p<0.05$)	LOAEL: ND NOAEL: ND	Snyder et al., 1980
C57BL/6 mice (40 males, test; 40 males, control)	RBC and WBC counts (Coulter), and differential count; microscopic examination of lung, liver, spleen, kidney, and bone marrow	958 mg/m^3 benzene, 6 hr/day, 5 days/wk for life	Decreased survival (median time 41 wk, test animals; 75 wk, controls); lymphocytopenia (WBC counts, ~3000/mm^3 or 15% of normal); anemia (RBC counts, $8\times10^6/mm^3$ or ~80% of normal); neutrophilia appearing at 17 wk; abnormal blood cell morphology; neutrophilic shift to left; bone marrow hyperplasia in 33% of test animals, none in controls; hematopoietic neoplasms	LOAEL: ND NOAEL: ND	Snyder et al., 1980
CD-1 mice (40 males, test; 40 males, control)	RBC and WBC counts (Coulter), and differential count; microscopic examination of lung, liver, spleen, kidney, and bone marrow	958 mg/m^3 benzene, 6 hr/day, 5 days/wk for life	Decreased median survival time (25.5 wk for benzene-exposed animals, 52.7 wk for controls); depressed peripheral RBC and lymphocyte counts (±2 SEM); neutrophilia; abnormal blood cell morphology; and a shift to immature myeloid cells (at 217 days); tumor incidence (5/40) not significant; acute myeloblastic leukemia in one exposed animal, chronic myelogenous leukemia in one; bone marrow hyperplasia in 9/35 animals without neoplasia, bone marrow hypoplasia in 11, splenic hemosiderin pigments in 6, and splenic hyperplasia in 19	LOAEL: ND NOAEL: ND	Snyder et al., 1982

Table 4-4. Peripheral blood and hematopoietic effects of benzene in animals—inhalation exposure (continued)

Strain/Species	Endpoints	Exposure	Effects	LOAEL/NOAEL (mg/m^3)	Reference
Sprague-Dawley rats (10 males and 10 females/group)	HCT; Hgb; WBC, RBC, platelet, reticulocyte, and differential counts; MCV; MCH; myeloid/erythroid ratio from bone marrow; leukocyte alkaline phosphatase; erythrocyte glycerol lysis time; and other more general parameters of subchronic toxicity	0, 3.2, 32, 319, or 958 mg/m^3 6 hr/day, 5 days/wk for up to 91 days; serial sacrifices after 7, 14, 28, 56, and 91 days of exposure	Statistically significant ($p<0.05$) decrease in WBC counts (males on day 14 and females on days 14–91) and slightly decreased femoral marrow cellularity at 958 mg/m^3 (300 ppm)	LOAEL: 958 NOAEL: 319	Ward et al., 1985
Sprague-Dawley rats (45 males, test; 27 males, control)	WBC and RBC counts (Coulter), reticulocyte counts, differential counts, Hgb, HCT, LDH, RBC acetylcholinesterase, and reduced glutathione, cytogenetic analyses of bone marrow, and microscopic examination of lung, liver, spleen, and kidney	958 mg/m^3 benzene, 6 hr/day, 5 days/wk for life	Mild weight depression, lymphocytopenia, trend to anemia, fatty changes in 77% of bone marrow samples	LOAEL: ND NOAEL: ND	Snyder et al., 1978
Female BDF1 mice (4/group)	WBCs, reticuloctyes, differential counts, and assays for CFU-S, BFU-E, CFU-E, and GM-CFU	0, 319, 958, or 2875 mg/m^3, 6 hr/day, 5 days/wk for 16 wk	Reduced lymphocyte count at $\geq$319 mg/m^3, decreased CFU-E, BFU-E, CFU-S, and GM-CFU	LOAEL: 319 NOAEL: NA	Seidel et al., 1989
Male C57BL/6J mice (5/group)	Peripheral blood counts, spleen and bone marrow BFU-E and CFU-E	32 mg/m^3, 6 hr/day, 5 days/wk for up to 178 days	Reduced circulating RBCs, lymphocyte counts, bone marrow CFU-E and BFU-E, and splenic CFU-E	LOAEL: 32	Baarson et al., 1984
Male and female C57/6 BNL mice	WBC, RBC, differential counts, hematocrits, bone marrow cellularity, and CFU-S in bone marrow and spleen	0, 32, 80, or 1278 mg/m^3, 6 hr/day, 5 days/wk for 2 wk	Reduced lymphocyte counts after 2 wk exposure at 80 mg/m^3 but no effect at 32 mg/m^3	LOAEL: 80 NOAEL: 32	Cronkite et al., 1985

Table 4-4. Peripheral blood and hematopoietic effects of benzene in animals—inhalation exposure (continued)

Strain/Species	Endpoints	Exposure	Effects	LOAEL/NOAEL (mg/m^3)	Reference
Male Sprague-Dawley rats	Erythrocyte and lymphocyte counts	0 or 319 mg/m^3 6 hr/day, 5 days/wk for life	No significant reductions in erythrocyte or lymphocyte counts	NOAEL: 319	American Petroleum Institute, 1983
Male B6C3F1 mice (24/group exposed, bone marrow tests on 3–16/group)	Bone marrow; cell counts, CFU-E, CFU-GM, and B- and T- lymphocytes	0, 3.2, 32, 319, or 639 mg/m^3, 6 hr/day, 5 days/wk for 1, 2, 4, or 8 wk	Reduced bone marrow cellularity, progenitor cells, and differentiating hematopoietic cells, at $\geq$319 mg/m^3, but no effects observed at $\leq$32 mg/m^3	LOAEL: 319 NOAEL: 32	Farris et al., 1996, 1997a
Male B6C3F1 mice (3 or 9/group)	Splenic, thymic, and femoral lymphocytes and labeling index of femoral lymphocytes	0, 3.2, 16, 32, 319, or 639 mg/m^3 6 hr/day, 5 days/wk for 1, 2, 4, or 8 wk	Persistent and rapid reductions in splenic and femoral lymphocytes at $\geq$ 319 mg/m^3, transient reduction of thymic cell count and splenic B-lymphocytes at 32 mg/m^3 after 2 wk, but comparable to controls by 4 wk	LOAEL: 319 NOAEL: 32	Farris et al., 1997b
Male Sprague-Dawley rats (16/group)	Thymus and spleen weight, spleen and bone marrow cellularity, spleen $CD4^+/CD5^+$, $CD8^-/CD5^+$ and $Kappa^+$ lymphocytes	0, 96, 639, or 1278 mg/m^3 6 hr/day, 5 days/wk for 2 or 4 wk	Reduced spleen B-$CD4^+/CD5^+$ and CD5+ T-lymphocytes at 1278 mg/m^3, no effects observed at 639 mg/m^3	LOAEL: 1278 NOAEL: 639	Robinson et al., 1997

[a]ND = not determined because only one concentration was used; NA = not applicable, adverse effects were observed at the lowest dose tested

exposure. The parameters of toxicity evaluated included behavior, body weight, organ weights, clinical pathology, gross pathology, and histopathology. Chamber analyses of benzene concentrations calibrated daily by gas chromatography and monitored continuously by infrared analyzer were within 10% of the target concentrations.

Clinical observations and body weight data revealed no signs of exposure-related toxicity. Hematological effects were not observed in either species at 1, 10, or 30 ppm (3.2, 32, or 96 mg/m^3). The mice exposed to 300 ppm (958 mg/m^3) for 91 days, however, exhibited decreased hematocrit, and reductions in total Hgb concentration, RBC count, WBC count, platelet count, myeloid/erythroid ratios, and percentage of lymphocytes; the RBCs of the exposed mice also displayed increases in MCV, mean cell Hgb, and glycerol lysis time, and in the incidence and severity of red cell morphologic changes. The values for RBC, motor conduction velocity, mean corpuscular hemoglobin (MCH), and glycerol lysis time were statistically significant ($p<0.05$) for both males and females, whereas the remainder of the values were statistically significant for males only.

Many of the effects occurred (and were statistically significant) by 14 days of exposure and most persisted throughout exposure, but the data provided indicated that the effects did not increase in severity with duration of exposure. Microscopic examination of the high-concentration group revealed changes in the thymus, bone marrow, lymph nodes, spleen, ovaries, and testes. The changes in the males occurred more often and with greater severity than those in the females. The most common compound-related histopathologic findings included myeloid hypoplasia of the bone marrow, lymphoid depletion of the periarteriolar sheath in the spleen and mesenteric lymph node, increased extramedullary hematopoiesis in the spleen, and plasma cell infiltration of the mandibular lymph node. The incidence and/or severity of thymic atrophy and myeloid hypoplasia appeared to increase with duration of exposure.

The rats, not as severely affected as the mice, exhibited significantly ($p<0.05$) decreased WBC counts (males on day 14 and females on days 14–91) and slightly decreased femoral marrow cellularity at 300 ppm (958 mg/m^3).

The investigators concluded that the hematological effects observed in this study are related to concentration and are similar to those reported by other investigators, but that they are statistically significant only at 300 ppm (958 mg/m^3). In addition, the mouse is more sensitive to the effects of benzene than the rat, and male mice appear to be more sensitive than female mice. The experiment provided a definitive LOAEL of 300 ppm (958 mg/m^3) and a NOAEL of 100 ppm (319 mg/m^3) for peripheral blood abnormalities in both rats and mice. The large numbers of animals used in the study and the evaluation of several parameters of hematotoxicity strengthen the conclusions of the investigators.

To further characterize the hematotoxicity of benzene, Snyder et al. (1978, 1980, 1982, 1984, 1988) conducted a series of studies using either 100 or 300 ppm (319 or 958 mg/m^3) benzene. These studies addressed mainly the influence of benzene concentration and animal species on toxic responses. The 300 ppm (958 mg/m^3) concentration was selected because it had produced severe hematotoxicity in AKR mice exposed for life, but had produced only lymphocytopenia in Sprague-Dawley rats exposed under the same conditions. Also of interest

was whether benzene would affect the incidence of lymphoma in the different strains of rodent. The AKR strain carries a virus that produced a high incidence of spontaneous lymphoma, killing the mice within 1 year, and the C57BL strain carries a virus that yields a high incidence of lymphoma following exposure to radiation, carcinogens, and immunosuppressive agents (Snyder et al., 1980).

Exposures were similar in all the studies. Essentially, test animals and control animals were placed in identical stainless steel inhalation chambers and exposed to benzene or filtered air, respectively, 6 hr/day, 5 days/week for life. Benzene concentrations, monitored every 30 minutes by UV absorbance at 255 nm, were read from a calibration curve. Tail bleeding of test and control animals took place at about the same time of day, at least every 3–5 weeks during exposure, for analysis of the following endpoints: WBC and RBC counts (Coulter), reticulocyte counts, differential counts, Hgb, and hematocrit. Evaluations of lactic acid dehydrogenase levels, RBC acetylcholinesterase levels, and reduced glutathione levels were conducted less frequently. The evaluation also included cytogenetic analyses of bone marrow. Other tissues taken for microscopic examination included lung, liver, spleen, and kidney.

In the first study of the series, Snyder et al. (1978) exposed AKR/J mice (60 test and 60 control) and Sprague-Dawley rats (45 test and 27 control) to 300 ppm (958 mg/m^3) of benzene. The rats had mild body weight depression that started at 30 weeks and persisted throughout life. The only significant hematological findings in the rats (±2 SE) were lymphocytopenia and a trend to anemia. Lymphocyte counts for the exposed animals were approximately 75% of normal at 2 weeks and 53% of normal at 20 weeks. Because of increased mortality, blood analyses were discontinued after 1 year of exposure. The investigators also noted fatty changes in 77% of the bone marrow samples taken from the benzene-exposed animals compared with 42% of the controls.

The last mouse in the study (Snyder et al., 1978) died after 28 weeks of exposure, probably of aplastic anemia (mean survival time, 11 weeks for test animals, 39 weeks for control animals). The treated animals suffered severe weight loss (59% weight change at 16 weeks), marked lymphocytopenia (e.g., lymphocyte values that dropped to <1000/mm^3 were ~3% of control levels after 36 days of exposure), bone marrow hypoplasia (81% of exposed mice and 6% of controls, p<0.001 by chi-square), granulocytosis (fivefold increase after 9 weeks of exposure), and reticulocytosis. The blood analyses were suspended after 92 days of exposure due to early mortality. Neither species exhibited any signs of leukemia or preleukemia (the absence of overt signs of leukemia; characterized by bone marrow dysfunction, usually low numbers of certain cell types). The investigators suggested that this might be related to early mortality from aplastic anemia in the mice and a lack of opportunity for recovery of the bone marrow (a possible factor in leukemogenesis) in the rats.

The investigators concluded from this study that inhalation of 300 ppm (958 mg/m^3) of benzene causes significantly decreased survival, severe lymphocytopenia, and anemia, accompanied by reticulocytosis and granulosis in AKR/J mice, and lymphocytopenia, mild anemia, and moderately decreased survival in Sprague-Dawley rats. They further concluded that AKR/J mice are more susceptible to benzene-induced hematotoxicity than are Sprague-Dawley rats.

The severity of hematologic effects induced by 300 ppm (958 mg/m^3) of benzene in AKR/J mice prompted Snyder et al. (1980) to conduct a similar study with 50 AKR/J mice exposed to a lower concentration (100 ppm [319 mg/m^3]) of benzene. The investigators also exposed 40 C57BL/6 mice to 300 ppm (958 mg/m^3) of benzene to compare the susceptibility of a different strain of mice with that of rats. Hematologic parameters were measured in both strains at regular intervals during exposure.

The AKR/J mice demonstrated no statistically significant differences between test and control mice in median survival (39 and 41 weeks, respectively), rate of weight gain, or in type and occurrence of lymphoma. Effects that did occur were not as severe as those observed at 300 ppm (958 mg/m^3). But even at 100 ppm (319 mg/m^3), the mice exhibited statistically significantly reduced lymphocyte counts after 1 week of exposure (cell counts were depressed to 65% of control levels), and the depression persisted throughout exposure. RBC counts were also consistently depressed relative to controls, and the depression was statistically significant (±2 SE) at 9/19 monitoring periods. Neutrophils were elevated (±2 SE) in 3/19 monitoring periods. Ten (20%) of the treated mice, but only one control, had bone marrow hypoplasia (p<0.05).

C57BL/6 mice exposed to 300 ppm (958 mg/m^3) benzene had a median survival time of 41 weeks, compared with 75 weeks for the controls. Starting 1 week after the beginning of exposure, mice exposed to benzene exhibited a statistically significant increase in incidence of lymphocytopenia at 29/30 monitoring periods (WBC counts were about 3000/mm^3 or ~15% of control values), and of anemia in 30/30 monitoring periods (RBC counts were ~80% of control values). After 17 weeks, the incidence of neutrophilia was significantly increased in 15 of the 22 remaining monitoring periods. Throughout exposure, the peripheral RBC exhibited anisocytosis and poikilocytosis. A neutrophilic shift to the left, characterized by the appearance of metamyelocytes, indicated immaturity. At the end of the first year, myelocytes, promyelocytes, and giant platelets began to appear. Blood analyses were discontinued after 61 weeks because of decreased survival.

Examination of the bone marrow revealed hyperplasia, mainly of granulopoietic elements, in 13 (33%) of the exposed mice; hyperplasia was not present in the controls. There was a significant increase in the incidence of hematopoietic neoplasms including six cases (15%) of thymic lymphoma. Two control mice died with nonthymic lymphoma. The investigators concluded from this study that the two strains of mice are more sensitive to the hematologic effects of benzene than the Sprague-Dawley rat, and that the AKR/J mouse is more sensitive than the C57BL/6 mouse. The response of the C57BL/6 mouse producing a proliferative effect on the myeloid cell line is significant because this is the cell line that undergoes leukemic transformation in humans exposed to benzene.

In the next study, Snyder et al. (1982) examined the effects of 300 ppm (958 mg/m^3) of benzene on CD-1 mice, a strain not known to harbor any endogenous lymphoma virus. The effects were similar to those observed in the AKR/J and C57BL/6 strains. These included decreased median survival time (25.5 weeks for benzene-exposed animals, 52.7 weeks for controls), statistically significantly (±2 SE) depressed peripheral RBC and lymphocyte counts after the first week of exposure, statistically significant neutrophilia (±2 SE) after 29 weeks (~209

days) of exposure, and the appearance of Howell-Jolly bodies at 7 days, anisocytosis at 22 days, poikilocytosis at 92 days, and a shift to immature myeloid cells at 217 days.

Tumor incidence was not significant. One benzene-exposed animal developed acute myeloblastic leukemia, one had chronic myelogenous leukemia, one had a benign lung adenoma, and two had malignant lymphoma with thymic involvement. Of the 35 benzene-exposed animals that did not have neoplasia, 9 had bone marrow hyperplasia, 11 had bone marrow hypoplasia, 6 had splenic hemosiderin pigments (indicating hemolysis or ineffective erythropoiesis), and 19 had splenic hyperplasia. The investigators noted that "exposed mice dying with bone marrow hypoplasia survived, on the average, 75 fewer days than mice dying with bone marrow hyperplasia. This may indicate that bone marrow hypoplasia is an early response to exposure followed by bone marrow hyperplasia. It may also indicate two different responses to the exposure."

Snyder et al. (1988) examined the influence of modifications in inhalation protocol on benzene-induced hematotoxicity and tumor development in C57BL/6 and CD-1 mice. Exposure was by two protocols, one representing intermittent occupational exposures (1 week of exposure to 300 ppm [958 mg/m^3] benzene followed by 2 weeks of nonexposure alternately for life), and the other representing intense but short-term exposures (1200 ppm [3834 mg/m^3] benzene for 10 weeks); all exposures were 6 hr/day, 5 days/week. The long-term intermittent exposures produced earlier mortality in both strains of mice than did the short-term, 10-week exposures. Both types of exposure produced severe lymphocytopenia and moderate anemia in both strains of mice, but peripheral blood values in the 1200 ppm (3834 mg/m^3)/10-week exposure groups returned to normal after termination of exposure. The 300 ppm (958 mg/m^3) exposure induced lymphocytopenia and anemia throughout the course of exposures. Tumor incidences were significantly increased (p<0.05–0.001) with both protocols in CD-1 mice and with the intermittent, long-term exposure in C57BL/6 strain. A puzzling finding was that neither leukemia nor lymphoma occurred in either strain by either protocol. The investigators concluded that benzene was cytotoxic by either protocol, especially to circulating lymphocytes (but recovery took place when exposure to the high concentration ceased) and that both protocols did induce tumors. The Snyder et al. (1978, 1980, 1982, 1984, 1988) studies were designed to answer specific questions, and used only one concentration at a time (except for the study on exposure protocol), and therefore were not sufficient for the derivation of LOAEL or NOAEL.

4.2.2.2. *Effects on Stem Cell Populations*

The benzene-induced peripheral blood abnormalities reflect a disruption of hematopoiesis in the bone marrow; thus many experimental studies have focused on this process, analyzing the total cellularity (the total number of bone marrow cells obtained from a femur), as well as the individual cellular components of the bone marrow. The pluripotent hematopoietic stem cells are capable of self-renewal and can differentiate along at least three lineages: erythrocytic, granulocytic/macrophagic, and thrombocytic (Snyder, 1987). These cells are quantitated by the spleen colony assay in which the colonies formed by the cells in the spleens of lethally irradiated host animals are counted; these are called colony-forming units-spleen (CFU-S). The specific progenitor cells, in contrast to the pluripotential stem cells, are committed to differentiate, and

they also have some capacity for self-renewal (cell division). These cells are enumerated by counting the colonies formed in cell cultures grown in the presence of specific growth factor stimuli (Snyder, 1987). The two types of erythroid progenitor cells are the less-differentiated burst-forming unit-erythroid (BFU-E), which responds to a factor called "burst-promoting activity" or to interleukin 2; and the more differentiated erythroid progenitor, the colony-forming unit-erythroid (CFU-E), which responds to erythropoietin. The granulocyte/macrophage colony-forming unit-culture (GM-CFU-C) responds to conditioned media from human leukemia cells or from organs of animals treated with plant lectins. The T- and B-lymphocytes arise from the pluripotential hematopoietic stem cells in the bone marrow. The cells progress to the recognizable blast cells and their progeny, and further mature to the functional forms normally observed in peripheral blood (Snyder, 1987).

Seidel et al. (1989) examined the effects of benzene on peripheral blood cell counts and the hematopoietic stem cell compartments of the bone marrow. Female BDF1 mice were exposed to 100, 300, or 900 ppm (319, 958, or 2875 mg/m^3) of benzene, 6 hr/day, 5 days/week for 16 weeks. Benzene concentrations were monitored twice a day. Control mice, placed in chambers, were exposed to air. The evaluation of hematopoietic effects included WBC (Coulter), reticulocyte, and differential counts, and assays for CFU-S, BFU-E, CFU-E, and GM-CFU (CFU-C) using standard procedures. The assays, conducted about every 2 weeks, usually took place about 66 hr after the end of the exposure period of the preceding week.

There was a concentration-dependent reduction in lymphocyte count after both 4 and 8 weeks of exposure. Lymphocyte counts of the animals exposed to 300 and 900 ppm (958 and 2875 mg/m^3) returned to control values 1–2 weeks after cessation of exposure, whereas the lymphocytes in the 100 ppm (319 mg/m^3) group remained low during that time. The animals exposed to 300 and 900 ppm (958 and 2875 mg/m^3) developed a slight anemia that did not worsen with longer exposure. Three-fourths of the animals with elevated CFU-E in the bone marrow also showed reticulocytosis at 13 weeks. The number of granulocytes was apparently not affected. The investigators noted that control values for the 300 ppm (958 mg/m^3) groups were approximately twice those for the 100 and 900 ppm (319 and 2875 mg/m^3) groups, possibly because the 300 ppm (958 mg/m^3) experiment had been performed 1 year earlier.

