EPA/635/R-02/001F

TOXICOLOGICAL REVIEW

OF

BENZENE

(NONCANCER EFFECTS)

(CAS No. 71-43-2)

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

October 2002

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 published relevant 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 U.S. Environmental Protection Agency's standing guidance of several relevant risk assessment guidelines dealing with reproductive, developmental, and neurotoxic effects. This draft has undergone internal peer review and an expert external peer-panel review in October 1998 as well as a 90-day public comment period.

The emphasis of this document is a discussion of the noncancer adverse health effects of benzene, including the no-observed-adverse-effect levels, the lowest-observed-adverse-effect levels, benchmark dose analysis, uncertainty factors, and any other considerations used to develop the RfDs and RfCs for benzene.

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LIST OF ACRONYMS AND ABBREVIATIONS

AChE	acetyl cholinesterase
ALC	absolute lymphocyte count
AML	acute myelogenous leukemia
ANOVA	analysis of variance
ASA	acetylsalicylic acid
AUC	area under the curve
BFU-E	burst-forming units–erythroid
BMC	benchmark concentration
BMCL	95% lower confidence limit on the BMC
BMD	benchmark dose
BMDL	95% lower confidence limit on the BMD
BMR	benchmark response
CFU-C	colony-forming units–culture
CFU-C/tibia	colony-forming granulopoietic stem cells
CFU-E	colony-forming units–erythroid
CFU-HPP	high proliferative potential colony-forming units
CFU-S	colony-forming units–spleen
CHO-M-OH	6-hydroxy-*trans,trans*-2,4-hexadienal
CI	confidence interval
Con A	concanavalin A
COOH-M-CHO	6-oxo-*trans,trans*-2,4-hexadienoic acid
COOH-M-OH	6-hydroxy-*trans,trans*-2,4-hexadienoic acid
CYP2E1	cytochrome P450 2E1
EMG	electromyographical
EPA	U.S. Environmental Protection Agency
F344	Fischer 344
GD	gestation day

LIST OF ACRONYMS AND ABBREVIATIONS (continued)

GM-CFU-C	granulocyte/macrophage colony-forming unit–culture
GM-CFU-G	granulocyte/macrophage colony-forming unit
GPA	glycophorin A
GSH	glutathione
GTP	guanosine triphosphate
HCT	hematocrit
Hgb	hemoglobin
HSDB	Hazardous Substance Data Bank
IC_{50}	Inhibition concentration 50%
i.p.	intraperitoneal
IL-1	interleukin-1
IL-1α	interleukin-1alpha
IL-1β	interleukin-1beta
IRIS	Integrated Risk Information System
Kd	kilodalton
LOAEL	lowest-observed-adverse-effect level
LPS	lipopolysaccharide
MA	*trans,trans*-muconic acid
MCH	mean corpuscular hemoglobin
MCV	mean corpuscular volume
MDS	myelodysplastic syndrome
MPO	myeloperoxidase
MUC	muconaldehyde
NADPH	nicotinamide adenine dinucleotide phosphate
NCEA	National Center for Environmental Assessment
NCEA-W	National Center for Environmental Assessment–Washington Division
NK	natural killer

LIST OF ACRONYMS AND ABBREVIATIONS (continued)

NOAEL	no-observed-adverse-effect level
NQO1 NAD(P)H	NAD(P)H quinone oxidoreductase
NTP	National Toxicology Program
OR	odds ratio
PBPK	physiologically based pharmacokinetic
PCE	polychromatic erythrocytes
PC-PFC	polyclonal plaque-forming cells
PCV	packed cell volume
PHA	phytohemaglutinin
RBC	red blood cell
RfC	reference concentration
RfD	reference dose
SD	standard deviation
SE	standard error
SRBC	sheep red blood cell
TWA	time-weighted average
UF	uncertainty factor
U.S. EPA	U.S. Environmental Protection Agency
WBC	white blood cell
YAC	yeast artificial chromosome

EXECUTIVE SUMMARY

The U.S. Environmental Protection Agency's (EPA's, the Agency's) Office of Mobile Sources, Office of Air and Radiation, requested that the National Center for Environmental Assessment (NCEA) provide an updated characterization of human health risk from exposure to benzene. Recently, EPA published *Carcinogenic Effects of Benzene: An Update* (EPA/600/P-97/001F, April 1998 [U.S. EPA, 1998a]) and *Extrapolation of the Benzene Inhalation Unit Risk Estimate to the Oral Route of Exposure* (NCEA-W-0517, April 1999). These documents serve as a source of information regarding the carcinogenic effects of exposure to benzene.