Assays performed after 4 and 8 weeks of exposure demonstrated decreases in stem cell numbers that were concentration-related, particularly at 8 weeks. At 4 weeks, the CFU-E count was depressed more than other stem cell counts. At 100 ppm (319 mg/m^3), the CFU-E were depressed to 48% of control, whereas the BFU-E, CFU-S, and CFU-C were depressed to 88%, 92%, and 94% of control, respectively. At 8 weeks, the values for CFU-E in animals exposed to 100, 300, and 900 ppm (319, 958, and 2875 mg/m^3) were 99%, 73%, and 35% of controls, respectively. Similar patterns of depression were observed for BFU-E and CFU-C numbers of animals exposed to benzene for 8 weeks; and CFU-S numbers were depressed to 65% of control at 300 ppm (958 mg/m^3), the only concentration tested after 8 weeks of exposure. Recovery studies demonstrated a gradual regeneration of progenitor cell numbers, and animals exposed to 300 (958 mg/m^3) for 16 weeks had completely recovered by 73–185 days post exposure. The investigators concluded that the erythroid system is more sensitive to the effects of benzene than the myeloid cell system, with the CFU-E showing more sensitivity than other components of the erythroid system under the conditions of this study.

Two studies were designed to examine the effects of low concentrations of benzene on the erythroid and myeloid progenitor cells. Baarson et al. (1984) examined the effects of subchronic exposure to a low concentration of benzene on the erythroid progenitor cells in the bone marrow and spleen of mice. Male C57BL/6J mice inhaled 10 ppm (32 mg/m^3) of benzene vapor 6 hr/day, 5 days/week for as long as 178 days. Peripheral blood counts and bone marrow and spleen BFU-E and CFU-E assays were performed on days 32, 66, and 178 of exposure. The numbers of circulating RBC in the benzene-treated groups were significantly decreased (p<0.05) at both 66 and 178 days. Lymphocyte values were decreased (p<0.05) at all three time points. Benzene did not affect levels of circulating neutrophils, bone marrow cellularity, and numbers of nucleated red cells in the bone marrow. The numbers of bone marrow CFU-E gradually declined during exposure to only 5% of control values after 178 days. The decline, statistically significant (p<0.01) at all three time points, was apparently exponential with a half-life of about 2 months. Bone marrow BFU-E colonies were significantly depressed in benzene-exposed animals (to about 55% of control; p<0.01) at 66 days, but recovered to control values by 178 days.

Depressions in splenic nucleated red cell numbers (to about 15% of control values; p<0.05) and in splenic nucleated cellularity (p<0.05) occurred at day 178. Splenic CFU-E colonies in benzene-exposed mice were decreased to 10% of control values at 178 days (p<0.05). In contrast, splenic BFU-E colonies were increased (but not significantly) at all time points. The spleen is a site of extramedullary erythropoiesis in the rodent, particularly under stressful conditions. The reduction in the numbers of nucleated red cells and CFU-E in the spleen following exposure to benzene indicated that the compensatory mechanism of the spleen was also affected. The investigators concluded that low concentrations of benzene are hematotoxic, and that further studies to examine this would be warranted. In a later study, Baarson and Snyder (1991) reported that the ingestion of ethanol in combination with exposure to benzene may enhance benzene-induced toxicity of the erythroid progenitor cells in C57BL/6J mice. These studies identify a LOAEL of 10 ppm (32 mg/m^3) for depressed hematopoiesis in mice.

Female C57BL/6xDBA/2F1 hybrid mice were exposed to 0 or 300 ppm benzene (0 or 958 mg/m^3), 6 hr/day, 5 days/week for 6–7 weeks (Vacha et al., 1990). Indices of hematopoiesis were measured in peripheral blood (RBC and WBC count, hemaglobin, hematocrit, reticulocyte, and leukocyte count), in addition to ^{59}Fe accumulation in the erythropoietic organs (spleen and bone marrow) and in the peripheral RBC. The distribution of developmental classes of erythroblasts was also determined. This study found that animals became anemic after 6–7 weeks of benzene exposure. The number of erythroblasts in the bone marrow was not different; however, exposure to benzene shifted the population to a less mature class of cells. The number of colonies derived from BFU-E and CFU-E were decreased to 70% and 34% of controls, respectively. A LOAEL of 300 ppm (958 mg/m^3) was established for hematotoxic effects.

Toft et al. (1982) evaluated the adverse effects of occupationally relevant levels of benzene on the bone marrow of mice. Male NMRI mice inhaled concentrations of benzene that ranged from 1–200 ppm (3.2–639 mg/m^3). Exposures were either continuous (24 hr/day for 4 to 10 days) or intermittent (8 hr/day, 5 days/week for 2 weeks), and endpoints (using five mice per group) included the number of nucleated cells per tibia, the number of colony-forming granulopoietic stem cells (CFU-C/tibia), and frequency of micronuclei in polychromatic erythrocytes (PCE). Mice exposed continuously to $\geq$21 ppm (67 mg/m^3) benzene exhibited

significant and concentration-dependent alterations in all three parameters. The values after 4 days of exposure to 21 ppm (67 mg/m^3) of benzene (estimated from a graph) are as follows: cells/tibia, ~24% of control; CFU-C/tibia, ~32% of control; micronuclei/500 PCE, 6 (control value, 0.41/500).

Intermittent exposure to 1.0, 10.5, 21, 50, 95, and 107 ppm (3.2, 33, 67, 160, 303, and 342 mg/m^3) for 2 weeks produced concentration-dependent decreases in CFU-C/tibia and increases in the frequency of micronuclei, significant at $\geq$21 ppm (67 mg/m^3), and concentration-dependent increases in cellularity, significant at $\geq$50 ppm (160 mg/m^3). Animals exposed intermittently to 95 or 201 ppm (303 or 642 mg/m^3) of benzene for 2–8 hr/day, 5 days/week for 2 weeks had significant decreases in cellularity and CFU-C/tibia (95 ppm [303 mg/m^3] for 6 and 8 hr/day) and increases in the frequency of micronuclei (95 ppm [303 mg/m^3] for 4 hr). At 201 ppm (642 mg/m^3), all exposure protocols, except for the 2 hr/day exposure, produced significantly adverse effects on all parameters. Statistical significance was determined by the Student's *t* test for cellularity and CFU-C and by the Wilcox-Whitney method for micronuclei which was set at $p \leq 0.05$. Statistical values were not given for the individual data points.

The investigators concluded that the CFU-C/tibia was suppressed to a greater extent than was the overall cellularity for most exposures and that CFU-C are more sensitive to prolonged exposure to low levels of benzene than are the majority of bone marrow cells. However, higher exposures for short durations produced the reverse, suppressing cellularity but not CFU-C. This could indicate that short exposures cause rapid injury to the bone marrow but that the injury could be offset by a compensatory increase in the proliferation rate of the bone marrow. They further concluded that intermittent exposure is less effective than is continuous exposure, that the induction of micronuclei is important because somatic mutations precede most chemically induced cancers, and that the cytotoxicity observed in the study could lead to aplastic anemia.

Because of the relationship between exposure to benzene and the development of myeloblastic leukemia, Dempster and Snyder (1990) evaluated the possibility that exposure to benzene provides a growth advantage for granulocytic progenitor cells over erythroid progenitor cells. Basically, DBA/2 male mice inhaled benzene concentrations of 0, 10, 30, or 100 ppm (0, 32, 96, or 319 mg/m^3), 6 hr/day, 5 days/week for 5 days and were evaluated for BFU-E, CFU-E, and GM-CFU-C on days 1 and 5 after exposure. To determine the effects of benzene on stem cells during increased erythropoiesis, subgroups of five exposed mice received injections of hemolytic doses of phenylhydrazine during or after exposure. Controls were exposed to air and/or injected with saline. Brief descriptions of treatment and the effects, significant at $p<0.05$, are as follows.

Benzene only. One day after exposure, there was a dose-dependent depression in bone marrow BFU-E and CFU-E, but GM-CFU-C were not affected (except for a spurious increase at 30 ppm [96 mg/m^3]); splenic CFU-E and BFU-E were increased at 10 ppm (32 mg/m^3) and CFU-E were depressed at 100 ppm (319 mg/m^3); splenic granulocytic cells were unaffected by benzene. Five days after exposure, BFU-E and CFU-F were the same as control values, but GM-CFU-C were decreased at 10 and 100 ppm (32 and 319 mg/m^3); splenic CFU-E exhibited a concentration-related increase, significant at 30 and 100 ppm (96 and 319 mg/m^3), whereas the numbers of the other two progenitor cell types were normal.

Benzene + phenylhydrazine. One day after exposure, bone marrow BFU-E were depressed at 30 and 100 ppm (96 and 319 mg/m^3), and CFU-E were depressed only at 30 ppm (96 mg/m^3); there was no effect on the number of GM-CFU-C. Splenic BFU-E and GM-CFU-C showed concentration-dependent depressions, and CFU-E were depressed, but only at 100 ppm (319 mg/m^3). Five days after exposure, bone marrow CFU-E were elevated in mice exposed to 10 ppm (32 mg/m^3), and GM-CFU-C exhibited a concentration-dependent depression; bone marrow BFU-E counts were similar to control counts. Splenic GM-CFU-C were elevated at all concentrations, CFU-E were elevated at 10 and 30 ppm (32 and 96 mg/m^3) and depressed at 100 ppm (319 mg/m^3), and BFU-E were elevated only at 10 ppm (32 mg/m^3).

The investigators concluded that acute exposure to benzene has different effects on erythroid and granulocytic progenitor cell populations that resulted in a growth advantage for granulocytic cells in both the bone marrow and spleen of exposed mice. This observed shift toward granulopoiesis occurred even in mice treated with the erythropoietic stimulus, phenylhydrazine, but the effects were short-lived. The bone marrow erythroid progenitor cells had recovered from their depression 5 days after exposure, and at that time the granulocytic progenitor cells had become depressed. The dose-dependent increase in splenic CFU-E 5 days after exposure probably reflects the spleen's attempt to repopulate the erythron.

In a series of studies, Luke et al. (1988a,b) and Tice et al. (1989) examined the effect of sex, route, schedule, and duration of exposure on benzene-induced cytotoxicity and genotoxicity of the bone marrow in mice. The effects on percentage of PCE in peripheral blood, PCV, and bone marrow cellularity (all considered by the investigators to be measures of bone marrow cytotoxicity) are reviewed below.

Groups of 6-10 male and female DBA/2 mice, male B6C3F1 mice, and male C57BL/6J mice inhaled either 300 ppm (958 mg/m^3) of benzene or ambient air 6 hr/day for 13 weeks. Exposures took place on either 3 consecutive days/week or 5 consecutive days/week. For comparison with the oral route of exposure, one group of B6C3F1 males received doses of 400 mg/kg by gavage on 5 consecutive days/week for 14 weeks. The oral dose of 400 mg/kg was estimated to exceed the total amount of benzene absorbed by a mouse during a 6-hr exposure to 300 ppm (958 mg/m^3). Peripheral blood smears, prepared weekly, were used to examine PCE. PCVs and bone marrow cellularity were determined at the end of the study. The data for PCE, PCV, and bone marrow cellularity were statistically analyzed using temporal averages. The data were then evaluated for differences related to sex, strain, regimen, and route of exposure using a two-way Brown Forsythe analysis of variance (ANOVA). Group mean data were compared using Student's *t* test.

Benzene initially induced a significant depression in the number of PCE in the peripheral blood of all three strains exposed by either route and both inhalation regimens. However, the extent and duration of the depression varied, depending on sex, strain, and exposure regimen. Female DBA/2 mice exhibited the smallest initial decrease in PCE and values returned to control levels by week 3 of exposure. The extent of depression was not dependent on exposure regimen. Among the males of the different strains, the DBA/2 mouse was the most severely affected, particularly when exposed to benzene 3 days/week. ANOVA revealed significant differences in the ability of benzene to suppress erythropoiesis that were related to both strain (for C57BL/6 and

DBA/2 mice) and exposure regimen. Oral treatment produced an initial suppression in PCE levels in the male B6C3F1 mice that persisted for only 2 weeks and was not revealed in the temporal average.

The study also demonstrated a significant depression in the PCV and bone marrow cellularity (determined at the end of the study) of the exposed mice. With regard to the PCV values, the depression was independent of the inhalation regimen in B6C3F1 and C57BL/6 mice. After correcting for differences in PCV levels in controls, the data showed that inhalation produced a greater effect than oral exposure to benzene, the extent of which was strain-dependent for all three strains (B6C3F1 ≈C57BL/6 >DBA/2; $p<0.0163$). In DBA/2 mice the extent of depression was sex-dependent (male > female; $p<0.025$).

With regard to bone marrow cellularity, the depression observed after inhalation exposure depended on sex (male > female; $p<0.0002$) and strain (DBA/2 < B6C3F1 ≈ C57BL/6; $p<0.0012$). Among the male mice, the extent of depression was dependent on route (inhalation > oral; $p<0.012$), and was not dependent on regimen. These studies used rather small numbers of animals, but otherwise appear to have been thoroughly conducted and evaluated; however, the information provided is for exposure to benzene that is significantly above the most recent occupational limits. The investigators suggest that further studies are needed to evaluate the influences on benzene toxicity relative to regimen and route of exposure at lower doses.

In a series of experiments, Cronkite et al. (1985, 1989) examined the hematotoxicity of inhaled benzene in mice. The study examined the effects on and recovery of peripheral blood, bone marrow, and progenitor cells, as well as the development of neoplasia and the influence of exposure regimen on toxicity. In the first study, male and female C57BL/6 BNL mice were exposed to benzene concentrations of 10, 25, 100, or 400 ppm (32, 80, 319, or 1278 mg/m^3); intermittent exposure(6 hr/day, 5 days/week); and duration of exposure ranging from 2 to 16 weeks. The endpoints of toxicity included WBC, RBC (Coulter), and differential counts; hematocrit; bone marrow cellularity; CFU-S in the bone marrow and spleen; and tumor development. Assays were performed 18–22 hr after termination of exposure or after various periods of recovery up to 16 weeks.

Inhalation of 25, 100, or 400 ppm (80, 319, or 1278 mg/m^3) of benzene for 2 weeks did not affect granulocyte counts but resulted in a concentration-related depression in the number of lymphocytes in the peripheral blood; there was no effect on lymphocytes at 10 ppm (32 mg/m^3). Hematocrit values were decreased at 100 and 400 ppm (319 and 1278 mg/m^3); RBC counts were also decreased, but no data were given. At the end of exposure, lymphocyte depression ranged from 21%–48% of control for the four groups. For the group exposed for 2 weeks, recovery was complete by 4 weeks after the end of exposure, and recovery of the remaining groups was complete by 8 weeks after exposure. This experiment provided an estimated LOAEL of 25 ppm (80 mg/m^3) and a NOAEL of 10 ppm (32 mg/m^3) for lymphocyte depression in mice exposed to benzene for 2 weeks (Cronkite et al., 1985). This differed from the results of Ward et al. (1985) that suggested a LOAEL of 300 ppm (958 mg/m^3) for all hematologic parameters tested in both mice and rats. The main difference in the two studies was the use of different strains of mouse.

Cronkite et al. (1985) also determined bone marrow cellularity, the percentage of bone marrow stem cells synthesizing DNA, numbers of pluripotent stem cells in bone marrow, and colony-forming units in the spleen. Cellularity was determined in animals inhaling 0, 10, 25, or 100 ppm (0, 32, 128, or 319 mg/m^3) (the 400 ppm [1278 mg/m^3] concentration was not tested as it was for the other parameters of the study). Benzene levels of 100 ppm (319 mg/m^3) administered for 2 weeks, significantly depressed bone marrow cellularity and stem cell CFU-S numbers (p<0.003 and <0.001, respectively) in exposed mice. In contrast, 10 and 25 ppm (32 and 128 mg/m^3) had no effect on these parameters. A single assay demonstrated a depression in the fraction of stem cells in DNA synthesis in mice exposed to 10 ppm (32 mg/m^3). There was no effect at 25 ppm (128 mg/m^3), and slight increases in the fraction in DNA synthesis at 100 and 400 ppm (319 and 1278 mg/m^3) (p<0.17 and <0.08, respectively). These results for DNA synthesis are of uncertain relevance.

In another experiment, the recovery of bone marrow CFU-S population was tracked after exposure of the animals to 300 ppm (958 mg/m^3) for 2, 4, 8, or 16 weeks. In animals exposed for 2 and 4 weeks, bone marrow CFU-S numbers were depressed to 90% of control values at the end of exposure but had recovered to >100% and >95% of control values, respectively, 4 weeks later. In animals exposed for 8 weeks, CFU-S levels were decreased to 50% of control 4 weeks after the last exposure, but recovered 8 weeks later (after 16 weeks, the values were >100% of control). In animals exposed for 16 weeks, CFU-S values were depressed to 27% of control, and although recovery was occurring by week 2 after the last exposure, it was still not complete by 16 weeks after termination of exposure. The investigators concluded that exposure to 300 ppm (958 mg/m^3) of benzene for 16 weeks significantly depressed the hemopoietic stem cells and that recovery was incomplete 16 weeks after termination of exposure.

In reviewing the effects of benzene on the CFU-S compartment, Snyder (1987) pointed out that under normal circumstances the CFU-S cells are nondividing and in that state are probably resistant to benzene toxicity; however, in response to benzene-induced injury of more differentiated cell types, the CFU-S may be drawn into the cell cycle and thereby become susceptible to benzene.

Cronkite et al. (1985) also noted that animals exposed to 300 ppm (958 mg/m^3) benzene for 16 weeks began to die from thymic and non-thymic lymphomata and solid tumors, beginning at 330 days of age. In a continuation of the Cronkite et al. (1985) study, Cronkite et al. (1989): (1) evaluated the effects of benzene on erythrocyte progenitors (BFU-E, CFU-E) and on neutrophil and macrophage progenitors (CFU-GM); (2) compared the effects of two different exposure regimens on peripheral blood cell counts and progenitor cell numbers; (3) evaluated mortality and neoplasia; and (4) tested the functional capacity of the stem cells using a "rescue assay." Hale-Stoner mice, aged 12–14 weeks, were used in the progenitor cell assays. Bone marrow cell suspensions, harvested from mice exposed to 400 ppm (1278 mg/m^3) of benzene 6 hr/day, 5 days/week for 9.5 weeks, and their corresponding controls, were counted and incubated to form plasma clots. The cells used in the CFU-E assay were cultured in 2% fetal calf serum; the cells used in the BFU-E assay were cultured in erythropoietin and pokeweed-mitogen (PWM) conditioned medium. The clots were scored for erythrocytic colonies (CFU-E) or megakaryocytes (BFU-E). The cells used in the CFU-G and CFU-GM assays were cultured in agar for subsequent scoring. Progenitor cell assays resulted in the following: CFU-E numbers

did not change after 4 days of exposures, but had decreased significantly (to approximately 50%–75% of control values) by assay days 29, 48, and 65. Twelve days after exposure ended, the cell numbers had recovered to 200% of control values. The BFU-E values also decreased significantly (to approximately 90% of control values) at 29, 48, and 65 days, but had recovered to only about 40% of control values on day 12 after exposure. During the time of this assay, the peripheral RBC counts were decreased to about 50%–75% of control values.

Granulocyte-macrophage aggregates, also observed in the clots, were decreased to about 10%–15% of control by day 29 and had recovered to about 85% of control values by 12 days after exposure. The CFU-GM cells in an agar assay decreased to approximately 40% of control after 30 days of exposure, increased slightly after termination of exposure, decreased to approximately 60% of control value by 30 days, then recovered to almost 80% of control by 80 days. The numbers of granulocytes in the blood also fluctuated during this time.

Influence of exposure protocols. CBA/Ca BNL male mice were exposed either to 316 ppm (1010 mg/m^3) of benzene administered 6 hr/day, 5 days/week for 19 exposures or to 3000 ppm (9584 mg/m^3) of benzene administered 6 hr/day for 2 successive days (Cronkite et al., 1989). After 19 days, 316 ppm (1010 mg/m^3) of benzene had reduced lymphocyte counts from 7500/µL to 300/µL, and 3000 ppm (9584 mg/m^3) for 2 days had resulted in a reduction from 6700/µL to 3300/µL. The differences in the effects of the two regimens persisted for 214 days after exposure. Neutrophil counts of the group exposed to 316 ppm (1010 mg/m^3) were also depressed, more than those exposed to 3000 ppm (9584 mg/m^3). By 214 days after exposure, the neutrophil counts in the group treated with 3000 ppm (9584 mg/m^3) had recovered, while those in the group treated with 300 ppm (958 mg/m^3) had not. All cell types were decreased in number compared with sham-exposed mice on day 1, and all absolute counts were depressed on 32, 67, and 214 days after the last exposure. These data were not given for the animals exposed to 3000 ppm (9584 mg/m^3).

On day 1 after exposure, both treatments had induced a reduction in bone marrow cellularity; this was significantly greater in the animals exposed to 316 ppm (1010 mg/m^3) for 19 days (depression was ~31% of control value) than in those exposed to 3000 ppm (9584 mg/m^3) for 2 days (depression was ~53% of control value). Cellularity had returned to control values by 32, 67, and 214 days after exposure. The CFU-S values followed the same response pattern: except by day 214 after exposure, recovery was incomplete for the group exposed to 3000 ppm (9584 mg/m^3).

In another experiment, exposure to 300 ppm (958 mg/m^3) for 80 weeks produced a severe depression in CFU-S (to 40% of control) that had not completely recovered 180 days after exposure ended. Exposure to 3000 ppm (9584 mg/m^3) for 8 weeks produced a mild depression in CFU-S that had recovered by 60 days. The inhalation of 3000 ppm (9584 mg/m^3) did not increase the incidence of leukemia or shorten its latency; however, 300 ppm ([958 mg/m^3] 6 hr/day for 16 weeks) significantly increased the incidence of myelogenous neoplasms.

Rescue assay. The injection of normal bone marrow cells into lethally irradiated rodents enhances their survival. The serial rescue assay tested the effect of benzene on this particular function of the bone marrow. C57BL/6BNL male and female mice were irradiated with 850 rad,

then injected with bone marrow cells from mice that had been exposed to 300 ppm (958 mg/m^3) of benzene 6 hr/day, 5 days/week for 16 weeks. Thirty days after the initial transplants some of the survivors' marrow was injected into another group of irradiated mice for a secondary transplant, and 30 days later those recipients served as donors in the third transplant to still another group of irradiated mice. The recipients of bone marrow in each transplantation of the series were observed for up to 594 days. In the first rescue, the 30-day survival was 100%; at 290 days, survival of the mice receiving cells from control mice was 93% and of those receiving cells from benzene-exposed mice, 64%.