The scope of the present report is limited to an assessment of the noncancer effects of 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 syntheses, and as a component of gasoline; hence, the potential for human exposure is great. Inhalation exposure is the major route of exposure to benzene, although oral and dermal 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 among 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. 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 in humans is via exhalation. 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 in humans following dermal exposure. Physiologically based pharmacokinetic models have been developed and are being improved upon to better define interactions of benzene metabolism, toxicity, and dosimetry. These interactions for humans exposed to low concentrations can be assessed only when the mode of action is understood at a quantitative level and is incorporated within a physiological modeling framework. However, the current models are insufficiently developed and validated to allow them to predict with certainty the relationship between metabolism and toxicity of benzene.

Benzene exposure results in adverse noncancer health effects by all routes of administration to test animal species. Hematotoxicity has been consistently reported to be the most sensitive indicator of noncancer toxicity both in limited studies in humans and experimental animals, with bone marrow as the principal target organ. Chronic exposure to benzene results in progressive deterioration of hematopoietic function. Whether the hematotoxic and carcinogenic effects of benzene are due to a common mechanism has not been established. Although leukocytopenia has been consistently shown to be a more sensitive indicator than anemia of benzene toxicity in experimental animals, lymphocytopenia has been shown to be even more sensitive than

leukocytopenia. A decrease in absolute lymphocyte count (ALC) was observed to be the most sensitive indicator of benzene exposure in several epidemiologic studies.

Benzene has also been shown to produce neurotoxic effects in test animals and humans after short-term exposure, but at relatively high concentrations; however, long-term neurotoxicity exposure studies are lacking. There is some evidence of adverse reproductive and developmental effects due to benzene exposure from human epidemiologic studies, but data are not conclusive enough to link low exposure levels to effects. No data from human studies were found to indicate that children are more sensitive than adults to benzene toxicity, nor were any significant gender differences found. The most frequently observed gender difference in test animals is a greater sensitivity of male mice to benzene exposure; in rats, females appear to be more sensitive than males. However, in the National Toxicology Program (NTP) study (NTP, 1986) the male was the most sensitive sex in the rat.

Although a large number of epidemiologic and experimental animal studies are available for evaluating noncancer health effects, there are few reports of human data with sufficiently reliable estimates of exposure to benzene and few long-term, repeated-dose experiments in test animals. Many of the epidemiologic studies are also complicated by exposure to other solvents, and some of the longer-term animal studies have employed exposure levels that were too high to establish reliable no-observed-adverse-effect level (NOAEL) values.

The epidemiologic occupational inhalation study by Rothman et al. (1996a) was chosen as the principal critical study for deriving both an RfC and an RfD. This study showed significant reductions in ALC, red blood cells (RBCs), and platelets in a subgroup of Chinese factory workers exposed to a median 8-hour time-weighted average (TWA) concentration of 13.6 ppm (43.4 mg/m^3). In a subgroup of 11 workers exposed to a median 8-hr TWA concentration of 7.6 ppm (24 mg/m^3), only ALC was still significantly reduced. Thus this concentration is a lowest-observed-adverse-effect level (LOAEL) for benzene immunotoxicity in humans. The findings from this study also indicate that white blood cells were significantly decreased and the mean corpuscular volume was significantly increased in the total exposed group of 44 workers occupationally exposed to a median 8-hour TWA of 31 ppm (99 mg/m^3) in comparison to an age- and sex-matched control group. These dose-response effects are consistent with the well-known hematotoxic effects of benzene in humans and experimental animals, as discussed in Sections 4.1.2.1 and 4.2.1 and summarized in Tables 1 and 6.

Reduction in ALC was the most sensitive endpoint in the Rothman et al. (1996a) study, and the ALC exposure-response data were used in benchmark dose (BMD) modeling to obtain a point of departure for the derivation of the RfC. It was necessary to transform the data to obtain an adequate fit with the models for continuous data; then, the continuous linear model provided the best fit. In the absence of a clear definition for an adverse effect for this continuous endpoint, a default benchmark response of one standard deviation change from the control mean response was selected, as suggested in EPA's draft Benchmark Dose Technical Guidance Document (U.S. EPA, 2000b). This default definition of a benchmark response for continuous endpoints corresponds to an excess risk of approximately 10% for the proportion of individuals below the 2^{nd} percentile (or above the 98^{th} percentile) of the control distribution for normally distributed effects. The BMD modeling thus resulted in a benchmark concentration (BMC) of 13.7 ppm (8-hour TWA) and a BMCL (95% lower confidence unit on the BMC) of 7.2 ppm (8-hour TWA).

As suggested in EPA's draft *Benchmark Dose Technical Guidance Document*, the BMCL was chosen as the point of departure for the RfC derivation. After converting to mg/m^3 and adjusting for continuous exposure, a $BMCL_{ADJ}$ of 8.2 mg/m^3 was obtained. Dividing this value by an overall uncertainty factor (UF) of 300 yields a chronic inhalation RfC of 3×10^{-2} mg/m^3. Because the BMC is considered to be an adverse-effect level, an effect-level extrapolation factor analogous to the LOAEL-to-NOAEL UF was used. EPA is planning to develop guidance for applying an effect-level extrapolation factor to a BMD. In the interim, a factor of 3 was used in this analysis. Additional factors of 10 for intraspecies variability, 3 for subchronic-to-chronic extrapolation, and 3 for database deficiencies—due to the absence of a two-generation reproductive/developmental toxicity study for benzene—comprise the remainder of the 300 composite UF.

For comparison, an RfC was also calculated from the LOAEL of 7.6 ppm (8-hour TWA) from Rothman et al. (1996a). After converting to mg/m^3 and adjusting for continuous exposure, a $LOAEL_{ADJ}$ of 8.7 mg/m^3 was obtained. Dividing this value by an overall UF of 1000 yields an RfC of 9×10^{-3} mg/m^3. The UF of 1000 was based on factors of 10 to account for the use of a LOAEL because of the lack of an appropriate NOAEL, 10 for intraspecies variability, 3 for subchronic-to-chronic extrapolation, and 3 for database deficiencies. This result of 9×10^{-3} mg/m^3 based on the LOAEL is in good agreement with the result of 3×10^{-2} mg/m^3 based on the BMCL.

Support for this chronic inhalation RfC has been provided by the experimental animal study of Ward et al. (1985). The subchronic inhalation study of Ward et al. (1985) was selected as a supporting study because it was the experimental animal study with the longest inhalation exposure duration and it provided good dose-response data. The exposure-response relationships for the different hematologic endpoints in male mice (the most sensitive sex/species in this study) were modeled using a BMD modeling approach, and decreased hematocrit (i.e., volume percentage of erythrocytes in whole blood) was chosen as the critical effect. The continuous linear model was selected because it provided the best fit. As above, a default level of one standard deviation change from the control mean response was used as the benchmark response. A BMC of 100.7 ppm and a BMCL of 85.0 ppm were obtained.

The BMCL was chosen as the point of departure for the RfC derivation. After converting to mg/m^3 and adjusting for continuous exposure, a $BMCL_{ADJ}$ of 48.5 mg/m^3 was obtained. As discussed above, a UF of 3 is used as an effect-level extrapolation factor, analogous to a LOAEL-to-NOAEL UF, because the BMC is considered an adverse-effect level. In addition, the standard UFs of 3 for interspecies extrapolation for inhalation studies and 10 for intraspecies variability are applied. A UF of 3 for database deficiencies is used, as above. Finally, a partial UF of 3 was used to extrapolate from subchronic to chronic exposure. Dividing the $BMCL_{ADJ}$ by the overall uncertainty factor of 1000 yields a chronic inhalation RfC of 5×10^{-2} mg/m^3. This value is in good agreement with the RfC of 3×10^{-2} mg/m^3 based on BMD modeling of the ALC data from the Rothman et al. (1996a) human study.

Similarly, for comparison purposes, a chronic inhalation RfC can be derived from the NOAEL of 30 ppm observed for hematologic effects in the Ward et al. (1985) study. First, the NOAEL is converted to mg/m^3 and adjusted to equivalent continuous exposure, yielding 17.1 mg/m^3. UFs are identical to those employed above, except that no NOAEL-to-LOAEL UF is used; thus, the overall UF is 300. Dividing 17.1 by 300 results in an RfC of 6×10^{-2} mg/m^3. This value is also in good agreement with the RfC derived from the Rothman et al. (1996a) study.