In the secondary transplant, no animals died by 30 days, but the animals receiving control cells started dying at day 171 and animals receiving cells from donors exposed to benzene started to die at 70 days. In the tertiary rescue, 50% of the recipients of bone marrow from benzene-exposed donors were dead by 30 days and 95% were dead by day 245. Among the animals receiving cells from the sham-exposed mice, only one died by day 30 and 50% died by day 375. The last animal died on day 594. The mechanism for the stem cell malfunction that led to increased mortality is not clear, but the investigators suggest that the observation could be associated either with genetic injury to the stem cells, exhaustion of the G_0 hematopoietic stem cells, or a radiation effect on the stroma of the recipient.

BDF1 mice were exposed to 0, 100, 300, or 900 ppm benzene (0, 319, 958, or 2875 mg/m^3) for up to 4 weeks (Seidel et al., 1990). The numbers of hematopoietic progenitor cells, early and late progenitors (BFU-E, CFU-E), and granuloid progenitors (CFU-C) were determined. A group was generated to establish the effect of ethanol (drinking water) on these effects. This study demonstrated that benzene decreased the number of CFU-E per femur in a concentration-dependent manner. This effect was evident at 300 and 900 ppm (958 and 2875 mg/m^3) concentration, however, the effect of the 100 ppm (319 mg/m^3) exposure group was uncertain, as the study focused on the effects of ethanol on benzene toxicity. The LOAEL/NOAEL was thus difficult to determine.

Male Sprague-Dawley rats (40/group) were exposed to vapor concentrations of 0 or 100 ppm benzene (0 or 319 mg/m^3), 6 hr/day, 5 days/week, for life (American Petroleum Institute, 1983). Blood samples were obtained at 2–4 week intervals throughout the treatment period. The erythrocyte and lymphocyte counts were depressed at nearly every sampling time in treated rats, but the extent of decrease was not statistically significant (p<0.05). Significantly increased incidences of splenic hyperplasia (p<0.005) and hemosiderin pigments (p<0.001) were observed in benzene-exposed rats. The incidence of normally rare tumors in treated rats were liver (4/40), Zymbal gland (2/40), and chronic myelogenous leukemia (1/40); the authors considered these tumors to be related to the benzene exposure. This study identifies a NOAEL of 100 ppm (319 mg/m^3) for hematological effects in rats. The observed decreases were not statistically significant and were therefore considered not to be toxicologically significant.

Decreases in bone marrow cellularity in the femur, hematocrit, and leukocytes were seen in DBA/2 mice (20/group) exposed to 300 ppm (958 mg/m^3) benzene in air for 6 hr/day, 5 days/week for 2 weeks (Chertkov et al., 1992). In most cases no erythroid or myeloid clonogenic cells could be recovered in bone marrow cultures started after the last benzene exposure. After 2

weeks of recovery, however, body weight, hematocrit, bone marrow cellularity, and committed hematopoietic progenitor cells had recovered to near normal values.

Neun et al. (1992, 1994) compared the in vivo sensitivity of Swiss-Webster and C57BL/6J mice to inhaled benzene with the in vitro sensitivity of culture bone marrow cells to benzene metabolites. Mice were exposed to benzene, 300 ppm (958 mg/m^3), 6 hr/day, 4 days/week for 2 weeks. Swiss Webster mice were more sensitive to benzene exposure as indicated by much greater reductions in femoral bone marrow cellularity and in the number of CFU-E per femur after in vivo exposure. Neither phenol nor muconic acid were toxic to cultured CFU-E from either mouse strain. CFU-E from Swiss-Webster mice were more sensitive to 1,4-benzoquinone or hydroquinone than CFU-E from C57B1/J6 mice. Thus, both in vitro and in vivo data indicate that Swiss-Webster mice were more sensitive to benzene toxicity.

Female BDF1 mice (C57BL/6xDBA/2F1 hybrids) were exposed to 0, 100, 300, or 900 ppm (0, 319, 958, or 2875 mg/m^3) benzene 6 hr/day, 5 days/week for up to 8 weeks (Plappert et al., 1994). Hematological studies included peripheral blood data, T4 and T8 lymphocytes counts in the blood and spleen, and hematopoietic stem and progenitor cell assays in the marrow CFU-S, CFU-C, and BFU-E, EFU-E. No significant changes were observed in the peripheral blood data of mice exposed to 900 ppm (2875 mg/m^3) benzene. Some perturbation of the reticulocyte numbers was observed, but values at days 3 and 5 did not differ from controls. Absolute numbers of lymphocytes and neutrophils did not differ from controls (no data shown). Slight anemia was observed at 4 and 8 weeks of treatment with 300 and 900 ppm (958 and 2875 mg/m^3) benzene. Minor changes occurred in the stem and progenitor cells. CFU-E depression after 4 days of exposure was significant. A dose-dependent depression of colony forming cell number appeared at 4 and 8 weeks of exposure with maximal effect at the level of CFU-E.

Farris et al. (1997a) exposed male B6C3F1 mice to 0, 1, 10, 100, or 200 ppm (0, 3.2, 32, 319, or 639 mg/m^3) benzene 6 hr/day, 5 days/week for 1, 2, 4, or 8 weeks. A separate 4-week experiment at benzene concentrations of 0, 1, 5, and 10 ppm (0, 3.2, 16, and 32 mg/m^3) was also conducted. Another group of animals was exposed to benzene for 4 weeks and then allowed to recover for up to 25 days. Bone marrow cell counts, CFU-HPP, percentage of CFU-HPP cells in S-phase, bone marrow CFU-E, bone marrow CFU-GM, labeling index, B- and T-lymphocytes, and hematology parameters were determined. Data sets for each parameter were individually evaluated with a univariate two-way ANOVA using contrasts to determine treatment effect. Statistical significance was determined at a level of $p<0.05$.

There were no significant effects on hematopoietic parameters from exposure to 10 ppm (320 mg/m^3) benzene or less. Exposure of mice to 100 or 200 ppm (320 or 640 mg/m^3) reduced the number of total bone marrow cells, progenitor cells, differentiating hematopoietic cells, and most blood parameters with a concentration-related effect. Replication of primitive progenitor cells in the bone marrow was increased during the exposure period, possibly in compensation for benzene cytotoxicity. Recovery after benzene exposure was rapid. Most hematopoietic parameters returned to control levels within 4 days following the 100 ppm (319 mg/m^3) exposure and within 11 days following exposure to 200 ppm (639 mg/m^3) benzene. The percentage of CFU-HPP in S-phase was elevated throughout the 25-day recovery period evaluated. Farris et al. (1996) reported that the frequency of micronucleated erythrocytes was increased in B6C3F1 male

mice exposed to 100 and 200 ppm (319 and 639 mg/m^3) benzene for 8 weeks. The authors suggested that the increased proliferation of primitive progenitor cells in concert with genetic damage provides the components for producing an increased incidence of lymphoma in mice. The effects on hematopoietic parameters indicate a LOAEL of 100 ppm (319 mg/m^3) and a NOAEL of 10 ppm (32 mg/m^3).

Farris et al. (1997b) exposed male B6C3F1 mice to 0, 1, 10, 100, or 200 ppm (0, 3.2, 32, 319, or 639 mg/m^3) benzene 6 hr/day, 5 days/week for 1, 2, 4, or 8 weeks. A separate 4-week experiment at benzene concentrations of 0, 1, 5, and 10 ppm (0, 3.2, 16, and 32 mg/m^3) was also conducted. Spleen and thymic lymphocyte counts were determined for three mice in each treatment. Femoral B-lymphocyte counts were evaluated in 10 mice per group. Labeling index of bone marrow B-lymphocytes was determined by BrdU incorporation. To minimize the potential for recovery, mice were sacrificed and sampled within 2 hr of the end of the last exposure. The spleen, thymus, and bone marrow data sets were individually evaluated with a univariate two-way ANOVA using contrasts to determine treatment effect within each time point. Statistical significance was determined at a level of 0.05.

Exposure to 100 or 200 ppm (319 or 639 mg/m^3) benzene induced rapid and persistent reductions in femoral B-, splenic T- and B-, and thymic T-lymphocytes; total nucleated thymus cells count; and total nucleated spleen cells. There was a significant decrease in thymic cell count and B-lymphocytes in the spleen at week 2 in mice exposed to 10 ppm (32 mg/m^3) in the 8-week study and no effects at 1 ppm (3.2 mg/m^3). These parameters, however, were comparable to the controls by week 4 of the 8-week exposure experiment, and in the separate 4-week experiment there were no significant decreases in these parameters at any time in the mice exposed to 10 ppm (32 mg/m^3) benzene or less. Thus, the 100 ppm (319 mg/m^3) level was considered a LOAEL and 10 ppm (32 mg/m^3) a NOAEL for these effects. The percentage of femoral B-lymphocytes and thymic T-lymphocytes in apoptosis was increased 6- to 15-fold by 200 ppm (639 mg/m^3). Replication of femoral B-lymphocytes was increased during the exposure period in the bone marrow apparently in compensation for the lymphocyte loss induced by 100 or 200 ppm (319 or 639 mg/m^3) benzene exposure.

Male Sprague-Dawley rats (16/group) were exposed to 0, 30, 200, or 400 ppm (0, 96, 639, or 1278 mg/m^3) benzene for 6 hr/day, 5 days/week for either 2 or 4 weeks. Of the 16 animals in each group, 8 were challenged intravenously with sheep red blood cells (SRBC) at 4 days before the end of the exposure to allow an assessment of humoral immunocompetence using the enzyme linked immunosorbent assay technique (Robinson et al., 1997). Thymus and spleen weights were determined and total cell counts were determined for the spleen and femur. Spleen $CD4^+/CD5^+$, $CD8^-/CD5^+$, and $Kappa^+$ lymphocytes were determined by immunostaining. Statistical evaluations were performed using Dunnett's test.

Total spleen cell counts were significantly reduced (29%) in animals exposed to 400 ppm (1278 mg/m^3) after 4 weeks, and thymus weight was also significantly reduced (28%). The spleen weight and cellularity were comparable to the controls at both 2 and 4 weeks in the 200 ppm (639 mg/m^3) exposure group. After 4 weeks at 400 ppm (1278 mg/m^3) there was a significant reduction in spleen B-, $CD4^+/CD5^-$, and $CD5^+$ T-lymphocytes. Rats exposed to 30, 200, or 400 ppm (99, 639, or 1278 mg/m^3) benzene for 2 or 4 weeks and then challenged with SRBC

developed a humoral response comparable to the controls, and only rats exposed to 400 ppm (1278 mg/m^3) for 2 weeks showed a significant reduction in spleen B-lymphocytes. These results indicate that 400 ppm (1278 mg/m^3) is a LOAEL and 200 ppm (639 mg/m^3) is a NOAEL for immunotoxicity in rats.

4.2.3. Other Routes of Exposure

Studies using parenterally administered benzene are not relevant to human exposure, which occurs most often by inhalation; however, below, certain of these studies with benzene and its metabolites are considered briefly because of their value in the study of the mechanisms of benzene toxicity.

Tunek et al. (1982) examined the hematotoxicity of benzene and two of its phenolic metabolites, hydroquinone and catechol, in male NMRI mice. Daily doses of 440 mg/kg of benzene to body weight were injected subcutaneously on 1–6 days and CFU-C/tibia, cells/tibia, and micronuclei/2000 PCE were assayed on the day after the last injection. Micronuclei appeared after one injection, peaked after three injections, then decreased with injections four, five, and six. The investigators presumed that the numbers of micronuclei decreased as a result of toxicity and cell death before the cells reached the PCE stage. By day 4, the CFU-C/tibia and number of cells/tibia were reduced to about 5%–10% of control values.

In another experiment, the animals received doses ranging from 0.7–440 mg of benzene/kg/day on 6 consecutive days and were killed on day 7. At doses of 28 to ~55 mg/kg, micronuclei were 10%–20% of controls. Doses higher than 55 mg/kg gradually increased the micronuclei until they were >100% of control at the highest dose. CFU-C/tibia and bone marrow cellularity were suppressed by about 40% at doses as low as 3.5 mg/kg. At 100–440 mg/kg both values dropped to 5%–10% of control values.

Six injected doses of hydroquinone ranging from 20–100 mg/kg produced micronuclei, with a sharp increase occurring at 6 × 80 mg/kg. Bone marrow cellularity was slightly elevated at low doses and was suppressed at higher doses, with the number of CFU-C following a similar pattern. Catechol, injected at doses ranging from 5–42 mg/kg for 6 consecutive days, had no effect on any of the three parameters examined. The toxic effects of benzene were alleviated by ~30%–40% when toluene (which competitively inhibits benzene metabolism) was injected simultaneously. The toxic effects of hydroquinone were somewhat reduced by simultaneous administration of toluene, but not nearly to the extent observed with benzene. The investigators calculated that a dose of 440 mg/kg of benzene would yield an excretion of ~20 mg/kg of hydroquinone. However, 20 mg/kg of hydroquinone was virtually nontoxic, whereas 440 mg/kg of benzene produced severe hematological effects. Based on these differences and the differences in the sequence of the responses to the two chemicals, Tunek et al. speculated that the hematotoxicity of benzene at low doses is due to agents other than hydroquinone, but that effects at and above the threshold dose result from the metabolic formation and accumulation of hydroquinone.

MacEachern et al. (1992) evaluated the effects of benzene on the morphology and function of bone marrow phagocytes. Male Balb/c mice received intraperitoneal injections of either benzene (660 mg/kg) or a combination of hydroquinone and phenol (50 mg/kg each) once per day for 3 days. Control animals received corn oil or phosphate-buffered saline. The animals were killed after the last injection and the following assays were performed: characterization and quantitation of subpopulations of bone marrow cells using monoclonal antibody techniques, measurement of chemotaxis (the process by which activated phagocytes migrate to an injured site in a tissue), and measurement of the oxidative metabolism of phagocytes (to evaluate maturation and activation of phagocytes). The bone marrow contained three distinct populations of cells: (population 1) a larger more dense population (41%), (population 2) a population of intermediate size and density (23%), and (population 3) a smaller, less dense population (33%). Population 1 consisted of 85%–90% granulocytes, including neutrophils, basophils, and eosinophils; population 2 contained a mixture of mononuclear phagocytes (35%–40%) and immature precursor cells (55%–65%); and population 3 contained lymphocytes (86%) and immature precursor cells (14%). This pattern of distribution is similar to that observed for human peripheral blood leukocytes and bone marrow cells (Landay and Bauer, 1988; Lund-Johansen et al., 1990, both cited in MacEachern et al., 1992). Differential staining of sorted mononuclear phagocytes revealed an increase in the number of mature, morphologically activated macrophages in the bone marrow of benzene-treated mice.

Granulocytes and mature macrophages were the only cells to migrate toward chemoattractants C5a or TPA, respectively, and cells from benzene-treated mice exhibited increased chemotactic activity, compared with controls ($p \leq 0.02$). The migration of bone marrow granulocytes from mice treated with hydroquinone and phenol toward C5a was depressed ($p \leq 0.02$) whereas the migration of the macrophages from these mice toward TPA was enhanced ($p < 0.02$).

Benzene treatment also induced a significant increase in basal oxidative metabolism in bone marrow granulocytes (population 1), but treatment with phenol and hydroquinone did not. The investigators concluded that phagocytes and granulocytes from the bone marrow of benzene-treated mice are activated and stimulated to differentiate. Further studies are in progress to determine whether these phagocytes or their mediators contribute to the development of benzene hematotoxicity.

Groups of four C57BL/6 male mice were given two daily doses of hydroquinone (100 mg/kg) or catechol (75 mg/kg), either intravenously or i.p., for 3 consecutive days (Wiedra and Irons, 1982). Spleen and bone marrow cells were collected for culture 1 day later, and effects on the development of polyclonal, plaque-forming cells (PC-PFC) from progenitor B-lymphocytes in response to challenge from LPS or dextran sulfate were evaluated. Both benzene metabolites were cytotoxic to spleen cells, and hydroquinone also reduced bone marrow cellularity. Both metabolites reduce the frequency of PC-PFC, but only catechol selectively inhibited maturation of LPS-activated marrow progenitors.

Eastmond et al. (1987) demonstrated that coadministration of 75 mg/kg of phenol and 25–75 mg/kg of hydroquinone 2 times/day for 12 days i.p. to mice resulted in a significant, dose-

related depression in bone marrow cellularity; the compounds alone produced neither a significant nor a dose-related response; catechol had no effect, either alone or in combination.

Hydroquinone (10 mg/kg-day), benzoquinone (2 mg/kg-day), or benzenetriol (6.25 mg/kg-day) administered i.p. to rats produced significant decreases in bone marrow cell counts, RBC, and Hgb (Rao et al., 1988); *trans,trans*-mucondialdehyde administered to CD-1 mice i.p. daily for 10 and 16 days produced hematotoxicity similar to that of benzene (Witz et al., 1985); and benzene metabolites added to macrophage cultures significantly and selectively inhibited macrophage function (Lewis et al., 1988).

Using an iron-uptake method, Snyder et al. (1989) demonstrated that i.p. administration of benzene, hydroquinone, *p*-benzoquinone, and muconaldehyde, singly and in combination with each other, inhibited erythropoiesis in female mice. The combination of hydroquinone plus muconaldehyde was most effective in decreasing iron uptake. Toluene alleviated the effects of benzene, but not the effects of hydroquinone or *p*-benzoquinone.

Irons et al. (1992) observed an enhanced colony-forming response by mouse bone marrow cells treated in vitro, first with hydroquinone and then with recombinant granulocyte/macrophage colony-stimulating factor. However, treatment with phenol, catechol, or *trans,trans*-muconaldehyde plus the colony-stimulating factor did not enhance colony formation. The combination of hydroquinone and the stimulating factor also appeared to recruit a segment of the myeloid progenitor cell population that was not normally responsive to the stimulating factor. These alterations in the differentiation of the myeloid progenitor cell population may be relevant in the pathogenesis of chemically-induced acute myelogenous leukemia.

Gaido and Wierda (1984) demonstrated that in vitro treatment with hydroquinone and *p*-benzoquinone decreased the ability of stromal cells to support granulocyte/macrophage colony formation; catechol and 1,2,4-benzotriol inhibited colony formation, but only at very high concentrations.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES

4.3.1. Reproductive Toxicity

Multiple-generation reproductive toxicity studies of benzene were not found in the available literature. Information pertinent to the reproductive toxicity of benzene in animals consists of the results of subchronic and chronic general toxicity studies, one dominant lethality study, and two female fertility studies.

4.3.1.1. *Oral Exposure*

The National Toxicology Program (NTP, 1986) evaluated the 90-day and 2-year toxicity of benzene in mice and rats. Other than ovarian effects, there were no signs of reproductive toxicity. Benzene was administered by gavage to B6C3F1 mice and F344/N rats. In the 90-day

study, the doses of benzene, administered in corn oil 5 days per week, ranged from 25–600 mg/kg; in the 2-year study, doses were 50, 100, or 200 mg/kg for male rats and 25, 50, or 100 mg/kg for female rats and all mice. There were no ovarian effects in either the rats or mice treated for 90 days or in the rats treated for 2 years; mice treated for 2 years exhibited ovarian lesions ranging from atrophy to neoplasia. The incidence of nonneoplastic lesions was definitely increased but were not dose-related. The nonneoplastic lesions, and their incidence, based on the total number of animals examined for each group, for mice administered 0, 50, 100, or 200 mg/kg of benzene were ovarian atrophy (32%, 79%, 65%, and 46%) and epithelial hyperplasia (26%, 89%, 63%, and 60%). The lack of a dose response, in addition to the use of the oral route of exposure (instead of inhalation, the most common route of human exposure), precludes the use of these data for quantitative risk assessment.

Spano et al. (1989) examined the cytotoxic action of benzene on mouse germ cells using flow cytometric DNA content measurements. Testicular monocellular suspensions were obtained from (C57BL/Cne × C3H/Cne) F1 male mice receiving single doses, by gavage, of 0, 1, 2, 4, 6, or 7 mL/kg of benzene to body weight. The effects were measured in three animals per group 7, 14, 21, 28, and 70 days after treatment. Testicular cells can be classified as mature haploid, immature haploid (haploid is split into two parts because of different staining on the elongated and round spermatids), diploid, or tetraploid, depending on DNA content.

Treatment did not affect body weight or testes weight, but did alter the ratio of testicular cell types. DNA histograms of mouse testis cells obtained at different times after benzene exposures showed a dose-related decrease in the tetraploid cell fraction (mainly primary spermatocytes); 7 days after treatment the tetraploid cell number was depressed to ~80% of control values in animals exposed to 6 and 7 mL/kg of benzene. The percentage of round spermatids was also decreased to ~80% of control values in animals exposed to 7 mL/kg of benzene; however, in this case the dose relationship was not distinct. These effects indicate cytotoxicity of differentiating spermatogonia. Dose-dependent recovery processes for the tetraploid cells had begun 21 days after treatment, simultaneously with reduction of other cell subpopulations, but was still incomplete by 70 days. Recovery of the round spermatids began by day 28 post treatment and was complete by day 70. There was also a time and exposure-dependent reduction (to 60% of control values for the 4, 6, and 7 mL/kg doses) in the percentage of elongated spermatids at 28 days after treatment; recovery was complete by 70 days. The investigators conclude from this study that benzene can induce acute cytotoxicity in mouse germ cells. The Spano et al. (1989) study is not suitable for quantitative risk analysis because it is an acute study and was conducted by the oral route; however, both the NTP (1986) and Spano et al. (1989) studies provide valuable descriptive information regarding the effects of benzene on the reproductive system.