To derive the RfD, the same BMCL of 7.2 ppm (8-hour TWA) for the default benchmark response of one standard deviation change from the control mean response for the critical effect of reduced ALC in Rothman et al. (1996a) was used as the point of departure. After converting the units, correcting for continuous exposure, and adjusting for the route-to-route extrapolation from inhalation to oral exposure, a $BMCL_{ADJ}$-equivalent oral dose rate of 1.2 mg/kg/day was obtained. Dividing the $BMCL_{ADJ}$-oral of 1.2 mg/kg/day by an overall UF of 300 yields a chronic oral RfD of 4×10^{-3} mg/kg/day. As above, a UF of 3 is used as an effect-level extrapolation factor, analogous to the LOAEL-to-NOAEL UF, because the BMC is considered to be an adverse-effect level. Additional factors of 10 for intraspecies variability, 3 for subchronic-to-chronic extrapolation, and 3 for database deficiencies comprise the remainder of the 300 composite UF.

For comparison, an RfD was also calculated from the LOAEL of 7.6 ppm (8-hour TWA) from Rothman et al. (1996a). After unit conversion, correction for continuous exposure, route-to-route extrapolation, and division by a combined UF of 1000, an RfD of 1×10^{-3} mg/kg/day was derived from the LOAEL. The combined UF of 1000 was based on factors of 10 to account for using a LOAEL because of the lack of an appropriate NOAEL, 10 for intraspecies variability, 3 for subchronic-to-chronic extrapolation, and 3 for database deficiencies. This RfD value of 1×10^{-3} mg/kg/day is in good agreement with the value of 4×10^{-3} mg/kg/day calculated from the BMCL.

For further comparisons, RfDs were calculated on the basis of the NTP's chronic experimental animal study (NTP, 1986). In this study, Fischer 344 (F344) rats and B6C3F1 mice of both sexes were administered benzene by gavage, and blood was drawn from subgroups of animals at various time points. This study identified a LOAEL of 25 mg/kg for leukopenia and lymphocytopenia in female F344 rats and male and female B6C3F1 mice; no NOAEL was identified. Reduction in lymphocyte count was selected as the critical effect, and the dose-response relationships at different time points were modeled using a BMD modeling approach. As above, a default benchmark response of one standard deviation change from the control mean was used to define the BMDs. The modeling results suggested that the male rat was the most sensitive sex/species in this NTP gavage bioassay, and a BMDL (95% lower confidence limit on the BMD) of 1 mg/kg was selected as the point of departure for deriving the RfD.

The BMDL was first adjusted for exposure 7 days/week and then divided by a composite UF of 1000. This composite UF reflects a UF of 3 used as an effect-level extrapolation factor, analogous to LOAEL-to-NOAEL UF, because the BMD is considered to be an adverse effect; a UF of 10 for interspecies extrapolation for oral studies; a UF of 10 for intraspecies variability; and a UF of 3 for database deficiencies. The resulting RfD was 7×10^{-4} mg/kg/day, which is in reasonably good agreement (within an order of magnitude) with the RfD of 4×10^{-3} mg/kg/day derived from the Rothman et al. (1996a) human inhalation study.

An RfD was also derived from the LOAEL of 25 mg/kg observed in the NTP (1986) study. The LOAEL was adjusted to a continuous exposure level of 17.9 mg/kg/day and then divided by a UF of 3000 to derive an RfD of 6×10^{-3} mg/kg/day. The combined UF of 3000 is based on factors of 10 for the absence of a NOAEL, 10 for interspecies extrapolation, 10 for intraspecies variability, and 3 for database deficiencies. This value of 6×10^{-3} mg/kg/day is in good agreement with the value 4×10^{-3} mg/kg/day derived from the Rothman et al. (1996a) human study.

1. INTRODUCTION

The overall purpose of this document is to provide a toxicological review of noncancer health effects of exposure to benzene, including the no-observed-adverse-effect levels (NOAELs), the lowest-observed-adverse-effect levels (LOAELs), benchmark dose (BMD) modeling, and uncertainties in derivation of an inhalation reference concentration (RfC) and an oral reference dose (RfD). This toxicological review document may serve as the scientific basis for establishing a national air quality standard for ambient air exposure to benzene by the U.S. Environmental Protection Agency's (EPA's) Office of Mobile Sources, Office of Air and Radiation, under the Clean Air Act Amendments of 1990, Subchapter II (42 U.S.C. 7521-7590) for hazardous air pollutants.