4.3.1.2. *Inhalation Exposure*

Subchronic toxicity studies have provided information relevant to the reproductive toxicity of benzene. In an early inhalation study, Wolf et al. (1956) demonstrated moderately increased testicular weights in groups of 10–25 rats exposed to 6600 ppm (21,084 mg/m^3) of benzene for 13 weeks, but not in rats exposed to 88 ppm ([281 mg/m^3] for 30 weeks), 2200 ppm ([7028

mg/m^3] for 30 weeks), 4400 ppm ([14,056 mg/m^3] for 5 weeks), or 9400 ppm ([30,030 mg/m^3] over 1–19 days). Groups of 5–10 male guinea pigs exposed to 88 ppm (281 mg/m^3) of benzene for ~9.6 months had a slight increase in testicular weights, but guinea pigs exposed to 88 ppm (281 mg/m^3) for 4 weeks did not. Groups of 1–2 rabbits exposed to benzene concentrations of 88 ppm (281 mg/m^3) for ~8.5 months had slight histopathological changes in the testes, described by the authors as degeneration of the seminiferous tubules (no other concentrations were tested in rabbits). Exposures were routinely 7 to 8 hr/day, 5 days/week. Air-exposed or unexposed animals served as controls. The vapor concentrations were maintained within 10% of the desired concentration. No further details were given for the results of this study, and quantitation was not possible. Although the study was not especially designed to detect reproductive effects, the gonadal effects noted did stimulate further interest in the potential reproductive effects of benzene.

Ward et al. (1985) exposed male and female CD-1 mice and Sprague-Dawley rats to benzene concentrations of 1, 10, 30, or 300 ppm (3.2, 32, 96, or 958 mg/m^3) 6 hr/day, 5 days/week for 13 weeks. Animals exposed to filtered air served as controls. Groups of 10 males and 10 females per species were sacrificed after 7, 14, 28, 56, and 91 days of treatment. Endpoints of the investigation that were relevant to reproductive toxicity included body and organ weights and gross and microscopic pathology. Organs were preserved in 10% neutral buffered formalin. Body weight of both species was unaffected by exposure. The mice exposed to 300 ppm (958 mg/m^3) of benzene exhibited statistically significant decreases in testes weights at days 28, 56, and 91, and decreased testes/body weight ratios on days 56 and 91 (data not given). Testicular weight for the rats was comparable to control values. The mice exposed to 300 ppm (958 mg/m^3) for 91 days had testicular lesions that included minimal to moderately severe bilateral atrophy and degeneration (7/10 mice), moderate to moderately severe decreases in spermatozoa in the epididymal ducts (6/10 mice), and minimal to moderate increases in abnormal sperm forms (9/10). Four of 10 female mice had bilateral ovarian cysts after 91 days of exposure to 300 ppm (958 mg/m^3). These testicular and ovarian lesions were not observed at earlier sacrifices, but similar lesions (considered by the investigators to be of doubtful biological significance) did appear at lower concentrations. Microscopic findings in the control animals were not mentioned. The rats had no signs of reproductive toxicity; however, hematotoxicity occurred at 300 ppm (958 mg/m^3), giving the overall study a LOAEL of 300 ppm (958 mg/m^3) and a NOAEL of 30 ppm (96 mg/m^3). The experiment appeared to be a carefully performed subchronic toxicity study, and the appearance of microscopic changes in the testes and ovaries of the 300 ppm (958 mg/m^3) group and similar, but only occasional, findings at 10 and 30 ppm groups suggest a concentration response; however, the 10-fold difference between the NOAEL and the LOAEL could cause an underestimation of the NOAEL, thus compromising quantitative risk assessment. The available studies on the reproductive toxicity of inhaled benzene to test animal species is summarized in Table 4-5.

Kuna et al. (1992) assessed the effects of benzene on female fertility in Sprague-Dawley rats. The study tested occupational exposure levels, as well as higher exposures that previously demonstrated developmental toxicity. Groups of 26 Sprague-Dawley female rats inhaled benzene vapor concentrations of 0, 1, 30, and 300 ppm ([0, 3.2, 96, and 958 mg/m^3] benzene purity, 99.96%) 6 hr/day 5 days/week for a 10-week premating and mating period, and daily from gestation days (GD) 0 to 20 and lactation days 5 to 20. Daily vaginal smears were examined to

Table 4-5. Reproductive toxicity of inhaled benzene in test animals

Species	No./group	Treatment	Reproductive effects	LOAEL/NOAEL (mg/m^3)	Reference
Male rat	10–25	21,084 mg/m^3, 7 hr/day, 5 days/wk for 13 wk	Moderate changes in testes weight (no quantitative data)	LOAEL: NA[a] NOAEL: NA	Wolf et al., 1956
Rabbit	1–2	256 mg/m^3, 7 hr/day, 5 day/wk for ~8.5 mo.	Slight change in histopathology of the testes (no details)	LOAEL: NA NOAEL: NA	Wolf et al., 1956
Male guinea pig	5–10	281 mg/m^3, 7 hr/day, 5 days/wk for ~9.6 mo.	Slight change in weight of testes (no quantitative data)	LOAEL: NA NOAEL: NA	Wolf et al., 1956
Male and female Sprague-Dawley rat	50/sex	0, 3.2, 32, 96, 958 mg/m^3 6 hr/day, 5 days/wk for 13 wk; 10/sex/group sacrificed after 7, 14, 28, 56, and 91 days of treatment	No microscopic lesions of ovaries and testes	LOAEL: 958[b] NOAEL: 96	Ward et al., 1985
CD-1 mouse	150/sex	0, 3.2, 32, 96, 958 mg/m^3 6 hr/day, 5 days/wk for 13 wk; 30/sex/group sacrificed after 7, 14, 28, 56, and 91 days of treatment	Cystic ovaries (4/10), testicular atrophy (7/10), decreased sperm count (6/10), and increased abnormal sperm forms (9/10) only at 958 mg/m^3 for 91 days	LOAEL: 958 NOAEL: 96	Ward et al., 1985
Female Sprague-Dawley rat	26	0, 3.2, 32, 96, 958 mg/m^3 6 hr/day, 5 days/wk for 10-wk period before and during mating, during gestation to GD 20, and from day 5 to day 21 of lactation	Reduction in body and liver weight in the female pups at 958 mg/m^3 ($p<0.05$); no effect on maternal mortality, body weight, physical parameters, pregnancy rate, length of gestation, number of live and dead pups at birth, and sex distribution data; no effect on pup survival and growth, and gross postmortem manifestations	LOAEL: 958 NOAEL: 96	Kuna et al., 1992

[a]ND = no determined, data not sufficient.
NA = not applicable, only one concentration tested.
[b]Effects on hematological parameters place the LOAEL for the overall study in rats at 958 mg/m^3, the NOAEL at 96 mg/m^3.

determine whether estrus was affected by treatment. Parameters of maternal toxicity (statistical tests used for a particular parameter are in parentheses) were monitored throughout the study. These included body weight changes (one-way ANOVA), mortality, and gross clinical signs. Physical examinations were performed weekly throughout the study. Dams and pups were sacrificed on day 21 of lactation, and those and any others that died before the end of the study were examined for gross effects. On day 4 of lactation, litters of more than 10 pups were randomly culled to 10 with an equal number per gender where possible.

The reproductive/developmental parameters evaluated (and statistical tests used) included percentage pregnant (Chi square), number pregnant/number mated, number of litters, length of gestation (one-way ANOVA), number of pups/litter (one-way ANOVA), gender ratio, mean postimplantation loss, viability index (Chi square), number of pups alive at 4 days pre-cull/number pups born alive, lactation index (Chi square), number pups alive at 21 days/number alive at 4 days post-cull, and body and organ weights of the pups (nested design).

Neither strain had any treatment-related effects as evidenced by data on maternal mortality, body weight, physical parameters, pregnancy rate, length of gestation, number of live and dead pups at birth, and sex distribution data. No effect was observed on pup survival and growth, or on gross postmortem findings. In the Sprague-Dawley rats there was a trend toward reduced body and organ weights in the 21-day-old pups exposed to 30 and 300 ppm (96 and 958 mg/m^3) levels; however, the differences were statistically significant ($p<0.05$) only for reduced body weight (89% of control value) and liver weight (80% of control value) in the female pups at 300 ppm (958 mg/m^3). Increases in relative kidney weight for the female Sprague-Dawley pups were attributed to differences in body weight. The CD rats exhibited no such changes. The investigators concluded that benzene at concentrations as high as 300 ppm (958 mg/m^3) did not induce reproductive toxicity in CD rats. The study in CD rats does not identify a LOAEL or NOAEL. The study in Sprague-Dawley rats identifies a LOAEL of 300 ppm (958 mg/m^3) and a NOAEL of 30 ppm (96 mg/m^3), based on the statistically significant reduction in body and liver weights, and based on past studies showing similar effects at similar concentrations of benzene.

4.3.2. Developmental Toxicity

4.3.2.1. *Oral Exposure*

Seidenberg et al. (1986) evaluated benzene, along with 54 other chemicals of known and unknown developmental toxicity potential, to validate the Chernoff/Kavlock assay as a screen for developmental effects. Benzene doses of 1300 mg/kg-day were administered in cottonseed oil by gavage to pregnant ICR/SIM mice on GD 8 through 12, and the dams were allowed to deliver. Maternal toxicity was assessed using body weights, mortality, or other clinical signs of overt toxicity as endpoints. Fetuses were counted, weighed, and examined on the day of birth. A toxic response was defined as a decrease in fetal body weight or an increase in the incidence of minor skeletal anomalies. Benzene had no effect on maternal body weight, but did produce significantly lower neonatal body weight (to ~95% of control value, $p<0.05$), as measured on days 1 and 2 after birth. This is a screening study that used only one dose and therefore does not provide dose-response information; also the oral dose does not correspond to the most common route of human

exposure, inhalation. However, the study does demonstrate weak fetal toxicity in the absence of observable maternal toxicity.

Exxon Chemical Company (1986) treated bred female Sprague-Dawley rats (20–22/group) by gavage with 0, 50, 250, 500, or 1000 mg/kg-day on GD 6–15. No dose-related mortality was observed. Significant ($p<0.05$) findings in the treated dams as compared with controls were decreased food consumption at ≥250 mg/kg, decreased body weight, body weight gains at ≥500 mg/kg, and increased incidence of alopecia at 1000 mg/kg. Developmental toxicity was limited to decreased ($p<0.05$) fetal body weights at ≥500 mg/kg. Fetuses were examined only for external malformations, not for skeletal and visceral malformations. This study identified a NOAEL of 50 mg/kg-day and a LOAEL of 250 mg/kg-day for maternal toxicity and NOAEL of 250 mg/kg-day and LOAEL of 500 mg/kg-day for developmental toxicity in Sprague-Dawley rats.

4.3.2.2. *Inhalation Exposure*

In a review of some of the earlier, mostly unpublished developmental toxicity studies on benzene, Brief et al. (1980) observed that the developmental effects in animals were characterized mainly by fetal toxicity in rats exposed to 40, 50, 500, and 2200 ppm (128, 159, 1597, and 7028 mg/m^3) (Dow Chemical Co., 1992b; Green et al., 1978; Litton Bionetics, 1977). Maternal toxicity and some fetal malformations occurred at 500 ppm (1597 mg/m^3); concentrations of 10 ppm (32 mg/m^3) produced conflicting results (Brief et al., 1980).

This section summarizes the data for the developmental toxicity of inhaled benzene in animals. The studies included experiments with three species (rats, mice, and rabbits) and intermittent (6 or 7 hr/day) and continuous (24 hr/day) exposures. Some studies tested more than one concentration of benzene and demonstrated a concentration response and LOAEL and/or NOAEL; some studies used only one concentration and were not useful for determining dose-response relationships, but they did provide supporting evidence for fetal toxicity; three studies evaluated fetal hematological parameters; and one study examined the possible synergistic effects of benzene with other chemicals. The available studies on the developmental toxicity of inhaled benzene are summarized in Table 4-6.

Kuna and Kapp (1981) exposed pregnant Sprague-Dawley rats (14–15/group) to benzene concentrations of 0, 10, 50, or 500 ppm (0, 32, 160, or 1597 mg/m^3) 7 hr/day on GD 6–15 and killed the dams on day 20 for the evaluation of maternal and developmental effects. Eleven pregnant control animals were exposed to air. Mean body weights and mean body weight gains were determined for the dams for GD 0–5, 5–15, 15–20, and 0–20. Hematologic evaluation of the dams, performed on GD 5 and 20, included RBC, WBC, and differential counts. Fetal observations per litter included numbers of corpora lutea, resorption sites, and live and dead fetuses. Fetal weight, crown–rump distance, and sex were noted. One-third of the fetuses were processed for visceral changes, while two-thirds were prepared for skeletal examination. Tests used to determine statistical significance of changes in body weights and lengths were the F test, Student's *t* test, Cochran's approximation of t (t'), analysis of covariance, and the method of Gomes and Howell. Analysis of the reproduction indices and the number of fetal variants of the

Table 4-6. Developmental toxicity of inhaled benzene in test animals

Strain/species	No. of dams/group	Exposure	Effects		Effect levels maternal/developmental (mg/m^3)	Reference
			Maternal	Developmental		
Sprague-Dawley rat	14–15 exposed 11 controls	0, 32, 160, or 1597 mg/m^3, 7 hr/day on GD[b] 6–15; sacrifice on day 20	Decreased body weight and body weight gain at 160 and 1597 mg/m^3, day 5–15; dose-related[c]	Decreased mean live body weight at 160 and 1597 mg/m^3, day 20[d]; decreased crown–rump distance, 1597 mg/m^3 [d]; skeletal and visceral (brain) abnormalities at 160 and 1597 mg/m^3, increased incidence of malformations at 1597 mg/m^3	LOAEL: 160/160 NOAEL: 32/32	Kuna and Kapp, 1981
Sprague-Dawley rat	35–37 exposed 32–34 controls	0, 3.2, 32, 128, or 319 mg/m^3, 6 hr/day on GD 6–15; sacrifice on day 20	None in any group	Decreased body weight at 319 mg/m^3 ($p \leq 0.05$); variants in all but one group (including controls), not dose-related; no increase in incidence of malformations	LOAEL: NA/319 NOAEL: NA/128	Coate et al., 1984
Sprague-Dawley rat	14–18	0, 319, 958, or 7028 mg/m^3, 6 hr/day on GD 6–15; killed on day 21	Decreased body weight gain at 7028 mg/m^3 ($p < 0.01$)	Decreased body weight and length at 7028 mg/m^3 ($p < 0.05$); increased skeletal variants all exposure groups ($p < 0.05$ at 319 and 7028 mg/m^3; females more sensitive than males); no increase in incidence of malformations	LOAEL: 7,028/319 NOAEL: 958/NA	Green et al., 1978
Sprague-Dawley rat	No data	32 or 128 mg/m^3, 6 hr/day on GD 6–15	None observed	Increased resorptions at 32 and 128 mg/m^3; no increase in incidence of malformations	LOAEL: NA/32[e] NOAEL: 128/NA	Litton Bionetics, 1977
Rat	5–12	1.0–670 mg/m^3 24 hr/day, 10–15 days before mating and throughout pregnancy	No data	Tendency toward decreased litter sizes at 64 mg/m^3; complete absence of litters at 670 mg/m^3	LOAEL: ND NOAEL: ND	Gofmekler, 1968

Table 4-6. Developmental toxicity of inhaled benzene in test animals (continued)

Strain/species	No. of dams/group	Exposure	Effects		Effect levels maternal/developmental (mg/m^3)	Reference
			Maternal	Developmental		
CFY rat	19 exposed 28 controls	0 or 1000 mg/m^3, 24 hr/day on days 9–14 of pregnancy	Decreased body weight gain ($p< 0.01$)	Decreased body weight ($p<0.01$) and growth retardation ($p< 0.05$); retarded skeletal development and increased incidence of extra ribs and fused sternebrae ($p<0.05$ for both); no increase in incidence of malformations	LOAEL: NA NOAEL: NA	Hudak and Ungvary, 1978
CFY rat	20–22 exposed 48 controls	0, 150, 450, 1500, or 3000 mg/m^3, 24 hr/day on GD 7–14; killed on day 21	Decreased body weight gain at ≥150 mg/m^3 ($p<0.001$), somewhat dose-related; liver/body wt. increased ($p<0.05$ or 0.01)	Decreased body weight at ≥150 mg/m^3 ($p<0.001$), increased resorptions and skeletal and weight retardation ($p<0.01$–0.05); effects not dose-related; no increase in incidence of malformations	LOAEL: 150/150 NOAEL: NA	Tatrai et al., 1980
CFY rat	17	400 mg/m^3, 24 hr/day on GD 7–15	Decreased body weight gain ($p<0.001$); increased relative liver wt. ($p<0.05$)	Retarded weight gain ($p< 0.01$); skeletal growth retardation ($p<0.001$)	LOAEL: NA NOAEL: NA	Ungvary, 1985

Table 4-6. Developmental toxicity of inhaled benzene in test animals (continued)

Strain/species	No. of dams/group	Exposure	Effects		Effect levels maternal/developmental (mg/m^3)	Reference
			Maternal	Developmental		
Swiss-Webster mouse	5	16, 32, or 64 mg/m^3, 6 hr/day on GD 6–15	None observed	16-day-old fetus: no effect on hematological parameters 2-day-old neonates: reduced circulating erythroid precursor cells (all concentrations) ($p<0.05$ at 64 mg/m^3); increased hepatic hematopoietic blast cells, lymphocytes, and granulopoietic precursor cells and decreased hepatic erythropoietic precursor cells (all $p<0.05$ at 64 mg/m^3); 6-week-old adult: similar pattern of enhanced granulopoiesis (64 mg/m^3)	LOAEL: NA/64 NOAEL: 64/32	Keller and Snyder, 1988
Swiss-Webster mouse	8	(a) 32 mg/m^3 benzene GD 6–15 (b) 5% ethanol in drinking water ad lib (c) 32 mg/m^3 benzene + 5% ethanol (d) air + distilled water	No data	Bone marrow samples from 6-week-old offspring: protocols (a) and (b) caused changes in CFU-E counts, males only; protocol (c) caused changes in CFU-E counts, females only[f]	LOAEL: NA NOAEL: NA	Corti and Snyder, 1996
CF-1 mouse	35–37	0 or 1597 mg/m^3 ppm, 7 hr/day on GD 6–15; killed on day 18	None	Decreased body weight ($p<0.05$), "significantly" increased skeletal variants of fetuses; no increase in incidence of malformations but was toxic to fetuses	LOAEL: NA NOAEL: NA	Murray et al., 1979

Table 4-6. Developmental toxicity of inhaled benzene in test animals (continued)

Strain/species	No. of dams/group	Exposure	Effects		Effect levels maternal/developmental (mg/m^3)	Reference
			Maternal	Developmental		
CFLP mouse	15 exposed 115 controls	0, 500 or 1000 mg/m^3, 24 hr/day on GD 6–15	Not mentioned	Weight and skeletal retardation, both concentrations ($p< 0.05$), somewhat dose-related	LOAEL: ND/500 NOAEL: NA/NA	Ungvary and Tatrai, 1985
New Zealand rabbit	20	0 or 1597 mg/m^3, 7 hr/day on GD 6–18; killed on day 29	None	Statistically significant decrease in minor skeletal variants, lumbar spurs and proportion with 13 ribs, in exposed fetuses	LOAEL: NA NOAEL: NA	Murray et al., 1979
New Zealand rabbit	11 or 15 exposed 60 controls	0, 500 or 1000 mg/m^3, 24 hr/day on GD 7–20	Decreased weight gain and increased relative liver wt. at 1000 mg/m^3 ($p< 0.05$)	Decreased body weight and increased abortions and skeletal variants at 313 ppm ($p< 0.05$ for all effects)	LOAEL: 1,000/1,000 NOAEL: 500/500	Ungvary and Tatrai, 1985

[a]Conversion factors, 1 ppm = 3.26 mg/m^3; 1 mg/m^3 = 0.31 ppm
[b]GD = gestation day
[c]Statistically different from control as determined by pairwise multiple comparison procedures
[d]Statistically different from control as determined by Cochran's approximation to *t (t')*
[e]ND = not determined; NA = not applicable
[f]Data from abstract; no other details available

control and treated groups was by the chi-square method or by regression analysis. Differences that would occur less than 5% of the time by chance were adopted as statistically significant.

There were no deaths, observable illness, or hematological changes for dams in any exposure group (Kuna and Kapp, 1981). During GD 5 through 15, maternal body weight gains were significantly decreased to 66% of control values in the 50 ppm (160 mg/m^3) group and to 63% of control in the 500 ppm (1597 mg/m^3) group during GD 5 through 15 (p<0.05 for both groups), whereas during GD 15–20, body weights in the dams exposed to 10 ppm (32 mg/m^3) and weight gain in the dams exposed to 10 and 500 ppm (32 and 1597 mg/m^3) were increased. Body weight corrected for gravid uterine weight was not determined. No differences were observed between exposed and control groups in the number of implantation sites per number of ovarian corpora lutea (implantation efficiency); the incidences of resorbed, dead or live fetuses; and the sex distribution. Fetal crown–rump lengths were decreased in the 500 ppm (1597 mg/m^3) group and mean body weight of live fetuses were decreased in both the 50 and 500 ppm (160 and 1597 mg/m^3) groups (control, 4.4±0.6 g; 50 ppm [160 mg/m^3], 3.8±0.7 g; 500 ppm [1597 mg/m^3], 3.6±0.8 g; all statistically significant, p<0.05). One fetus from each of four litters from dams treated with 500 ppm (1597 mg/m^3) of benzene displayed exencephaly (one fetus), angulated ribs (one fetus), or ossification of the forefeet out of sequence (two fetuses); these abnormalities were not observed in control fetuses. This group also exhibited delayed ossification in the skull, vertebral column, rib cage, pelvic girdle and extremities; significantly fewer tail bones than the controls (p<0.05). There was evidence of a dose-related (but not statistically significant) decrease in the mean number of phalanges and metacarpals. In summary, 13 litters and 142 fetuses were examined from the group exposed to 500 ppm (1597 mg/m^3); 30 fetuses from six litters had delayed ossification (p<0.05) and four fetuses from four litters had skeleta variants and abnormalities. In the group exposed to 50 ppm (160 mg/m^3), 125 fetuses from 15 litters were examined; 23 fetuses from six litters had variants (p<0.05). The investigators concluded that the effects observed in this study were benzene-induced and that the chemical toxic to the fetal rat and possibly to the dams at concentrations of 50 and 500 ppm (160 and 1597 mg/m^3) and is "teratogenic" (authors' description) at 500 ppm (1597 mg/m^3). The study appears to be conducted according to standard protocols and recommendations (U.S. EPA, 1991) for developmental toxicity. However, the statistical analyses for skeletal variants and abnormalities are based on the fetus rather than the litter, as is recommended in U.S. EPA (1991). The study establishes a LOAEL for the developmental toxicity of inhaled benzene of 50 ppm (160 mg/m^3) and NOAEL of 10 ppm (32 mg/m^3).