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

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 milligrams per kilograms per day (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 it provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for the respiratory system (portal-of-entry effects) and for systems peripheral to the respiratory system (extrarespiratory or systemic effects). It is generally expressed in units of milligrams per cubic meter (mg/m^3). The RfC and RfD do not represent a sharp dividing line between safe and unsafe. In establishing the RfC and RfD, all relevant biologically significant noncancer health effects in the published literature were reviewed and considered.

Development of these hazard identification and dose-response assessments for benzene has followed the general framework for risk assessment, as set forth by the National Research Council (1983). EPA guidelines that were used in the development of this assessment include *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998b), *Proposed Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1996a), *Draft Revised Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1999b), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996b), *Risk Assessment of Guidelines 1986* (U.S. EPA, 1987), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), and *Benchmark Dose Technical Guidance Document* (external review draft; U.S. EPA, 2000b).

Literature search strategies employed for this compound were based on the Chemical Abstracts Registry Number (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.

- CASRN: 71-43-2
- U.S. EPA Hazardous Waste No.: U019 (in commercial product)
  F005 (nonspecified source, spent solvent)
- Hazardous Substance Data Bank (HSDB, 1997) No.: 2554
- Synonyms: annulene, benzeen (Dutch), benzen (Polish), benzine, benzol, benzole, benzolo (Italian), bicarburet of hydrogen, coal naphtha, cyclohjexatriene, fenzen (Czech), mineral naphtha, motor benzol, NCI-C55276, phene, phenyl hydride, pyrobenzol, pyrobenzole
- Registered trade name: Polystream
- Empirical formula: C_6H_6
- Molecular weight: 78.11 (Budvari, 1989)
- Vapor pressure: 75 mm Hg at 20°C (NFPA, 1994)
- Water solubility: 1750 mg/L at 25°C (Banerjee et al., 1980). Miscible with ethanol, ethyl ether, acetone, and chloroform.
- Partition coefficients: Log K_{ow}: 2.13 (Hansch and Leo, 1985)
  Log K_{oc}: 1.8 to 1.9 (HSDB, 1997)
- Henry's law constant: 5.5×10^{-3} atm-m^3/mol (Mackay and Leinonen, 1975) at 25°C
- Conversion factor: 1 ppm = 3.24 mg/m^3 at 20°C; 1 mg/m^3 = 0.31 ppm; 1 mg/L = 313 ppm
- Melting point: 5.5°C (HSDB, 1997)
- Boiling point: 80.1°C at 760 mm Hg (HSDB, 1997)
- Odor: Aromatic (HSDB, 1997)
  Lower 0.84 ppm (HSDB, 1997)
  Upper 53 ppm (HSDB, 1997)

- Taste threshold: 0.5 to 4.5 mg/L (HSDB, 1997)
- PEL (permissible exposure limit) TWA = 1 ppm (OSHA, 1987)
- TLV (threshold limit value) TWA = 10 ppm (ACGIH, 1996)

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

The absorption, distribution, metabolism and excretion of benzene have been intensively investigated in several experimental animal species and in humans. Benzene is readily absorbed from oral and inhalation exposures. Dermal absorption is also rapid; however, quantitatively, dermal absorption is very low due to rapid evaporation from skin. Benzene is rapidly distributed throughout the body after exposure by all routes, and accumulation in fatty tissues is observed. Metabolism of benzene is necessary for the expression of the characteristic hematotoxic and carcinogenic effects of benzene. Despite extensive research, no single metabolite has been identified as responsible for all the toxic effects of benzene, and the weight of evidence points toward an interaction of several metabolites. At low doses, benzene is rapidly metabolized and excretion occurs primarily as conjugated metabolites; however, at higher doses metabolic pathways become saturated, and exhalation of unmetabolized benzene is observed to be the primary route of excretion. Therefore, extrapolation from results observed at high doses underestimates the potential toxic effects of low doses. The pathways of benzene metabolism appear to be qualitatively similar across species. Quantitative differences in metabolism among animal species are observed, however, and no good model for human metabolism has been established. Thus, despite the development of several physiologically based pharmacokinetic (PBPK) models, extrapolation of results from laboratory animal results to humans has proved difficult.

3.1. ABSORPTION

Benzene is readily absorbed by both test animals and humans from inhalation, oral, and dermal exposures (U.S. EPA, 2000a).

3.1.1. Gastrointestinal Absorption

Although only limited data are available on absorption of benzene in humans by the oral route, accidental or intentional poisoning case studies indicate that benzene is readily absorbed (Thienes and Haley, 1972).

Nearly complete absorption of orally administered benzene has been demonstrated in laboratory animal studies. Parke and Williams (1953) performed a mass-balance study using radiolabeled ^{14}C-benzene with gavage exposures in rabbits at doses of 340–500 mg/kg. Calculation of the mass balance 2–3 days after exposure showed that 84–89% of the administered ^{14}C could be accounted for as metabolites, CO_2, and exhaled unchanged benzene. Thus, approximately 90% of the dose was absorbed. Sabourin et al. (1987) similarly found that gastrointestinal absorption of ^{14}C-benzene administered to rats and mice at a dose range of 0.5 to 150 mg/kg was greater than 97%. Although this study used corn oil as a vehicle, it is reasonable

to assume that oral absorption from water solutions would also be close to 100%. One could expect that the presence of food in the stomach may delay absorption and an empty stomach may enhance it.

The presence of soil had only minor effects on absorption of orally administered ^{14}C-benzene (Turkall et al., 1988). Male rats were gavaged with 5% aqueous solutions of gum acacia with either benzene alone or with benzene preadsorbed onto a sandy soil or a clay soil. Presence of soil had no significant effect on the absorption of benzene, as less than 2% of the administered dose was excreted in the feces within 48 hours. Adsorption of benzene to soil apparently increased the rate of gastrointestinal absorption, as the peak plasma benzene concentrations were higher in both soil treatments, and the time to peak was reduced in the sandy soil. The presence of both soils also increased the proportion of ^{14}C excreted in the urine, as opposed to excretion as unmetabolized benzene in expired air. Increased excretion in the urine also indicated that the proportion of the benzene subject to metabolism was increased. Phenol was the primary metabolite detected in the acid-hydrolyzed urine in all treatment groups, followed by hydroquinone, catechol, and benzenetriol. No significant differences in the distribution of metabolites among the treatments were found. Because the urine extracts were acid-hydrolysed, conjugation products were not determined. Gastrointestinal absorption was apparently rapid and efficient, even in the presence of soil.

3.1.2. Respiratory Absorption

There is a significant experimental database on the respiratory absorption of benzene in humans (Nomiyama and Nomiyama, 1974; Pekari et al., 1992; Srbova et al., 1950; Yu and Weisel, 1998). Srbova et al. (1950) examined absorption of benzene by 23 human subjects exposed to a range of concentrations, from 47 to 100 ppm (150 to 320 mg/m^3), for 2–3 hours. Absorption was greatest in the first 5 minutes of exposure (70–80%) but declined rapidly over the next 15 minutes and varied between 20 and 60% after 1 hour and between 20 and 50% after 2 hours of exposure. Considerable variability between individuals was noted. Nomiyama and Nomiyama (1974) determined both retention and uptake of benzene by three female and three male subjects, 18–25 years of age, exposed to 52–62 ppm (166–198 mg/m^3) for 4 hours. Exhaled air was sampled every hour to determine respiratory excretion of benzene. Retention declined from approximately 50% in the first hour and stabilized at 30% after 3 hours. Respiratory uptake averaged 47%, with excretion of 17%. No significant differences between males and females were reported; however, as noted, only three subjects of each sex were examined.

Pekari et al. (1992) studied respiratory absorption of benzene in three males exposed to 1.7 and 10 ppm (5 and 39 mg/m^3) benzene for 4 hours each. Absorption of benzene was determined by measuring differences in concentration between inhaled and exhaled air. The average absorption was 52% at the low concentration and 48% at the high concentration. In a recent study, Yu and Weisel (1998) measured the uptake of benzene from sidestream tobacco smoke by three female subjects. Benzene concentrations were measured in inhaled and exhaled air. Smoke was generated by burning cigarettes, resulting in a variable benzene concentration ranging from 32 to 69 ppm (102 to 220 mg/m^3). Absorption in eight experiments averaged 64%, with a range of 48–73%. The exposure periods were either 30 or 120 minutes, but no significant decrease in absorption with the longer exposure period was observed.