Coate et al. (1984) conducted a similar study, in which pregnant Sprague-Dawley rats inhaled 0, 1, 10, 40, or 100 ppm (0, 3.2, 32, 128, or 319 mg/m^3) of benzene 7 hr/day on GD 6–15. The various exposure groups consisted of 35–37 animals, and two control groups consisted of 32 and 34 dams. Benzene levels in the chambers, monitored at least once per day using both infrared and gas chromatography methods, were within 10% of target concentrations by both analyses, except for the gas chromatography analysis of the 10 ppm (32 mg/m^3) concentration, which was within 17% of target. The maternal animals were weighed and observed on days 0, 5, 8, 10, 16, and 20 and on days 6–15 were observed prior to, once during, and after exposure for signs of toxicity. On day 20, the dams were sacrificed and the fetuses were removed.

Parameters of developmental toxicity included number of corpora lutea per ovary, numbers per placements of uterine implantations, number of resorptions, number of live and dead fetuses, gross external variation, body weight, and examination of 50% for visceral abnormalities and 50% for skeletal abnormalities. Statistical analyses of maternal and fetal body weight and fetal length were conducted using Bartlett's test and ANOVA, F-ratio and Scheffe's multiple pairwise-comparison procedure, and Tukey's test. Pregnancy rates were analyzed using a chi-square test, and implantation, resorption, fetal viability, and sex indices and incidence of visceral and skeletal variants were analyzed using the Kruskal-Wallis non-parametric test for comparison of group means. All group differences were evaluated using the 0.05 one-tailed probability level as the criterion of significance.

Maternal body weight and mortality rate did not differ between treated and control animals (other than a slight, but insignificant decrease in body weight of the 100 ppm [319 mg/m^3] group), and there were no clinical signs or gross pathology, suggestive of maternal toxicity during gestation. Body weight corrected for gravid uterine weight was not determined, and hematological assays were not performed. The average number of implantations, number of resorptions, resorption incidence, number of live fetuses, and the incidence of dams with one or more resorbed implantations were comparable in all control and exposed groups.

The fetuses in the 100 ppm (319 mg/m^3) group exhibited a significant decrease ($p \leq 0.05$) in average male and female fetal body weights and a statistically insignificant decrease in fetal length. These values were within 10% of control values. There were no significant differences in the percentage of fetuses per litter with one or more variants, although slight increases in variants (dilation of the renal pelvis and ureters) occurred in the 1 and 100 ppm (3.2 and 319 mg/m^3) groups. Soft tissue anomalies occurred in all exposed groups, but were not dose-related. Delays in ossification of the skull, vertebral centra and extremities occurred at 100 ppm (319 mg/m^3), but were not concentration-related. The investigators concluded that benzene is weakly toxic to the fetus at 100 ppm ([319 mg/m^3] a concentration that was not toxic to the dams) and is not toxic to the fetus at 40 ppm (128 mg/m^3). This study appears to establish a LOAEL of 100 ppm (319 mg/m^3) and a NOAEL of 40 ppm (128 mg/m^3) for the fetal toxicity of benzene; however, the long-term effects of reduced fetal body weight are unknown. The study did not include an evaluation of hematologic parameters for the dams (an important factor in benzene toxicity), otherwise it appears that recommended procedures were followed.

Green et al. (1978) exposed groups of pregnant Sprague-Dawley rats to benzene concentrations of 100, 300, and 2200 ppm (319, 958, and 7028 mg/m^3) 6 hr/day on GD 6–15. Daily body weights were measured as an indicator of maternal toxicity; peripheral blood cell counts were not performed. The dams were sacrificed on day 21, and developmental toxicity was evaluated. One-third of the litters was processed for the examination of soft tissues, and all litters were processed for skeletal examination. Statistical analyses included the Student's *t* test for the evaluation of mean fetal weights and crown–rump length (the litter was the experimental unit and the level of significance was $p<0.05$); the Fisher exact probability test for the litter incidence of fetal anomalies; and the chi-square test for preferential sex effects.

Maternal weight gain was similar to control values for the rats exposed to 100 and 300 ppm (319 and 958 mg/m^3); however, body weight for the females exposed to 2200 ppm (7028

mg/m^3) was significantly reduced from GD 8–20 to ~44%–83% (p<0.01 for all days) of control values (Green et al., 1978). There were no differences between all exposure groups and controls with regard to implantation sites/litter, live fetuses/litter, percentage resorption/implantation site, percentage litters with resorption, litters totally resorbed, and resorption/litter with resorption. At concentrations of 100 and 300 ppm (319 and 958 mg/m^3), fetal sex ratio, mean fetal body weight, and mean fetal crown–rump length were comparable to control values; at 2200 ppm (7028 mg/m^3), mean fetal weight and mean crown–rump length were significantly lower than controls (p<0.05). The incidence of delayed ossification was similar for the exposed groups and controls; however, among the fetuses exposed to 300 and 2200 ppm (958 and 7028 mg/m^3) and exhibiting delayed ossification, the incidence was increased significantly (p<0.05) for females over males. The number of litters displaying an increased incidence of unossified sternebrae was increased at 100 ppm (319 mg/m^3) (9/18 litters) and 2200 ppm (7028 mg/m^3) (11/15 litters), and at 2200 ppm (7028 mg/m^3) the number of females with the abnormality was significantly increased (p<0.05) over males. The investigators concluded that these concentrations of benzene administered to the rat are toxic to the fetus, but do not induce malformations. The concentration of 2200 ppm (7028 mg/m^3) benzene was maternally toxic; however, fetal toxicity manifested as skeletal abnormalities was observed at concentrations that were not maternally toxic (and were statistically significant). The apparently increased sensitivity of the female fetus to the effects of benzene is supported by other observations that female rabbits, mice, and rats are more sensitive to these effects of benzene (Desoille et al., 1961; Ito, 1962a,b,c,d; Sato et al., 1975). The investigators tentatively suggested that the differences in the responses of the males and females may be related to hormonal differences.

Benzene concentrations of 10 or 40 ppm (32 or 128 mg/m^3), administered to groups of female Sprague-Dawley rats (26–31/group) during GD 6–15 for 6 hr/day, did not induce maternal toxicity (assayed by behavior, appearance, body weight gain) and did not induce malformations in the fetuses (Litton Bionetics, 1977). However, both concentrations increased the incidence of resorption of the fetuses. The report was brief and provided no details, but does support other findings of fetal toxicity in animals exposed to low concentrations of benzene. This study identifies a LOAEL of 10 ppm (32 mg/m^3) for developmental effects in rats.

In an early study, Gofmekler (1968) exposed female rats (5 controls, 10–12 exposed) to benzene concentrations ranging from 0 to 670 mg/m^3 (210 ppm) for 24 hr/day for 10 to 15 days prior to mating and all during pregnancy. The rats exhibited a concentration-related decrease in litter size and produced no litters at the highest concentration of 210 ppm (670 mg/m^3). This effect was not observed in the experiments of Green et al. (1978) at higher benzene concentrations administered over a different exposure period (6 hr/day on GD 6 through 15). This may suggest that the rat embryo is more sensitive to continuous exposure than to intermittent exposure only during organogenesis. However, females in that study were exposed before mating and ovulation rate and fertility may have been affected independently of developmental effects. The Gofmekler (1968) study demonstrated significant differences in the weight of liver, lung, spleen, kidney, and thymus from fetuses of benzene-treated dams. The weights were mostly depressed, but the effect was not concentrated-related. At 63.6 mg/m^3, organ weight, except fors the adrenals, was higher than control values. Gofmekler (1968) concluded that exposure of pregnant rats to relatively low concentrations of benzene can disturb normal fetal development.

However, the lack of a concentration response in the organ weight data for fetal rats precludes the determination of a LOAEL or NOAEL for the study.

Hudak and Ungvary (1978) conducted an experiment in CFY rats exposed to 313 ppm (1000 mg/m^3) of benzene 24 hr/day on GD 9 through 14. Exposure to benzene resulted in statistically significant decreases in maternal body weight gain (p<0.01), fetal weights (p<0.01) and percentage of weight retarded fetuses (p<0.05), and increased incidences of extra ribs and fused sternebrae (p<0.05 for both). The use of only one concentration precluded the definition of a LOAEL and/or NOAEL for the study.

A later study (Tatrai et al., 1980) examined the developmental toxicity of benzene using several concentrations. Pregnant CFY rats (48 controls, groups of 20–22 exposed) inhaled 0, 150, 450, 1500, or 3000 mg/m^3 (47, 140, 465, or 930 ppm), 24 hr/day on GD 7–14. Animals were sacrificed on day 21 for the evaluation of developmental effects. The dams exhibited significantly decreased body weight gain at all concentrations (p<0.001, ANOVA); the effect was concentration-related at 47, 140, and 465 ppm (150, 450, and 1500 mg/m^3), but not at 930 ppm (3000 mg/m^3). For example, the body weight gain for dams exposed to 465 ppm (1500 mg/m^3) of benzene was 28.5% of the starting body weight compared with the control value of 62.8% for the controls, whereas the body weight gain for dams exposed to 930 ppm (3000 mg/m^3) of benzene was 37.0%. A footnote in the report of Tatrai et al. (1980) indicates that maternal body weight was corrected for the total weight of fetuses and placentas. Liver/body weight ratios were significantly increased in the dams exposed to 140, 465, or 930 ppm (450, 1500, or 3000 mg/m^3), with a response pattern similar to the body weights (p<0.05 or p<0.01, ANOVA). Fetal body weights were decreased at ≥47 ppm (150 mg/m^3) (p<0.001, all concentrations); fetal loss in the percent of total implantation sites was increased 4–7 times control values at ≥140 ppm (450 mg/m^3) (p<0.05 or p<0.01, Mann Whitney U test); and skeletal and weight retardation were observed in the groups exposed to the three highest concentrations (p<0.01 or p<0.05). For these effects, there was no distinct relationship between concentration and response, and there was no increase in the incidence of malformations, even at concentrations ≥47 ppm (150 mg/m^3) that were maternally toxic. The main difference observed for the days 7–14 exposure regimen (Tatrai et al., 1980) study versus the days 9–14 regimen (Hudak and Ungvary, 1978) was the increase in fetal mortality (30%–40% of the implants) that occurred when exposure was started on GD 7.

To test the hypothesis that the congenital effects of industrial solvents may be attributed to simultaneous exposure to a combination of two or more chemicals, Ungvary and Tatrai (1985) conducted a study using combinations of benzene and its methyl derivatives, toluene and xylene, with each other or with acetylsalicylic acid (ASA), a known developmental toxin. Groups of 11–20 pregnant CFY rats inhaled combinations of benzene (400 mg/m^3) and toluene (1000 mg/m^3) or xylene (600 mg/m^3) 24 hr/day on GD 7–15. Additional groups of 7–14 animals were exposed to 2600 mg/m^3 of benzene on GD 10–12 and given oral doses of either 250 or 500 mg/kg of ASA on GD 12. Controls were exposed by inhalation to air or were given an oral dose of physiological saline on day 12. The animals were killed on day 21.

At 400 mg/m^3 benzene produced maternal toxicity as evidenced by decreased weight gain (47% gain in the benzene treated dams versus 69% gain in the controls [p<0.001]) benzene alone induced retardation of fetal and skeletal growth, but did not increase the incidence of skeletal

anomalies; of skeletal, internal, or external malformations; or the percentage of abnormal survivors. The combined developmental effects of the solvents were not additive. However, when each solvent was given in combination with orally administered ASA these solvents (administered by inhalation) enhanced maternal as well as fetal toxicity. In addition, benzene and its methyl derivatives significantly increased the frequency of ASA-induced malformations. The induction of most malformations was not solvent-dependent; but cleft lip and palate and abnormalities of the spinal column occurred only in response to the combined treatment with solvents and ASA. The investigators concluded that under the conditions of this experiment, neither benzene nor its alkyl derivatives induced malformations, alone or in combination; and that the fetal toxicity observed with all three solvents was not additive when the solvents were combined; however, all three solvents potentiated the toxic effects of ASA, including the induction of malformations.

Pregnant Swiss-Webster mice (five per exposure level per progeny age group; initial age 8–12 weeks) were exposed via inhalation to nominal vapor concentrations of 0, 5, 10, or 20 ppm benzene (0, 16, 32, or 64 mg/m^3) for 6 hr/day on GD 6–15 (Keller and Snyder, 1986). On GD 16 (two fetuses/litter/sex), 2 days after birth (two neonates/litter/sex), and 6 weeks after birth (one adult/litter/sex), were sacrificed for measurement of hematopoietic progenitor cells (CFU-E, BFU-E, and GC-CFU-C) from the liver (fetuses and neonates), and bone marrow and spleen (adults). In addition, 10-week-old progeny from litters in the control and mid-exposure group were exposed for 2 weeks to 10 ppm (32 mg/m^3) benzene, then sacrificed for measurement of hematopoietic progenitor cells from the bone marrow and spleen. There was no evidence of maternal or non-hematopoietic developmental toxicity in benzene-exposed mice. There was a significant (p<0.05) increase in the numbers of BFU-E from livers of male and female fetuses exposed to the low- and mid-exposure levels. The following significant (p<0.05) changes were observed with respect to CFU-E: in fetuses, there were increases in liver CFU-E at the low- and mid-exposure levels, and decreases at the high-exposure level; in male neonates, there were increases and decreases in liver CFU-E at the mid-exposure level, and increases at the high-exposure level; in adult mice there were decreases in bone marrow CFU-E and increases in spleen CFU-E in males exposed to 10 ppm (32 mg/m^3) in utero. Liver GM-CFU-C in neonates was significantly (p<0.05) decreased at the mid-exposure level (males only) and increased at the high-exposure level. Mice exposed to 10 ppm (32 mg/m^3) benzene in utero and for 2 weeks as adults had significantly (p<0.05) decreased bone marrow CFU-E (males only) and splenic GM-CFU-C; mice exposed to air in utero and 10 ppm (32 mg/m^3) benzene for 2 weeks as adults had no changes in bone marrow or splenic CFU-E, but had a significant (p<0.05) decrease in splenic GM-CFU-C (females only). The authors concluded that benzene treatment in utero induced hematopoietic alterations in fetuses, persisting until at least 10 weeks after birth. This study could be interpreted as identifying a LOAEL of 5 ppm (16 mg/m^3) for developmental hematopoietic effects in mice because statistically significant changes in BFU-E and CFU-E, were observed in the 16-day fetuses from dams exposed to 5 ppm (16 mg/m^3). The responses observed, however, were typically biphasic in nature, showing increases in BFU-E and CFU-E at 5 and 10 ppm (16 and 32 mg/m^3) followed by decreases at 20 ppm (64 mg/m^3). Only five pregnant animals were used in the study per exposure dose level, and limited numbers (two fetuses, two neonates or one adult/litter/sex) of animals were used for the evaluation of hematotoxic effects. Also, the responses did not establish a consistent pattern in the different ages of progeny examined.

Therefore, there is a high degree of uncertainty associated with determining whether the effects observed at 5 ppm (16 mg/m^3) are truly adverse effects.

Keller and Snyder (1988) examined the effects of in utero benzene on the developing recognizable hematopoietic precursor cells, peripheral blood cells, and Hgb production in Swiss-Webster mice. Three separate exposure experiments were performed in which pregnant mice (five per group) were exposed to benzene concentrations of 0, 5, 10, or 20 ppm (0, 16, 32, or 64 mg/m^3) in chambers 6 hr/day on GD 6–15. Maternal toxicity was evaluated based on morbidity, mortality, or weight loss, but maternal peripheral blood count was not determined. In experiment 1, the fetuses of the benzene-exposed dams were assayed for signs of hematotoxicity on GD 16 (two fetuses/sex/litter). In experiment 2, the offspring were assayed at 2 days (two neonates/sex/litter). In experiment 3, progeny were examined at 6 weeks (one/sex/litter) of age. Parameters of toxicity for the fetuses and 2-day-old neonates included: the number of live, dead, and resorbed fetuses, body weights, gross abnormalities, RBC, WBC, blood cell differentials, Hgb levels, and the number of liver cells in the hematopoietic differentiating, proliferating pool (DPP). The responses of 6-week-old progeny were evaluated based on peripheral WBC and RBC counts, Hgb, and smears made from femur bone marrow or spleen for the determination of the number of cells in the DPP. Although the authors did not state it, the experimental animals may have been the same for both studies (Keller and Snyder, 1986, 1988) , as all the details of the exposures were identical. Differences in cell counts were statistically evaluated by the Student's *t* test; differential counts and DPP cells were evaluated using a two-way ANOVA; and total litter responses (male and female) versus treatment and ratios of Hgb A major to Hgb A minor were assessed by one-way ANOVA followed by Dunnett's tests. Differences between treatments were considered significant when they were greater than the two-tailed, $p<0.05$ Dunnett's critical value.

Benzene exposures monitored hourly were well within target range (Keller and Snyder, 1988). There was no evidence of maternal toxicity at any exposure level. For the fetuses, there were no effects on litter size, male/female ratio, or body weight, as well as the numbers of dead, resorbed, or malformed. Significant findings ($p<0.05$) relevant to hematopoietic toxicity in the three groups of progeny included the following:

Peripheral blood. Hgb levels of exposed and control mice were similar for all groups. Peripheral blood cell and differential cell counts of the 16-day fetuses and differential cell counts of the 6-week-old progeny were comparable to those of the controls. The 2-day-old neonates exposed in utero to 5 ppm (16 mg/m^3) of benzene exhibited significant increases in RBC counts and decreases in mean corpuscular Hgb and the 6-week-old adults exposed in utero to 5 and 20 ppm (64 mg/m^3) of benzene had increased RBC counts; however, these did not occur with a distinct pattern and the investigators concluded that they probably had no toxicological significance. Peripheral blood cell differentials from the 2-day-old neonates revealed significantly decreased numbers of early nucleated red cells (statistically significant at all concentrations); the effect was concentration-dependent. The 2-day-old neonates exposed in utero to 20 ppm (64 mg/m^3) also had decreased numbers of late nucleated red cells and increased numbers of nondividing granulocytes; these effects were not concentration-dependent.

Hematopoietic organs. The 16-day fetuses from dams exposed to benzene exhibited no changes in hematopoietic parameters of the liver at any benzene concentration. The 2-day-old

neonates exposed in utero to 20 ppm (64 mg/m^3) of benzene had changes in the liver that were significant at 20 ppm (64 mg/m^3). These included: increase in the number of blast cells to 3.2 times that of the controls, depression in the number of late nucleated red cells (polychromatic normoblasts and their nucleated progeny) to 70% of the control value, an increase in the number of lymphocytes to 1.4 times the control value, an increase in the number of nondividing granulocytes to 1.9 times that of the control value, and an increase in the numbers of dividing granulocytes to 2.5 times the control value. These effects were not statistically significant at lower concentrations. The effects on the early red cells and granulocytes were concentration-related, the others did not show a distinct dose-response. The 6-week-old adults, exposed in utero to 20 ppm (64 mg/m^3) of benzene, also exhibited statistically significant changes in the cells of hemopoietic organs. These included fewer early nucleated red cells in the liver (to 55% of the control value), more splenic blast cells (to 6.5 times the control value), and increased dividing and non-dividing splenic granulocytes (to 2.9 and 3.2 times the control value, respectively). The effects were not dose related, and with the exception of one cell count at 5 ppm (16 mg/m^3) were not statistically significant at lower concentrations.

Keller and Snyder (1988) concluded that benzene induces hematotoxicity in the offspring of mice exposed during pregnancy, as evidenced by reductions in the numbers of early nucleated red cells in the peripheral blood of 2-day-old neonates at all exposure levels, and the increase in the number of dividing granulocytes in the liver of 2-day-old neonates and in the spleen of 6-week-old adults. The toxicological significance of these results, however, is unclear. The only clearly dose related response was the decrease in early nucleated red cells in the peripheral blood of 2-day-old neonates. Early nucleated red cells, however, are present in the peripheral blood only in very young animals, as clearly indicated by the near absence of such cells in the 6-week-old adult mice. A limited number of pregnant animals (five per group) and progeny (two/litter/sex) were tested. Therefore, biological significance and confidence in the data is questionable. Thus, a NOAEL or LOAEL cannot be derived based on the results of the report of Keller and Snyder (1988).

Corti and Snyder (1996) examined the influence of gender, development, pregnancy, and ethanol consumption on the hematotoxicity of inhaled benzene. They exposed age-matched male, virgin female, and pregnant Swiss-Webster mice (number of animals in each group was not given, but there were 12 or 13 dams) to 10 ppm (32 mg/m^3) for 6 hr/day for 10 consecutive days (GD 6–15 for the pregnant females). Half of the animals also received 5% ethanol in the drinking water during the exposure period. On day 11, bone marrow cells from the adults and liver cells from the fetuses were assayed for the numbers of CFU-E. CFU-E assays were also performed on bone marrow cells isolated from in utero-exposed 6-week-old males and females. Depressions in CFU-E numbers were seen only in males. CFU-E in adult males was depressed to ~70% of control values by exposure to benzene, ethanol, or ethanol plus benzene. The action of the two agents was neither additive nor synergistic. CFU-E from fetal livers was significantly decreased in males exposed to benzene or benzene plus ethanol, but not for ethanol alone. The CFU-E value for males exposed to both benzene and ethanol, however, was reduced by only 5% in comparison to the control as compared to 20% for ethanol alone. There were no significant differences for female fetuses. Assays of CFU-E in 6-week-old mice exposed to benzene *in utero* showed reduction of as much as 50% in comparison with controls for males, but no significant reductions for females. In fact females exposed to benzene and ethanol in utero showed a 40% elevation of

CFU-E in comparison to unexposed controls. For both ethanol and benzene, only a single concentration was used in these experiments. Thus, it is not possible to evaluate if the results are part of a dose-response relationship.