Considered together, these studies indicate that the respiratory absorption of benzene in humans is approximately 50%. The observed decline in absorption with increasing exposure time is apparently due to respiratory excretion of unmetabolized benzene. Animal studies indicate that the metabolism of benzene is saturated at exposure concentrations in excess of 10 ppm (32 mg/m^3). Thus, with the exception of the study by Pekari et al. (1992), benzene metabolism may have been saturated in the human respiratory absorption studies. This would have been expected to lead to greater respiratory excretion of unmetabolized benzene. Because absorption at low dose levels is most relevant for derivation of an RfD, the estimates of 48–52% retention derived by Pekari et al. (1992) are the most relevant values for evaluating absorption in humans.

A number of test animal studies have also been conducted on the absorption of benzene from inhalation exposure. 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 at these exposure concentrations. Exposure concentration appears to affect the retention of inhaled radioactivity, as demonstrated by Sabourin et al. (1987) in rats and mice. The retention of benzene by rats and mice during a 6-hour 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, uptake was higher in mice. At exposure concentrations below 350 mg/m^3, mice retained approximately 50% more radioactivity per kilogram body weight than did rats, but there was no significant difference at the highest (2500 mg/m^3) concentration. In general, mice inhaled greater amounts of benzene per kilogram body weight due to their higher relative minute volume per kilogram than other species.

Henderson (1996) reviewed species differences in absorption and retention of benzene given by either the oral or the inhalation route. Mice have both a higher respiratory rate and a faster rate of metabolism for benzene than either rats or monkeys. After a 6-hour exposure to 7–10 ppm benzene, mice retained 20% of the dose, compared with 3–4% for rats and monkeys. At higher exposure levels the metabolism becomes saturated and a sevenfold increase in exposure concentration, between 130 and 925 ppm, results in only a threefold increase in metabolism. When oral doses exceed the capacity for benzene metabolism, greater concentrations are then exhaled.

3.1.3. Dermal Absorption

Studies of both humans and experimental animals indicate that benzene is rapidly absorbed through the skin from both liquid and vapor phases. The percentage of absorption of the applied doses is generally higher in experimental animals than in humans. The percutaneous absorption of benzene has been studied in humans (Franz, 1984) and in laboratory animals (Maibach and Anjo, 1981; Franz, 1983; Susten et al., 1985). Dermal absorption is minimal when compared with inhalation or oral absorption; this is due in large part to benzene volatilizing rapidly from the skin. 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 can be underestimated in situations where contact with the benzene source is maintained for a prolonged period. 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.

Franz (1984) studied both in vivo and in vitro dermal absorption of benzene in humans. A dose of 0.0026 mg/cm^2 of ^{14}C-benzene was applied to the ventral forearm skin of four volunteers. Volatilization was rapid, as no free benzene on the skin was observed after 30 seconds. Absorption of the applied dose was estimated by urinary excretion of ^{14}C for 36 hours. No correction was made for volatilization, but a correction was made for the fact that not all absorbed benzene is excreted in the urine. Correction was determined by the fraction of ^{14}C from a subcutaneous injection in rhesus monkeys that was not excreted in the urine. Excretion was rapid, with 80% of the urinary excretion occurring by 8 hours after exposure. Total absorption was estimated to be 0.05% of the applied dose. Absorption using in vitro techniques with human skin was estimated to be 0.1% at a similar dose rate. Absorption in vitro increased linearly with dose level and also as a function of exposure time.

Laitinen et al. (1994) studied occupational exposure to benzene in eight 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 exposure showed much higher levels of exposure than expected, based on corresponding air concentrations in the workplace. Actual breathing zone concentrations of benzene varied from the Finish detection limit of 0.2 cm^3/m^3 to 3.7 cm^3/m^3, depending on whether it was in unleaded or leaded gasoline. Comparison of measured blood concentrations to predictions based on air measurements suggested that dermal exposure could have accounted for 68% of exposure. These mechanics had direct dermal contact with gasoline during the frequent changing of filters and fuel pumps. This suggests far more dermal exposure to benzene than exposure via the inhalation route.

In a series of experiments conducted in a residence with a benzene-contaminated water supply, Lindstrom et al. (1993) estimated, by modeling, that a dose of 281 μg benzene would be absorbed during a 20-minute shower, with 60% derived from dermal absorption and 40% from inhalation absorption, based on results from one individual only. The validity of this observation is highly questionable.

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). It may be noted the percent absorption of the applied dose of benzene in each of these test animals was approximately twofold to threefold higher than that of humans.