Murray et al. (1979) conducted developmental toxicity studies in mice that also included the evaluation of hematological parameters in the offspring. CF-1 mice were exposed to 500 ppm (1597 mg/m^3) of benzene 7 hr/day on GD 6 through 15 and were killed on GD 18. There were no significant signs of maternal toxicity and values for PCV, RBC, Hgb, and WBC were comparable to control values for the adult mice. There were no adverse effects on developmental parameters such as number of live fetuses, or number of resorptions/litter; however, body weight of the mouse fetuses was slightly but significantly decreased (to 94% of control values; $p<0.05$). The investigators described a "significant" increase in the incidence of skeletal variations of the mouse fetuses as evidenced by delayed ossification of sternebrae and skull bones and unfused occipital bones of the skull, but there was no significant increase in the incidence of malformations. Fetal mice could not supply sufficiently large blood samples for hematological assays. The investigators concluded that benzene did not induce malformations in mice at 500 ppm (1597 mg/m^3), but was toxic to the fetuses. This study used only one concentration, and statistical analysis for the variations were not clearly stated (significance was probably determined by the Wilcoxon test); however the study did demonstrate fetal toxicity at a concentration that was not maternally toxic.

Ungvary and Tatrai (1985) examined the developmental toxicity of benzene in CFLP mice. Groups of 11 to 15 pregnant animals inhaled 500 or 1000 mg/m^3 (156 or 313 ppm) of benzene 24 hr/day during GD 6–15. Unexposed and air-exposed (chamber) animals served as controls. The mice were killed on GD 18.

The authors mentioned in the abstract of the paper that all solvents caused moderate and concentration-dependent maternal toxicity. This was the only information provided for maternal toxicity, among the mice. Of the mouse fetuses from dams exposed to 156 and 313 ppm (498 and 1000 mg/m^3), 25% and 27% respectively, exhibited weight retardation and 10 and 11% had retarded skeletal development. All of these effects were statistically significant ($p<0.05$). There were no differences between control and exposed groups in the incidence of minor anomalies or malformations. The investigators concluded that under the conditions of this study, benzene does not induce malformations, but does induce fetal toxicity in mice exposed to 156 and 313 ppm (498 and 1000 mg/m^3), at maternally toxic concentration. The mouse data suggest a LOAEL of 156 ppm (498 mg/m^3).

Murray et al. (1979) conducted developmental toxicity studies in rabbits that also included the evaluation of hematological parameters in the offspring. New Zealand rabbits were exposed to 500 ppm (1597 mg/m^3) of benzene 7 hr/day on GD 6–18. The rabbits were sacrificed on GD 29. The rabbits exhibited no significant signs of maternal toxicity and there were no adverse effects on developmental parameters such as number of live fetuses or number of resorptions/litter. Body weight and length of the rabbit fetuses were comparable to control values. The rabbits exhibiting two minor skeletal variants, lumbar spurs and the proportion of fetuses with 13 ribs (the normal number is 12 or 13), occurred significantly less often among the exposed litters. Increases in the incidence of malformation.were not significant. Values for PCV, RBC, Hgb, and WBC were comparable to control values for adult and fetal rabbits. The

investigators concluded that benzene did not induce malformations in rabbits at 500 ppm (1597 mg/m^3), and was only weakly toxic to the fetuses. This study used only one concentration, and statistical analyses for the variations observed were not clearly stated (but significance was probably determined by the Wilcoxon test).

Ungvary and Tatrai (1985) examined the developmental toxicity of benzene and other solvents in New Zealand rabbits. Groups of 11–15 pregnant animals inhaled 500 or 1000 mg/m^3 (156 or 313 ppm) of benzene 24 hr/day on GD 7–20. Unexposed and air-exposed (chamber) animals served as controls. The rabbits were killed on GD 30.

The maternal rabbits exhibited concentration-related decreases in weight gain (to 37% of control values at 313 ppm [1000 mg/m^3]) and increases in relative liver weights (1.2 × control values at 313 ppm [1000 mg/m^3]); compared with control values, the effects were statistically significant at 313 ppm (1000 mg/m^3) (p<0.05). Two dams died and six aborted at the higher concentration. The fetuses exhibited concentration-related decreases in body weight (to 83% of control values, p<0.05 at 313 ppm [1000 mg/m^3]), concentration-related increases in the percent of dead or resorbed fetuses (3.1 × control values, p<0.05 at 313 ppm [1000 mg/m^3]), concentration-related increases in skeletal retardation (not statistically significant) and minor anomalies (2.5 × control values, p<0.05 at 313 ppm [1000 mg/m^3]), and concentration-related decreases in the percent of malformations. The investigators concluded that under the conditions of this study, benzene does not cause malformations, but induces fetal toxicity in rabbits at 313 ppm (1000 mg/m^3), a maternally toxic concentration. The evidence supporting the LOAEL and NOAEL in rabbits is weakened by a lack of experimental detail presented in the report, the small numbers of pregnant rabbits employed, and the use of the fetus, rather than the litter as the experimental unit.

4.3.3. Mechanisms of Developmental and Reproductive Toxicity

The mechanism(s) for the developmental and reproductive toxicity of benzene are not well understood. Summarized below are a few of the suggested mechanisms that pertain specifically to the developmental and reproductive toxicity of benzene.

- Following administration of benzene to pregnant rats, Pushkina et al. (1968) observed decreased ascorbic acid content in the whole fetus and maternal organs as the concentration of benzene increased, first in the maternal liver and later in the placenta and fetal liver. Benzene also increased the DNA content and decreased the RNA content in the placenta, fetal liver, and fetal brain, and decreased DNA content in the maternal liver. Pushkina et al. (1968) suggested that these alterations in ascorbic acid, RNA, and DNA content are possible mechanisms for fetal toxicity.

- Ungvary and Donath (1984) suggested that damage to the peripheral noradrenergic fibers observed in their study in pregnant rats may result in a disturbed control of ovarian and uterine blood flow and steroid production and may thus be instrumental in the embryotoxic action of organic solvents.

- Tatrai et al. (1980) suggested that several factors may be responsible for the embryotoxicity of benzene: first, because of its lipophilicity, benzene can pass the placental barrier and affect the embryonal cells directly; second, phenol (a major metabolite of benzene), shown to inhibit DNA synthesis in bone marrow in vivo, can also pass the placental barrier; third, benzene can damage maternal circulation and cause bone marrow depression, resulting in adverse nutritional conditions for the fetus. Benzene oxide was suggested as the toxic metabolite of benzene. Benzene oxide appears to be toxic only at the site of production, and the enzymes responsible for its production only appear in the rat fetus late in pregnancy. However, in the human fetus, the enzymes are present during weeks 9-13 of pregnancy. Tatrai et al. (1980) suggest that a transplacental effect is more plausible than any other mechanism.

- Reports indicating that paternal (as well as maternal) exposure to benzene is associated with increased risk for stillbirths, and the findings of increased incidences of testicular lesions in benzene-exposed animals, suggest that exposure of males may be important in the reproductive toxicity of benzene (Savitz et al., 1989; Spano et al., 1989; Ward et al., 1985).

4.4. OTHER STUDIES

4.4.1. Neurotoxicity

4.4.1.1. *Oral*

Sprague-Dawley rats given a single dose of 1870 mg of benzene/kg exhibited tremors and tonic-clonic convulsions and died within minutes. A dose of 352 mg/kg (for a 25 g mouse) produced slight nervous system depression (Cornish and Ryan, 1965). The LD_{50} for benzene in nonfasted rats is 0.81 g/kg.

In the only nonacute oral study of neurotoxicity found in the available literature, Hsieh et al. (1988a) evaluated the effects of benzene on neurotransmitters of the brain. CD-1 adult male mice (5/group) received benzene in the drinking water for 4 weeks ad lib. Controls received tap water. The nominal concentrations were 0, 40, 200, and 1000 mg/L. Treated water solutions were shaken frequently after preparation and were changed every 3 days. The original concentrations of benzene in the drinking water decreased approximately 27%-30% over a 3-day period. Based on water consumption, the daily estimated doses of benzene were 0, 8, 40, and 180 mg/kg-day.

After 4 weeks of continuous exposure, the animals were killed and their brains were rapidly removed and sectioned into different regions consisting of the hypothalamus, medulla oblongata, cerebellum, corpus striatum, cerebral cortex, and midbrain. The sections were homogenized individually and analyzed for catecholamines; norepinephrine and dopamine and their principal metabolites, vanillymandelic acid, 3,4-dihydroxyphenylacetic acid, and homovanillic acid; and for the indoleamine serotonin and its metabolite, 5-hydroxyindoleacetic acid. The levels

of the metabolites provided a measure of turnover of the catecholamines. Analysis was by high-performance liquid chromatography.

The benzene concentrations used in this study did not significantly alter behavior, body weights, or food and water consumption. Generally, the increases in levels of monoamine neurotransmitters were dose-related at 8 and 40 mg/kg-day, but in several cases there were no further increases at 180 mg/kg-day. The increases were greatest for norepinephrine in the hypothalamus (increased over control by approximately 38%, 55%, and 58% at 8, 40, and 180 mg/kg-day, respectively) and medulla oblongata (16%, 42%, and 20%). For serotonin, a similar pattern of increase was observed in the higher association center with increased exposure to benzene. In the hypothalamus, the serotonin levels were 21%, 86%, and 93% above the control value. A generalized increase was also observed in the medulla oblongata (5%, 25% and 19%) and the midbrain (8%, 46%, and 23%). The increases in the parent compounds were associated with increases in their corresponding metabolites, reflecting increased turnover of the amines. The data of Hsieh et al. (1988a) did not predict a no-effect level of benzene in drinking water. In fact, although the assay has potential as a biomarker for exposure, the biological significance of these findings is questionable. The findings, however, suggest that neurotoxicological effects in the hypothalamus and peripheral nervous system could be of particular concern for the developing CNS, resulting in permanent effects.

4.4.1.2. *Inhalation*

Ten rabbits exposed to ~ 45,000 ppm (143,760 mg/m^3) benzene exhibited light narcosis after 3.7 minutes of exposure, followed by tremors, chewing, excitement, and running movements after 5 minutes (Carpenter et al., 1944). There was a loss of pupillary reflex to strong light after 6.5 minutes, loss of blink reflex to tactile stimulus after 11.4 minutes, pupillary contraction after 12 minutes, involuntary blinking after 15.6 minutes, and death after 36.2 minutes (Carpenter et al., 1944). The inhalation LC_{50} for benzene vapors in the rat is 1600 ppm (5111 mg/m^3)/4 hr (Gerarde, 1960).

Andersson et al. (1983) examined the effects of high concentrations of benzene on dopamine and noradrenaline turnover within various parts of the hypothalamus that are involved in neuroendocrine regulation. Turnover of the catecholamines was assessed by measuring changes in the degree of catecholamine stores following tyrosine hydroxylase inhibition.

Sprague-Dawley rats exposed to 1500 ppm (4792 mg/m^3) of benzene 6 hr per day for 3 days were divided into two groups of 4 or 6 animals. In the first group (-inhibitor), which served as controls, sacrifice immediately followed exposure; in the second group (+inhibitor), the injection of tyrosine hydroxylase inhibitor immediately followed exposure and the animals were sacrificed 2 hr later. The extent of depletion of the catecholamines was determined by calculating the percentage depletion in the test group based on the levels of catecholamine present in the control group at the time of the injection of the hydroxylase inhibitor.

Quantitative microfluorimetry demonstrated that benzene induced statistically significant alterations in catecholamine content and turnover in various sections of the hypothalamus.

Benzene (-inhibitor) produced increases in the catecholamine fluorescence in the median and lateral palisade zones of the median eminence ($p<0.05$) and within the posterior periventricular hypothalamic region ($p<0.05$). Benzene (+inhibitor) enhanced the disappearance of catecholamine in the median palisade zone ($p<0.002$), within the posterior periventricular hypothalamic region ($p<0.05$), within the parvocellular part of the paraventricular hypothalamic nucleus ($p<0.01$), and within the dorsomedial hypothalamic nucleus ($p<0.01$).

The investigators concluded that benzene produced a pattern of discrete changes in noradrenalin and DA turnover in certain areas of the hypothalamus. The study included only one concentration of benzene and a small number of animals. Quantitatively, no LOAEL or NOAEL was established. Tyrosine hydroxylase is the key enzyme for biosynthesis of catecholamine and the hypothalamus is one of the major association centers in the central nervous system. It is clear that benzene will affect the functions of all these centers at an inhalation dose of 500 ppm (1597 mg/m^3) as demonstrated in the changes in dopamine levels of various areas, probably including centers controlling respiration, hunger, and thirst.

Ungvary and Donath (1984) evaluated the effects of benzene on the noradrenergic innervation of reproductive organs. CFY rats were exposed to 1500 mg/m^3 of benzene 8 hr/day on GD 8-10. The abundance of fluorescent noradrenergic fibers normally found in the ovaries and uterus of the pregnant rat decreased while background fluorescence increased (interpreted by the investigators to indicate an increased release of noradrenalin). To confirm that the effect was selective to nerve fiber, Ungvary and Donath injected benzene into the anterior chamber of the eye. The benzene-induced damage to the sympathetic nerve-plexus of the iris was similar to that in the uterus and ovary. The density of fluorescent fibers was decreased (dose-related), and the iris exhibited substantial hyperemia and increased background fluorescence 72 hr after injection. The investigators concluded that benzene has a selective and differential toxic effect on postganglionic neurons, with potential embryotoxicity.

Dempster et al. (1984) examined the temporal relationship between the behavioral and hematological effects of inhaled benzene. Mice (30-45/group) were exposed to benzene concentrations of 100 to 3000 ppm (319 to 9584 mg/m^3) 6 hr/day for the number of days necessary to achieve a (concentration × time) product of 3000 ppm-days. The controls were age-matched and exposed to air. The behavioral parameters evaluated included the following: milk licking (an observation of the mice licking a spout protruding from a wall in order to obtain milk); hindlimb grip strength (a mouse was held by the tail and steadily pulled backward through a trough until both hind paws grasped a wire triangle; the mouse was then pulled until its grip was broken); and home cage food and water intake (measured by weighing water bottles and feeders daily). Lymphocyte numbers were reduced to 68% of control values after 5 exposures to 100 ppm (319 mg/m^3) benzene, to 50% of control values after 2 exposures to 300 ppm (958 mg/m^3), and to 50% of controls after 1 exposure to 1000 and 3000 ppm (3194 and 9584 mg/m^3). Maximal depression in the lymphocyte counts occurred after 10 days of exposure to 300 ppm (958 mg/m^3). The lymphocyte counts remained depressed throughout each exposure regimen.

RBC counts were not depressed as rapidly or to the same extent as the lymphocytes. At the end of exposure to 300 ppm (958 mg/m^3), for example, the RBC counts were reduced to 70% of control values at the end of exposure to 300 ppm (958 mg/m^3). Increased milk licking, the

most sensitive of the behavioral parameters, was statistically significant following 1 or 2 days of exposure to 100 ppm (319 mg/m^3) and after 4 or 5 days of exposure to 300 ppm (958 mg/m^3). The maximal increase in this behavior pattern occurred after 7-8 days of exposure to 300 ppm (958 mg/m^3), following the same time course as the hematological effects. Short-term exposures to high concentrations did not increase milk licking, but did increase food consumption. Thus the increased milk licking at 100 ppm (319 mg/m^3) was apparently not due to hunger. Exposure to 1000 ppm (3195 mg/m^3) for day reduced hindlimb grip strength. These effects disappeared following termination of exposure.

In a study designed to reflect occupational exposure, male CD-1 and C57BL/6 mice were exposed to 300 or 900 ppm (958 or 2875 mg/m^3) benzene 6 hr/day for 5 days followed by 2 weeks of no exposure, after which the exposure regimen was repeated for an unspecified amount of time (Evans et al., 1981). The following seven categories of behavioral activities were monitored in exposed and control animals: stereotypic behavior, sleeping, resting, grooming, eating, locomotion, and fighting. Only minimal and insignificant differences were observed between the two strains of mice. Increased behavioral activity in both strains of mice was observed after exposure to benzene. Mice exposed to 300 ppm (958 mg/m^3) of benzene had a greater increase than those exposed to 900 ppm (2875 mg/m^3), probably because of narcosis-like effects induced at the higher exposure level. It is not known if benzene induces behavioral changes by direct action upon the central nervous system.

Frantik et al. (1994) found that male albino SPF rats from a Wistar-derived strain exposed to benzene for 4 hr in glass chambers (dose not specified) exhibited depression of evoked electrical activity in the brain; the authors calculated the 30% effect level (depressed activity) as 929 ppm (2968 mg/m^3). When female H strain mice were exposed to benzene for 2 hr, the 30% effect level for depression of evoked electrical activity in the brain was 856 ppm (2735 mg/m^3).

Adult male Kunming mice (5/group)were exposed to 0, 0.78, 3.13, or 12.52 ppm (0, 2.5, 10, 40 mg/m^3) benzene for 2 hr/day, 6 days per week for 30 days (Li et al., 1992). Exposures were conducted under static conditions in 300 m^3 plexiglass chambers. Benzene was monitored by gas chromatography every 30 minutes for 3 days at the beginning of the experiment, but was apparently not monitored for the rest of the experiment. The animals were monitored for Y maze performance (rapid response); locomotor activity; forelimb grip strength; acetyl cholinesterase (AChE) activity in the blood and brain; brain, liver, spleen, and kidney weights; and bone marrow cellularity. Statistical significance was evaluated by *t* value for forelimb grip strength and locomotor activity and by U value for rapid response frequency.

Significantly increased grip strength was observed at 0.78 ppm (2.5 mg/m^3), but at higher doses, grip strength was significantly decreased. The frequency of rapid response (running Y maze in less than 3 seconds) followed a similar pattern to the forelimb grip strength. The frequency increased significantly ($p<0.05$) from 33.7% in controls to 43.8% in the 0.78 group but declined significantly to 29.4% and 25.5% in the 3.13 and 12.52 ppm (10 and 40 mg/m^3) groups, respectively. No statistically significant differences were observed in AChE activity in either blood or brain, or in locomotor activity at any dose level. Relative liver weight was significantly increased and relative spleen weight was significantly ($p<0.05$) decreased in the high-dose group. No statistical analysis of the bone marrow histological investigation was presented. There were

no apparent responses in the 0.78 or 3.13 ppm (2.5 or 10 mg/m^3) groups. In the high-dose 12.52 ppm (40 mg/m^3) group, however, there were reductions of 91% in myeloblasts, 64% in premyeloblasts, 77% in metamyelocytes, 100% in reticulum, and 100% in erythroblasts.

The neurological effects, forelimb grip strength and frequency of rapid response, reported in this study are unique and interesting. However, there are several limitations to this study that prevent its use to establish an RfD value. The number of animals used in each group (5) was low, exposures were performed under static conditions, and benzene concentrations were monitored on only the first 3 of 30 exposure days. The very large decreases in several blood parameters are in contrast to most other studies, which have found minimal or no response in bone marrow parameters at similar exposure concentrations. Most other studies on the hematotoxicity of inhaled benzene have used exposure durations of 6 hr/day, in contrast to the 2 hr/day in this study. Thus, the dramatic effects upon bone marrow parameters in this study suggest that actual benzene exposure may have been higher than reported.

4.4.2. Immunotoxicity

4.4.2.1. *Oral*

Male Charles River CD-1 mice (5/group; 6-7 weeks of age) were exposed to benzene in drinking water to 0, 31, 166, or 790 mg/L (providing doses of 0, 8, 40, or 180 mg/kg-day, as calculated by the authors) for 28 days (Hsieh et al., 1988b). The treatment had no adverse effects with respect to mortality, clinical signs, body weight change, liver weight, or gross necropsy. A dose-related decrease in relative spleen weight was observed, significant (p<0.05) at the high-exposure level. In one test, spleen cellularity was reported to be significantly decreased at all exposure levels, and in a separate test, only at the high-exposure level. Although relative thymus weights were decreased at all exposure levels, the values were not statistically significantly different from controls. Dose-related hematological effects (erythrocytopenia, leucocytopenia, lymphocytopenia, increased motor conduction velocity) were observed at all exposure levels. The authors indicated that the increased MCV and decreased hematocrit and numbers of RBC were indicative of severe macrocytic anemia. Biphasic responses were observed in immunological tests including mitogen-stimulated (lipopolysacharide [LPS], poke weed mitogen [PWM], concanavilin A [Con A], phytohemaglutinin [PHA]) splenic lymphocyte proliferation; mixed splenic lymphocyte culture response to allogenic yeast artificial chromosome (YAC)-1 cells; cytotoxic splenic T-lymphocyte response to allogenic YAC-1 cells with a significantly increased response at the low-exposure level; and significantly decreased responses at the mid- and/or high-exposure level. Using several methods to determine primary antibody response to sheep RBC, significantly decreased responsiveness was observed at the mid- and/or high-exposure levels; this response was either significantly increased or not different from controls in mice exposed to the low-exposure level. This study identified a LOAEL of 8 mg/kg-day (the lowest dose tested) for hematological and immunological effects in male mice exposed to benzene in drinking water for 30 days. No NOAEL was established.

In a subchronic study, groups of male C57BL/6 mice were exposed via drinking water to 0, 152, or 853 mg/L benzene for 7-28 days; using estimated daily water intakes, the authors

calculated benzene dosages of 27 and 154 mg/kg-day (Fan, 1992). Five mice per group were killed after 7, 14, 21, or 28 days of exposure. An unspecified number of mice were exposed to 152 mg/L for 28 days and killed 7, 14, or 21 days after the last dosage. The focus of this study was to determine the toxicity of benzene on natural killer (NK) cells involved in nonspecific host resistance, and on interleukin-2, which is the primary growth factor of T cells, a growth factor for B cells and NK cells, and is involved in the regulation of granulocyte and eosinophil production that occurs in response to NK cell activity and interleukin-2 production. No overt signs of toxicity were observed in the benzene-exposed mice. Significant decreases in the number of spleen cells were observed in both groups of benzene-exposed mice. This effect was observed after 21 days of exposure in the 152 mg/kg-day group and after 14 days in the 852 mg/kg-day group. After 21 days, a significant increase in splenic NK cell activity was observed in both groups; however, after 28 days, the activity was not significantly different from controls. Splenic interleukin-2 production was significantly depressed after 28 days in both groups. Spleen cell numbers and interleukin-2 production were also depressed in the mice exposed to 152 mg/kg-day for 28 days and killed 7 and 14 days (interleukin-2 levels only) after the end of the exposure. This study identified a LOAEL of 152 mg/kg-day (the lowest dose tested) for effects on the immune system in male mice. A NOAEL was not identified.

Female B6C3F1 mice (12/group; 6-7 weeks of age) were exposed to benzene in drinking water (containing emulphor to increase solubility of benzene) at levels of 0, 50, 1000, or 2000 mg/L (0, 12, 195, or 350 mg/kg-day, as calculated by the authors) for 30 days (White et al., 1984). Body weight was significantly (p<0.05) decreased at the high exposure level. A dose-related (p<0.01) decrease in absolute and relative spleen weight was observed. In one test, spleen cellularity was reported to be significantly (p<0.05) decreased at all exposure levels, and in a separate test at only the mid- and high-exposure levels. Dose-related (p<0.05) leukopenia and lymphocytopenia were observed. A dose-related (p<0.01) decrease in eosinophils was observed. At the high-exposure level, significant (p<0.05) decreases in levels of erythrocytes and Hgb were observed. No exposure-related effects were observed for levels of blood urea nitrogen, serum creatinine, serum glutamic oxaloacetic transaminase, or serum glutamic pyruvic transaminase, indicators of renal and hepatic damage. Dose-related (p<0.05) changes were observed in immunological tests on spleen cells and in assays of bone marrow; decreases were observed with respect to IgM antibody forming cells/spleen in response to sheep RBC, lymphocyte proliferation response to the T cell mitogen Con A and the B cell mitogen LPS, number of T lymphocytes, and femoral CFU-GM; an increase was observed in bone marrow cell DNA synthesis. These effects were not significant at 12 mg/kg-day but were dose related (p<0.05). Of all the immunological indices tested, only one endpoint (stimulation index for lymphocyte proliferation of spleen cells in response to medium containing 0.5 µg/mL of Con A) was significantly (p<0.05) decreased at 12 mg/kg-day. The number of B lymphocytes was not affected, but the investigators commented that the number of B lymphocytes in the controls was lower than for historical controls for their laboratory. This study identifies a NOAEL 12 of mg/kg-day and a LOAEL of 195 mg/kg-day for hematological effects in mice exposed to benzene in drinking water for 30 days, and a LOAEL of 12 mg/kg-day for immunological effects.

4.4.2.2. *Inhalation*

Male C57BL/6J mice (7-8/group) were exposed to benzene at a concentration of 0, 10.2, 31, or 301 ppm (0, 32.6, 99, 319, or 962 mg/m^3) in whole-body dynamic inhalation chambers for 6 hr/day for 6 days (Rozen et al., 1984). Mice were bled within 30-90 minutes of the last exposure for determination of peripheral blood counts. Five animals with blood counts closest to the group mean were selected for lymphocyte assays. Single-cell suspensions of bone marrow and spleen cells from these animals were used for T- and B-cell enumeration and mitogen-induced proliferative assays. Statistical significance (p<0.05) was determined by one-way ANOVA.

Lymphocyte counts were depressed at all exposure levels. Lymphocyte counts were significantly reduced to approximately 65% of controls in the 10.2 ppm (32.6 mg/m^3) exposure group and to 35% of controls at 100 or 300 ppm (319 and 962 mg/m^3). Erythrocyte counts were significantly stimulated (115% of control) at 10.2 ppm (32.6 mg/m^3) and were depressed only at 100 and 300 ppm (319 and 962 mg/m^3). At a exposures of 10.2 ppm (32.6 mg/m^3), the frequency of femoral B-lymphocyte colony-forming cells was reduced to approximately 30% of the control value. In contrast, the number of femoral B-lymphocytes was not significantly reduced in the low-exposure group, but was reduced to less than 10% of the control at 100 or 300 ppm (319 and 958 mg/m^3). Similarly, splenic PHA-induced blastogenesis was significantly depressed at 31 ppm (99 mg/m^3) without a concomitant depression in numbers of T-lymphocytes. The numbers of T-lymphocytes were reduced to less than 50% of controls in the 100 and 300 ppm (319 and 958 mg/m^3) exposure groups. These results demonstrate that short-term exposure to inhaled benzene even at low exposure concentrations can cause reductions in immune-associated processes. This study identifies a LOAEL for depression of lymphocytes of 10.2 ppm (32.6 mg/m^3), the lowest dose tested.

Rosenthal and Snyder (1985) investigated the effects of exposure to benzene at 0, 10, 30, 100, or 300 ppm on the immune response of male C57BL/6J mice (5-7/group) to challenge with the facultative intracellular pathogen *Listeria monocytogenes*. All mice (5-7/group) were exposed to benzene for 5 days (6 hr/day) before infection with *L. monocytogenes*. At this point benzene exposure was stopped for half of the groups and the other half continued to be exposed to benzene for 7 days after infection, for a total of 12 days exposure. Bacterial proliferation in the spleen was measured at 1, 4, and 7 days after infection as an index of the resistance of the mice to infection. Body and spleen weights were determined, T and B lymphocytes were enumerated, spleen cellularity was determined, and spleen monocyte/macrophage, polymorphs, lymphocytes, and nucleated red blood cells were scored. Data were evaluated by one-way ANOVA and pairwise comparisons were made by a two-tailed Student's t test (p<0.05). Counts of *L. monocytogenes* in the spleen were compared using the chi-square test of geometric means of bacteria counts.

None of the benzene exposure treatments affected *L. monocytogenes* counts in the spleen 1 day after infection. Pre-exposure to benzene at 300 ppm resulted in a sevenfold increase in spleen bacterial counts at 4 days after infection, but lower concentration had no significant effect. However, with continued benzene exposure after infection, concentration of 30 ppm or more resulted in dose-dependent increases in spleen bacterial counts at 4 days after infection. By 7 days after exposure, spleen bacterial counts had returned to control levels in all treatments. The

authors suggested that benzene exposure caused a delay in the cell-mediated immune response, since there was a temporary increase in spleen bacterial counts. Both T and B lymphocytes were particularly sensitive to benzene exposure. Lymphocyte counts were depressed at benzene exposure concentrations of $\geq$30 ppm, and counts did not return to control levels even after cessation of benzene exposure. This study identified a LOAEL of 30 ppm and a NOAEL of 10 ppm for effects on the immune system.

4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS AND MODE OF ACTION

Benzene exposure results in adverse noncancer effects by all routes of administration. Hematotoxicity and immunotoxicity have been consistently reported to be the most sensitive indicators of noncancer toxicity in limited studies in humans and experimental animals, and these effects have been the subject of several reviews (Aksoy, 1989; Goldstein, 1988; Snyder et al., 1993b; Ross, 1996). The bone marrow is the target organ for the expression of benzene hematotoxicity and immunotoxicity. Neither gastrointestinal effects from oral exposure nor pulmonary effects due to inhalation exposure have been reported.

Chronic exposure to benzene results in progressive deterioration in hematopoietic function. Anemia, leukopenia, lymphocytopenia, thrombocytopenia, pancytopenia, and aplastic anemia have been reported after chronic benzene exposure (Aksoy, 1989; Goldstein, 1988). In contrast to these blood cellularity depression effects, benzene is also known to induce bone marrow hyperplasia. Acute myelogenous leukemia has been frequently observed in studies of human cohorts exposed to benzene, and there is evidence linking benzene exposure to several other forms of leukemia (U.S. EPA, 1998c). Whether the hematotoxic/immunotoxic effects of benzene and its carcinogenic effects are due to a common mechanism is not yet known. This is in part due to the fact that although the bone marrow depressive effects of benzene in humans can be readily duplicated in several experimental animal model systems, a suitable experimental animal system for the induction of leukemia has not been found. The hematotoxicity/immunotoxicity of benzene leads to significant health effects apart from potential induction of leukemia, as several deaths due to aplastic anemia have been reported (ATSDR, 1997). Also, in a follow-up study of benzene-exposed workers, Aksoy et al. (1972) reported that 8 of 32 workers that had been diagnosed with pancytopenia died, mainly from infection and bleeding.

Leukocytopenia has been consistently shown to be a more sensitive indicator of benzene toxicity in experimental animal systems than is anemia, and lymphocytopenia has been shown to be an even more sensitive indicator of benzene toxicity than overall leukocytopenia (Snyder et al., 1980; Ward et al., 1985; Baarson et al., 1984). Rothman et al. (1996) also found that a decrease in absolute lymphocyte count was the most sensitive indicator of benzene exposure in a group of workers. Ward et al. (1996) also found a strong relationship between benzene exposure and decreased WBC counts in a rubber worker cohort, but no significant relationship with RBC counts was found. A common observation in experimental animal and human studies of lymphocytopenia has been the absence of a clear threshold.

In common with many other organic solvents, benzene has been shown to produce neurotoxic effects in test animals and humans after short-term exposures to relatively high concentrations. The neurotoxicity of benzene, however, has not been extensively studied and no systematic studies of the neurotoxic effects of long-term exposure were located. Benzene produces generalized symptoms such as dizziness, headache, and vertigo at levels of 250-3000 ppm (799-9584 mg/m^3) (Brief et al., 1980), leading to drowsiness, tremor, delirium, and loss of consciousness at 700 to 3000 ppm (2236-9584 mg/m^3) (ATSDR, 1997). These neurological symptoms are reversible upon removal of the subject from exposure. Kahn and Muzyka (1973) reported that workers exposed to benzene for 2-9 yr at 6-15.6 ppm (20-50 mg/m^3) complained of frequent headaches, became tired easily, had difficulties sleeping, and complained of memory loss. The limitations of this study were that the workers were exposed to both benzene and toluene and that the dose and duration of exposure were unknown.

There were also few studies in test animals examining the neurotoxicity of benzene. These studies used short-term exposures, usually to concentrations that have been shown to induce significant hematotoxicity. One exception is the study of Li et al. (1992). Li et al. (1992) observed biphasic responses in forelimb grip strength and frequency of rapid response in running a Y-maze following inhalation exposure to 0, 0.78, 3.13, or 12.52 ppm (0, 2.5, 10, or 40 mg/m^3) benzene for 2 hr/day, 6 days per week for 30 days. Both responses increased at the lower concentration and declined at the intermediate and high concentration. There were, however, a number of irregularities in the experimental protocol that need to be resolved before these observations can be used to establish NOAEL or LOAEL values. The toxicological significance of these responses is also not clear.

There is some evidence of reproductive and developmental benzene toxicity from human epidemiology studies, but the data did not provide conclusive evidence of a link between exposure and effect. In most cases, there was exposure to other chemicals as well, and the quantitative data were not sufficient to determine a NOAEL or LOAEL. Some test animal studies provide limited evidence that benzene affects reproductive organs; however, these effects were limited to high exposure concentrations that exceeded the maximum tolerated dose. Fertility studies that have shown adverse effects on the number of live fetuses have employed benzene concentrations that caused severe maternal toxicity, as indicated by large reductions in body weight gain. Studies that used lower benzene concentrations have shown no reduction in fertility (Coate et al., 1984; Green et al., 1978; Kuna et al., 1992; Murray et al., 1979).

Results of inhalation studies conducted in test animals are fairly consistent across species and demonstrate that at concentrations of greater than 47 ppm (150 mg/m^3) benzene is fetotoxic and causes decreased fetal weight and/or minor skeletal variants (Coate et al., 1984; Green et al., 1978; Kuna and Kapp, 1981; Murray et al., 1979). Exposure of mice to benzene in utero during development has been shown to cause changes in the hematogenic progenitor cells in fetuses, 2-day neonates, and 6-week-old adults (Keller and Snyder, 1986, 1988). However, the biological significance of these effects is questionable because of the experimental design limitations.

4.6. SUSCEPTIBLE POPULATIONS

4.6.1. Possible Childhood Susceptibility

No data from human studies were found to indicate that children are more sensitive to benzene toxicity than are adults.

Exposure of mice to benzene in utero during development has been shown to cause changes in hematogenic progenitor cells in fetuses, 2-day neonates, and 6-week-old adults (Keller and Snyder, 1986, 1988). These results indicate that lasting damage to the hematopoietic system can occur during development. It is not known whether the effect is the same as in adults or is unique to developmental exposures. Dose-related hematoxicity was observed in the absence of any apparent maternal effects in Swiss-Webster mice (Keller and Snyder, 1986, 1988). At high doses, significantly increased skeletal variations in CF-1 mouse fetuses (Murray et al., 1979) and in rabbits (Ungvary and Tatrai, 1985) were observed.

4.6.2. Possible Gender Differences

No human exposure data were found to indicate that benzene affects human males and females differently. In the occupational benzene exposure study of Rothman et al. (1996), 21 of the 44 workers in both the exposed and control groups were female. The study, however, did not indicate that either gender was more affected. Although there are no data for humans, there are limited indications of gender differences in studies with test animals.

The most frequently observed gender difference in test animals is a greater sensitivity of male mice to benzene. Ward et al. (1985) reported that microscopic examination revealed that changes in the thymus, bone marrow, lymph nodes, spleen, and reproductive organs occurred more often and with greater severity in males than in females, following inhalation exposure at 300 ppm (958 mg/m^3) of CD-1 mice for 13 weeks. Male DBA/2 mice showed greater depression in bone marrow cellularity than female DBA/2 mice in response to inhalation exposure to 300 ppm (958 mg/m^3) for 13 weeks (Luke et al., 1988a).

Male mice were also more sensitive to benzene by oral exposure (NTP, 1986). In a 17-week oral gavage study, the LOAEL for hematological effects in male B6C3F1 mice was 50 mg/kg as compared to 400 mg/kg for females. Female rats, in contrast, appear to be more sensitive than males to benzene toxicity. In the NTP (1986) 17-week oral gavage study, the LOAEL for hematological effects for female F344/N rats was 25 mg/kg as compared to 100 mg/kg for males.

Male mice are two- to threefold more sensitive to the genotoxic effects of benzene as measured by micronuclei induction and sister chromatid exchanges (Luke et al., 1988a). Available studies indicate that the differential susceptibility to benzene is subject to hormonal regulation of the CYP2E1. Immature mice do not differ in sensitivity to micronuclei induction, and castrated males are less sensitive than females (Siou and Conan, 1980). However, treatment of castrated males with testosterone restores the sensitivity to micronuclei induction. Renal levels

of CYP2E1 in males are 20-fold higher than in females, but studies have indicated that renal levels of CYP2E1 can be induced in females by testosterone treatment (Hu et al., 1993; Pan et al., 1992). Kenyon et al. (1996) found that male B6C3F1 mice have an almost twofold faster rate of benzene oxidation than females. Phenol disappearance from the blood was also faster in male mice, suggesting that phenol metabolism is faster in males. These differences in benzene metabolism correlated with the sensitivity to genotoxicity.

Corti and Snyder (1996) found that CFU-E levels were depressed in livers of male fetuses of Swiss-Webster mice exposed in utero to 10 ppm (32 mg/m^3) in comparison to controls. Similarly, bone marrow CFU-E was also depressed in 6-week-old adult male mice that had been exposed in utero. No depression in CFU-E was observed in female fetuses or adult females. This suggests that differences in the sensitivity of male mice can be expressed in utero and persist into adulthood. This result is in contrast to the results observed by Siou and Conan (1980), who found that immature male and female mice did not differ in sensitivity to benzene.

Green et al. (1978) reported that exposure of pregnant mice to 2200 ppm (7028 mg/m^3) benzene caused a greater increase in missing sternebrae in female fetuses than in male fetuses, but this was not observed at concentrations of 300 ppm (958 mg/m^3) or less. The dose causing this effect induced extreme maternal toxicity, complicating interpretation of this effect. The apparently increased sensitivity of the female fetus to the effects of benzene is supported by other observations that female rabbits, mice, and rats are more sensitive to these effects of benzene (Desoille et al., 1961; Ito, 1962a-d; Sato et al., 1975). The investigators tentatively suggested that the differences in the responses of the males and females may be related to hormonal differences.

4.6.3. Possible Genetically Susceptible Populations

Ross (1996) has suggested that NAD(P)H:quinone oxidoreductase (NQO1) may play a critical protective role in benzene toxicity. This enzyme detoxifies the reactive 1,4-benzoquinone generated by myeloid peroxidase (MPO) by reducing it back to hydroquinone. Ross (1996) summarized evidence suggesting that the target cells of benzene toxicity have a high ratio of MPO:NQO1 activities in vivo. Traver et al. (1992) characterized a point mutation in the NQO1 gene that leads to a total loss of NQO1 activity. This appears to be a true polymorphism in the NQO1 gene, as Rosvold et al. (1995) found that the frequency of this mutant allele in a reference population was 13% and Edwards et al. (1980) reported that NQO1 was absent in 4% of samples taken from a British population. If the postulated role of NQO1 in detoxification of benzene metabolites is correct, then individuals lacking this enzyme activity could be especially susceptible to benzene toxicity.

Seaton et al. (1994) reported differences of up to 13-fold in liver microsome CYP2E1 among individual humans. Hepatic metabolism of benzene by the CYP2E1 enzyme has been shown to be the first step in generation of reactive benzene metabolites that are responsible for the toxicity of benzene. Thus, differences in CYP2E1 between individual humans could indicate potential differential susceptibility to benzene toxicity.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

5.1.1. Choice of Principal Study and Critical Effect

The human occupational inhalation study of Rothman et al. (1996) and the 28-day drinking water exposure study of Hsieh et al. (1988b) have been selected as co-principal studies. The study of Rothman et al. (1996) was selected because it is a well-conducted human exposure study demonstrating a dose-response relationship for the sensitive indicator of decreased absolute lymphocyte counts. The study of Hsieh et al. (1988b), though of short duration, was selected as a co-principal study because it demonstrate the lowest available LOAEL of 8 mg/kg-day for hematological effects in mice and used drinking water as the exposure method, which is the most likely oral exposure pathway for chronic human exposure.

The human occupational study of Rothman et al. (1996) showed significant reductions in absolute lymphocyte counts in Chinese factory workers exposed to a median 8-hr TWA concentration of 7.6 ppm (24 mg/m^3). This was the lowest exposure level examined in the study, and thus represents a human LOAEL for hematotoxic effects. This study showed that absolute lymphocyte count, WBC, RBC, hematocrit, and platelets were all significantly decreased, and MCV was significantly increased in a group of 44 workers occupationally exposed to a median 8-hr TWA of 31 ppm (99 mg/m^3) in comparison to an age- and sex-matched control group. These effects are consistent with the well-known hematotoxic effects of benzene (Aksoy, 1989; Goldstein, 1988). Only the absolute lymphocyte count was reduced in the subgroup exposed to a median 8-hr TWA of 7.6 ppm (24 mg/m^3) benzene (p<0.03), suggesting that this exposure level may be at or near the point of departure for hematotoxic effects of benzene in humans. The study of Rothman et al. (1996) is notable among epidemiology studies because benzene exposures were monitored, exposure to toluene was minimal, and subjects were compared to matched controls. A dose-response relationship was established between the absolute lymphocyte count and benzene air level and also with benzene metabolites in the urine. The median 8-hr TWA of 7.6 ppm (24 mg/m^3) was the lowest exposure group examined, and is therefore a LOAEL for benzene immunotoxicity in humans.

The co-principal study of Hsieh et al. (1988b) demonstrated significant (p<0.05) dose-dependent hematological effects (erythrocytopenia, leukocytopenia, lymphocytopenia, and increased MCV) at all exposure levels in male Charles River CD-1 mice. The hematological effects were accompanied by a biphasic response in several immunological tests with significantly increased responses at the low-exposure level and significantly decreased responses at the mid- and high-exposure levels, and also by a significantly decreased hematocrit at the mid- and high-dose levels. In one test, spleen cellularity was reported to be significantly decreased at all exposure levels, and in a separate test, only at the high-exposure level.

Although the NTP (1986) study featured a 103-week chronic exposure regimen, the lowest dose tested (25 mg/kg-day) demonstrated significant leukopenia and was higher than the LOAEL of 8 mg/kg-day identified in the Hsieh et al. (1988b) study.

An alternative possibility for the principal study would be to use the NOAEL of 0.7 mg/kg-day identified in the 6-mo study of Wolf et al. (1956). This study was not chosen because the results were presented only as a summary, actual data and statistical analysis were not reported, and rats have been shown to be consistently less sensitive than mice to benzene toxicity (Snyder et al., 1978; Ward et al., 1985; NTP, 1986).

5.1.2. Methods of Analysis

Rothman et al. (1996) identified a median 8-hr TWA concentration of 7.6 ppm (24 mg/m^3) as a LOAEL for the immunological endpoint, decreased absolute lymphocyte counts. A NOAEL was not identified as this was the lowest exposure group examined in the study.

Calculation of an equivalent oral dose rate from the inhalation LOAEL of 7.6 ppm is shown below.

The LOAEL is first converted to milligrams per cubic meter assuming 25°C and 760 mm Hg:

LOAEL (milligrams per cubic meter) = 7.6 ppm × MW/24.45 = 24 mg/m^3.

The converted value is then adjusted from the 8-hr TWA to an equivalent ambient human exposure concentration using the default occupational minute volume (U.S. EPA, 1994b).

$LOAEL_{[HEC]}$ = LOAEL (mg/m^3) × (VEho/VEh) × 5 days/7 days;

$LOAEL_{[HEC]}$ = 24 mg/m^3 × (10 m^3/20 m^3) × 5 days/7 days = 8.6 mg/m^3;

where:

$LOAEL_{[HEC]}$ = the LOAEL dosimetrically adjusted to a ambient human equivalent concentration;

LOAEL = occupational exposure level (8-hr TWA);

VEho = human occupational default minute volume (10 m^3/8 hr); and

VEh = human ambient default minute volume (20 m^3/24 hr).

An inhalation absorption rate of 50% was used to calculate the absorbed benzene dose, and oral absorption was assumed to be 100%. These values were based on human inhalation absorption studies (Nomiyama and Nomiyama, 1974a,b; Pekari et al., 1992; Srbova et al., 1950) and the study of Sabourin et al. (1987), which compared inhalation and oral absorption in rats and mice. Sabourin et al. (1987) found that the retention of benzene by rats and mice during a 6-hr exposure decreased as exposure concentration increased; 33% ± 6% to 15% ± 9% for rats, and 50% ± 1% to 10% ± 2% for mice as exposure concentration increased from 26 to 2,600 mg/m^3

(10 to 1,000 ppm). In the same study, gastrointestinal absorption of benzene administered by gavage was >97% for doses between 0.5 and 150 mg/kg body wt. At oral doses below 15 mg/kg >90% of the ^{14}C excreted was in the urine as non-ethylacetate-extractable material. At higher doses an increasing percentage of the orally administered benzene was exhaled unmetabolized. Thus, in the dose range represented by the LOAEL from the study of Rothman et al. (1996), absorption of a comparable oral dose was assumed to be 100%.

To calculate an equivalent oral dose rate, the $LOAEL_{[HEC]}$ is multiplied by the default inhalation rate, multiplied by 0.5 to correct for the higher oral absorption, and divided by the standard default human body weight of 70 kg (U.S. EPA, 1988):

$$8.6 \text{ mg/m}^3 \times 20 \text{ m}^3\text{/day} \times 0.5 \div 70 \text{ kg} = 1.2 \text{ mg/kg-day}.$$

The study of Hsieh et al. (1988b) identified a LOAEL of 8 mg/kg-day, the lowest dose tested, for hematological responses. A NOAEL was not identified because a significant adverse response was observed at the lowest dose tested.

5.1.3. RfD Derivation

Uncertainty factors (UFs) are applied to account for recognized uncertainties in extrapolation from experimental conditions to the assumed human scenario (i.e., chronic exposure over a lifetime). UFs are typically applied as values of 10 in a multiplicative fashion (Dourson and Stara, 1983). EPA practice, however, also includes use of a partial UF of $10^{1/2}$ (3.333; U.S. EPA, 1994b) on the assumption that the actual values for the UFs are log-normally distributed. In practice, when a partial UF is applied, the value is rounded to 3, such that the total factor for UFs of 3.33 and 10, for example, would be 30 (3 × 10). When two partial UFs are applied, however, they are not rounded, so UFs of 3, 3, and 10 would result in a total uncertainty factor of 100 (actually $10^{1/2} \times 10^{1/2} \times 10^{1}$).

To calculate an RfD using the oral equivalent dose of 1.2 mg/kg-day derived from the study of Rothman et al. (1996), the following UFs were selected. A factor of 10 was used for intraspecies differences in response (human variability) as a means of protecting potentially sensitive human subpopulations. A factor of 10 was applied for use of a LOAEL because of the lack of an appropriate NOAEL. Two partial UFs of 3 were applied to account for extrapolation from subchronic exposure to chronic exposure and for database deficiencies to give a final UF of 1000. Use of a partial UF for exposure duration adjustment is necessary because the mean exposure duration in the study (6.4 yr, range 0.7-16 yr) is less than the Superfund interim guidance cutoff value of 7 yr for designation as a chronic exposure (Means, 1989). Uncertainty regarding deficiencies in the database is small because of an adequate oral toxicity database and the extensive supporting inhalation database. The database includes developmental toxicity studies; however, a two-generation reproductive study was unavailable. No UF was applied for route-to-route exposure because of benzene's well-documented target organ specificity irrespective of the route of administration. There is also little evidence of toxic effects in either the lungs or the gastrointestinal tract. No modifying factor is considered necessary.

RfD = 1.2 mg/kg-day ÷ 1000 = 1E-3 mg/kg-day.

To calculate an RfD using the LOAEL value of 8 mg/kg-day in male mice from the study of Hsieh et al. (1988b), the following uncertainty factors were selected. A factor of 10 was used for intraspecies differences in response (human variability) as a means of protecting potentially sensitive human subpopulations. A factor of 10 was applied for consideration of interspecies variation. A partial uncertainty factor of 3 was applied for extrapolation from subchronic (28 days) to chronic duration, because the subchronic LOAEL from the study of Hsieh was lower than the LOAEL of 25 mg/kg-day determined in a chronic oral exposure study (NTP, 1986). A second partial uncertainty factor of 3 was applied for database deficiencies because of the lack of a two-generation reproductive study. A factor of 10 was applied for use of a LOAEL because of a lack of an appropriate NOAEL, to give a final uncertainty factor of 10,000.

RfD = 8.0 mg/kg-day ÷ 10,000 = 8E-4 mg/kg-day.

Although derivation of an RfD value based on the study of Rothman et al. (1996) requires route-to-route extrapolation, the level of uncertainty associated with this value is lower than the value calculated from the Hsieh et al. (1988b) study. Therefore, the RfD value of 1E-3 mg/kg-day is designated as the RfD.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

5.2.1. Choice of Principal Study and Critical Effect

The occupational study of Rothman et al. (1996) is designated as the principal study. This cross-sectional study compared the hematological outcomes of 44 workers occupationally exposed to benzene and 44 age- and gender-matched unexposed controls from Shanghai, China. Absolute lymphocyte count, WBC, RBC, hematocrit, and platelets were all significantly decreased, and MCV was significantly increased in a group of 44 workers occupationally exposed to a median 8-hr TWA of 31 ppm (99 mg/m^3) in comparison to an age- and sex-matched control group. These effects are consistent with the hematotoxic effects of benzene (Aksoy, 1989; Goldstein, 1988). Only the absolute lymphocyte count was reduced in the subgroup exposed to a median 8-hr TWA of 7.6 ppm (24 mg/m^3) benzene (p<0.03). The study of Rothman et al. (1996) is notable among epidemiology studies because benzene exposures were monitored, exposure to toluene was minimal, and subjects were compared to matched controls. A dose-response relationship was established between the absolute lymphocyte count and benzene exposure monitored by organic vapor passive dosimetry, as well as with benzene metabolites in the urine. The median 8-hr TWA of 7.6 ppm (24 mg/m^3) was the lowest exposure group examined, and it is therefore a LOAEL for benzene immunotoxicity in humans.

The principal study is also supported by a large database of inhalation and oral exposure experiments in a range of test animal species. Ward et al. (1996) found a strong exposure response relationship between estimated benzene dose and low WBC count in a rubber worker cohort. The maximum daily benzene exposure estimate in this study was 34 ppm (109 mg/m^3). A number of subchronic laboratory studies using mice have also indicated similar LOAEL or

NOAEL values of 10-30 ppm (32-96 mg/m^3) for hematotoxic and immunotoxic effects (Baarson et al., 1984; Rozen et al., 1984; Farris et al., 1997b; Robinson et al., 1997). These studies, however, present a range of exposure duration of 5 days to 178 days. For example, Baarson et al. (1984) exposed mice for 178 days, but used only one exposure concentration and thus did not provide dose-response information. Snyder et al. (1978, 1980, 1982, 1984) conducted chronic duration experiments, but these studies employed benzene concentrations of either 100 or 300 ppm (319 or 958 mg/m^3) that are well above the concentrations known to produce serious health effects. Keller and Synder (1986) demonstrated increases in colony-forming hematopoietic cells in fetuses following exposure of pregnant females to 5 or 10 ppm (16 or 32 mg/m^3) and decreases after exposure to 20 ppm (64 mg/m^3). Although this can be interpreted as a LOAEL of 5 ppm (16 mg/m^3), the toxicological significance of these biphasic responses is unclear. Keller and Synder (1988) also showed that the numbers of early nucleated red cells in 2-day-old neonates decreased in a dose-related response following in utero exposure to 5, 10, or 20 ppm (16, 32, or 64 mg/m^3) benzene. The toxicological significance of this response; however, is also unclear as these cells disappear soon after birth and were essentially absent from 6-week-old progeny regardless of benzene exposure. Thus, the data provided by Rothman et al. (1996) provide a suitably conservative basis for establishing an RfD that will be protective of human health, since the LOAEL may be close to the point of departure for the onset of hematological effects as noted above.

5.2.2. RfC Derivation

The co-principal study of Rothman et al. (1996) identified a median 8-hr TWA concentration of 7.6 ppm (24 mg/m^3) as a LOAEL for the immunological endpoint, decreased absolute lymphocyte counts. A $LOAEL_{[HEC]}$ was calculated as described in Section 5.1.2 by adjusting for the occupational ventilation rate and for the intermittent work week schedule for 24-hr exposure, 7 days per week:

$$LOAEL_{[HEC]} = 24\ mg/m^3 \times (10\ m^3/20\ m^3) \times 5\ days/7\ days = 8.6\ mg/m^3.$$

To calculate an RfC using the adjusted $LOAEL_{[HEC]}$ value of 8.6 mg/m^3, the following UFs were selected. A factor of 10 was used for intraspecies differences in response (human variability) as a means of protecting potentially sensitive human subpopulations. A factor of 10 was applied for use of a LOAEL because of lack of an appropriate NOAEL. Two partial uncertainty factors of 3 were applied to account for extrapolation from subchronic exposure to chronic exposure and for database deficiencies to give a final uncertainty factor of 1000. Use of a partial uncertainty factor for exposure duration adjustment is necessary because the mean exposure duration in the study (6.4 yr, range 0.7-16 yr) is less than the Superfund interim guidance cutoff value of 7 yr for designation as a chronic exposure (Means, 1989). Uncertainty regarding deficiencies in the database is small because of an adequate oral toxicity database and the extensive supporting inhalation database. The database includes developmental toxicity studies; however, a two-generation reproductive study was unavailable. There is also little

evidence of toxic effects in either the lungs or the gastrointestinal tract. No modifying factor is considered necessary.

$$RfC = 8.6\ mg/m^3 \div 1000 = 9E\text{-}3\ mg/m^3.$$

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

Benzene is widely used as an industrial solvent, as an intermediate in chemical syntheses, and as a gasoline additive (NTP, 1994). Because of its widespread use, the potential for human exposure is great. The toxicity of benzene has been recognized for more than a century, and the biological impacts of benzene exposure have been extensively studied in humans and experimental animal models. Metabolism of benzene is necessary for development of its toxic effects. Evidence has accumulated indicating that oxidation of benzene by CYP2E1 in the liver is the first step in initiation of benzene toxicity. Convincing evidence of the importance of CYP2E1 was provided by Valentine et al. (1996), who showed that genetic knockout mice lacking expression of the CYP2E1 protein produced much lower levels of benzene metabolites and failed to develop signs of hematotoxicity following acute benzene exposure at dose levels that resulted in severe genotoxicity and cytotoxicity in both wild-type and B6C3F1 mice.

The majority of benzene metabolism occurs in the liver, but the bone marrow is the target organ where its toxicity is expressed. The major hepatic metabolites of benzene are phenol, catechol, and hydroquinone. Catechol and hydroquinone have been shown to accumulate in bone marrow after benzene exposure (Rickert et al., 1979). The bone marrow has high peroxidase activity, which results in oxidation of the phenolic metabolites produced in the liver to the highly reactive 1,4 benzoquinone (Smith et al., 1989). The metabolic basis for the toxicity of benzene has been extensively studied (Snyder and Hedli, 1996).

The most frequently observed toxic effect of benzene, both in humans and test animal models, has been bone marrow depression, leading to lymphocytopenia, leukocytopenia, thrombocytopenia, anemia, and aplastic anemia (Aksoy, 1991; Goldstein, 1988). The most sensitive effect observed in humans is the depression of absolute lymphocyte counts in peripheral blood. In test animal model studies the most sensitive effects observed have been depressions of the colony-forming ability of bone marrow progenitor cells. These cells are responsible for producing the blood cells needed to replace the aging blood cells in the circulatory system.

Although a large number of human and experimental animal studies have been conducted, there have been few human studies with reliable estimates of exposure to benzene and few long-term repeated dose experiments in test animals. The human studies are also complicated by exposure to other solvents. The long-term test animal studies conducted have employed exposure levels that were too high to establish a reliable NOAEL, as significant adverse effects were observed even in the lowest dose tested in all the long-term studies examined. Thus, the lack of

reliable NOAEL values from either human or test animal studies is an area of uncertainty in establishing RfD and RfC values that are protective of human health.

An area of scientific uncertainty in this assessment concerns the neurotoxic effects of benzene. In common with many other organic solvents, benzene has been shown to produce neurotoxic effects in experimental animals and humans after short-term exposures to relatively high concentrations. Benzene produces generalized symptoms such as dizziness, headache, and vertigo, leading to drowsiness, tremor, delirium, and loss of consciousness. In an occupational study, Kahn and Muzyka (1973) reported that workers complained of frequent headaches (usually at the end of the work day), became tired easily, had difficulties sleeping, and complained of memory loss. There is a lack of reliable information on dose-related neurotoxic effects under low-dose, chronic exposure conditions in either humans or experimental animal model systems. Li et al. (1992) reported that forelimb grip strength and the frequency of rapid response in Y-maze running in Kunming mice was increased following brief inhalation exposure to 0.78 ppm (2.5 mg/m^3) benzene, but both responses were decreased at concentrations of 3.13 ppm (10 mg/m^3) or higher. Several experimental deficiencies in this study prevent its use to calculate human toxicity values, but further investigation of these effects could reveal neurotoxic effects of concern to human health.

There have been a number of developmental and reproductive studies in humans and in test animal model systems. Several test animal studies have shown developmental effects exhibiting manifestations such as reductions in numbers of live fetuses, reductions in live weight, and minor skeletal variants, but the benzene concentrations used also caused severe maternal toxicity. The studies of Keller and Snyder (1986, 1988), however, demonstrated that exposure to low concentrations of 5, 10, or 20 ppm (16, 32 or 64 mg/m^3) benzene in utero during development can cause changes in colony-forming hematopoietic cells (Keller and Snyder, 1986, 1988). These studies were considered as critical because the LOAEL of 5 ppm is below the LOAEL of 7.6 ppm established for hematoxic and immunotoxic effects observed in humans (Rothman et al., 1996). However, the confidence in the human data is much higher than that in the limited hematotoxic endpoints measured in mice by Keller and Snyder (1986, 1988).

6.2. DOSE RESPONSE

6.2.1. Oral Reference Dose

Quantitative estimates of human risk as a result of low-level chronic benzene exposure by the oral route are based on human occupational inhalation exposure and on subchronic test animal experiments, because no suitable chronic oral human or test animal data are available. Hematotoxicity and immunotoxicity in humans and test animals are the critical effects observed in both humans and test animals.

The human chronic dose of ingested benzene considered to be without any appreciable risk (the RfD) is 1E-3 mg/kg-day. This is 1/1000 of the oral equivalent dose extrapolated from the $LOAEL_{[HEC]}$, using decreased absolute lymphocyte count observed in the lowest exposure group investigated in a subchronic human occupational study as the indicator of adverse effects

(Rothman et al., 1996). The RfD is supported by an RfD value of 8E-4 mg/kg-day, which is 1/10,000 of the LOAEL dose, using hematological effects (erythrocytopenia, leukocytopenia, lymphocytopenia, and increased MCV) in a subchronic test animal drinking water study as the indicator of adverse effects (Hsieh et al., 1988a,b).

The overall confidence in this RfD assessment is medium. The co-principal studies of Rothman et al. (1996) and Hsieh et al. (1988b) were both well conducted; however, neither study identified a NOAEL. The co-principal study of Rothman et al. (1996) is a subchronic human occupational study. A dose-response relationship was established among the absolute lymphocyte count, benzene air level, and benzene urine metabolites. Five blood elements measured (absolute lymphocyte count, WBC, RBC, hematocrit, platelets, and MCV) were significantly different in the high benzene-exposure group in comparison to controls. However, only the absolute lymphocyte count was reduced in a subgroup exposed to a median 8-hr TWA of 7.6 ppm (24 mg/m^3) benzene, suggesting that this exposure level may be at or near the point of departure for the hematotoxic effects of benzene in humans. Thus, it is expected that further studies at lower exposure levels may not reveal further adverse effects. However, since a NOAEL was not established, the RfD could be subject to future revision.

The human occupational study of Rothman et al. (1996) investigated the effects of inhalation exposure and requires route-to-route extrapolation for calculation of an RfD. In experiments conducted to compare the metabolite doses to the target organ following oral or inhalation exposure, Sabourin et al. (1987, 1989a) found that there was no simple relationship between the two routes of exposure. Oral doses and inhalation exposures that produced similar concentrations of one metabolite in the blood produced very different doses of another metabolite. The target specificity of benzene toxicity for the bone marrow progenitor cells irrespective of route of administration, however, is well documented both in humans and experimental animal models. Thus, the route-to-route extrapolation is justified and introduces a lower degree of uncertainty than extrapolating from test animals to humans. The assumption of 50% benzene absorption also adds scientific uncertainty to the derivation of the RfD. Srbova et al. (1950) found that initial inhalation of benzene at 47-100 ppm (150-319 mg/m^3) by humans was 70%-80%, but decreased to approximately 50% after 1 hr. Nomiyama and Nomiyama (1974a) reported an initial retention at 50%-60% that reached a steady state at 30%-40%. Pekari et al. (1992) exposed three volunteers to benzene at levels of 1.6 or 9.4 ppm (5-30 mg/m^3) for 4 hr. Estimates were 48% for the high dose and 52% for the low dose. Sabourin et al. (1987) found that the retention of benzene by rats and mice during a 6-hr exposure decreased as exposure concentration increased: 33% ± 6% to 15% ± 9% for rats and 50% ± 1% to 10% ± 2% for mice as exposure concentration increased from 26 to 2600 mg/m^3 (10 to 1000 ppm). In contrast, Sabourin et al. (1987) found that gastrointestinal absorption was >97% for oral doses up to 150 mg/kg. Thus, the assumption of 50% absorption from oral exposures in comparison to 100% absorption is well supported by experimental data and introduces little additional uncertainty into the route-to-route extrapolation.

Another area of scientific uncertainty in the use of the Rothman et al. (1996) study concerns the duration of exposure. The length of exposure in human studies that constitutes subchronic exposure is not defined (U.S. EPA, 1994b). Because a study that is 10% of a test animal's lifespan is considered subchronic, an exposure duration of 7 yr (one-tenth of the assumed

human lifespan of 70 yr) has been used as interim guidance for the Superfund program as a cutoff for deriving a subchronic human reference dose (Means, 1989). A partial UF was applied in the calculation of the RfC based on the Rothman et al. (1996) study because the mean length of exposure of 6.4 yr is less than the interim guidance level of 7 yr, and the weight of evidence indicates that although initial-development bone marrow depression is rapid in both human and several experimental animal model systems, the effects progress with chronic exposure. Ward et al. (1996) found that estimated cumulative benzene exposure in the past 30 days, 60 days, 180 days, or total all showed a significant relationship with incidence of low WBC counts in a rubber worker cohort. The strength of the association, however, increased with the length of exposure, and the strongest relationship was found with total cumulative exposure. Ward et al. (1996) did not find a significant relationship between the estimated benzene exposure in the past 30, 60, or 180 days and low RBC counts, but the relationship was statistically significant ($p<0.03$) for total cumulative exposure. The NTP (1986) oral gavage exposure study also showed that decreased lymphocyte counts in mice at the low-dose level only developed after 12 mo of exposure. These results suggest that the severity of hematotoxicity and immunotoxicity increase in severity at low exposure levels with prolonged exposure.

The co-principal study of Hsieh et al. (1988b) suffers from several deficiencies, including small group size (5/group), use of only one sex of one species, short exposure duration (28 days), and failure to identify a NOAEL. The observed hematological responses, however, are consistent with a large body of literature; the exposure route, drinking water, is appropriate for expected human oral exposure; male mice are the most sensitive sex of the most sensitive species; and the LOAEL of 8 mg/kg-day is lower than the LOAEL identified in the long-term gavage study of NTP (1986). Results from the other subchronic studies (White et al., 1984; NTP, 1986) and the chronic study (NTP, 1986; Huff et al., 1989) support the critical effects identified in the principal study.

6.2.2. Inhalation Reference Concentration

Quantitative estimates of human risk as a result of low-level chronic benzene inhalation exposure are based on subchronic human occupational inhalation exposure because no suitable chronic human data are available. Hematotoxicity and immunotoxicity are the critical effects observed in humans.

The human chronic air concentration (RfC) considered to be safe is 9E-3 mg/m^3. This is 1/1000 the $LOAEL_{[HEC]}$, using decreased absolute lymphocyte count observed in the lowest exposure group investigated in a subchronic human occupational study as the indicator of adverse effects (Rothman et al., 1996).

The overall confidence in this RfC assessment is medium. The principal study is a subchronic human occupational study (Rothman et al., 1996). A dose-response relationship was established between the absolute lymphocyte count and benzene air level and benzene urine metabolites. Five blood elements measured (absolute lymphocyte count, WBC, RBC, hematocrit, platelets, and MCV) were significantly different in the high benzene-exposure group in comparison to controls. However, only the absolute lymphocyte count was reduced in a

subgroup exposed to a median 8-hr TWA of 7.6 ppm (24 mg/m^3) benzene, suggesting that this exposure level may be at or near the point of departure for the hematotoxic effects of benzene in humans. Thus, it is expected that further studies at lower exposure levels may not reveal further adverse effects. However, since a NOAEL was not established, the RfC could be subject to future revision.

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APPENDIX A. BENCHMARK CONCENTRATION ANALYSIS OF DATA FROM ROTHMAN ET AL., 1996

The RfD and RfC values derived in this Toxicological Review of the Noncancer Effects of Benzene were based on the principal study by Rothman et al. (1996). Rothman et al. conducted a cross-sectional study of 44 workers exposed to a wide range of benzene concentrations and 44 age- and gender-matched unexposed controls from Shanghai, China. The median 8-hour TWA benzene exposure concentration for all exposed workers was 31 ppm (99 mg/m^3). The exposed group was subdivided into 2 equal groups of 22 of those exposed to greater than the median concentration and those exposed to less than the median concentration. The median (range) 8-hour TWA exposure concentration was 13.6 (1.6–30.6) ppm (43.4 [5.1–97.8] mg/m^3) for the low-exposure group and 91.9 (31.5–328.5) ppm (294 [101–1049] mg/m^3) for the high-exposure group. A subgroup of the low-exposure group composed of 11 individuals who were not exposed to >31 ppm (100 mg/m^3) at any time during the monitoring period was also examined in some comparisons. The median (range) 8-hour TWA exposure of these individuals was 7.6 (1–20) ppm (24 [3.2–64] mg/m^3).

Because the exposed individuals in this study were divided into only two primary exposure groups—those with mean 8-hour TWA exposure concentrations above the median and those below the median—there were not enough points in the dose-response curve to perform a benchmark dose analysis.