Dermal uptake of benzene from aqueous solutions is an important parameter for evaluating the risk due to exposure to benzene-contaminated ground or surface waters, for example, during showering, bathing, or swimming. Morgan et al. (1991) compared dermal

uptake of undiluted benzene with one-third, two-thirds, and completely saturated aqueous solutions of benzene. A capped skin depot (3.1 cm^2 surface area) was glued to the shaved skin of male Fischer 344 (F344) rats, and 2 mL of benzene or aqueous solutions were used. Blood samples were collected via an implanted jugular catheter at 0, 0.5, 1, 2, 4, 8, 12, and 24 hours after initiation of exposure. The blood concentration during exposure to undiluted benzene continued to rise throughout the 24-hour exposure and reached a level of 24.2 mg/L. Dermal absorption from aqueous solutions was rapid, and peak blood levels of benzene were reached after 1 hour. Benzene could not be detected in blood from the one-third saturated solution; it reached a peak level of approximately 0.18 mg/L in the two-thirds saturated treatment and 0.33 mg/L in the saturated treatment. Benzene was essentially completely absorbed, with less than 1% remaining in solution after 24 hours. Benzene was preferentially absorbed, because the volume of aqueous benzene solution absorbed did not differ significantly from absorption of distilled water. Benzene was rapidly and completely absorbed from aqueous solutions.

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. This is equivalent to an absorption rate of 0.31, 1.89, and 5.90 $\mu g/cm^2$/hour, respectively. The skin absorption coefficient was 0.619 cm/hour. Using the mouse dermal absorption data and human occupational exposure data, Tsuruta 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 were supplied air through latex masks and 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 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. On the basis of these findings, dermal exposure studies in rats probably provide a conservative estimate of the dermal absorption of benzene by humans. In an in vitro experiment using F344 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.

Adsorption of benzene to soils resulted in a small decrease in dermal absorption when compared with free benzene (Skowronski et al., 1988). Radiolabeled ^{14}C-benzene was applied to the shaved skin of male Sprague-Dawley rats either as free benzene or mixed with a sandy soil (4.4% organic matter) or a clay soil (1.6% organic matter). The application area was occluded by gluing a shallow glass cover onto the skin prior to application. Statistically significant decreases in the area under the plasma-concentration time curve were observed for both soils, but the effect of the clay soil was greater. No statistically significant differences in the absorption or elimination half-lives among treatments were observed. The greatest excretion of ^{14}C was observed in the urine (45–86% of applied dose), with lesser amounts eliminated by respiratory excretion (6–13%). Excretion in the feces was 0.2% or less. These values were corrected for volatilization losses during administration of 67%, 59%, and 39% of applied radioactivity for the free benzene, sandy soil, and clay soil treatments, respectively. Although these experiments suggested that soil may reduce benzene absorption, the use of occlusion of the

exposed skin is not realistic for the dermal exposure scenarios used in risk assessment. Thus, the small decreases in uptake have little meaning for evaluation of risks from soil-absorbed benzene.

3.2. DISTRIBUTION

There were no studies reported regarding the distribution of benzene in humans after either oral or dermal exposure. However, the animal data available provided some useful information. Both human and animal data were available following inhalation exposure.

3.2.1. Oral Exposure

Low et al. (1989, 1995) followed the distribution of ^{14}C-benzene administered by oral gavage in female Sprague-Dawley rats. This study sought to determine whether the distribution of benzene or benzene metabolites could explain the observations of tissue-specific induction of solid tumors in the Zymbal gland, oral and nasal cavities, and mammary glands associated with chronic oral exposure (NTP, 1986). Following oral doses of 0.15 or 1.5 mg/kg in olive oil, the concentrations of ^{14}C in various organs could be assigned to three groups, with the highest concentrations in liver and kidneys, intermediate concentrations in the blood, and lowest concentrations in the Zymbal gland, nasal cavity tissue, oral cavity tissue, mammary glands, and bone marrow. At dose levels of 15 mg/kg or higher, disproportionate increases were found in mammary glands and bone marrow. At the 0.15 mg/kg dose level, all of the ^{14}C activity found in the tissues or blood after 1 hour appeared as benzene metabolites, indicating that first-pass metabolism in the liver was very efficient. A high proportion of the metabolites extracted by ethyl acetate from the Zymbal gland and the nasal cavity tissue 1 hour after administration were present as unidentified metabolites. Similarly, a high proportion of the water-soluble metabolites from these tissues were also unidentified peaks that did not correspond to known metabolites of benzene. Elimination of benzene-derived ^{14}C in all organs was biphasic. Benzene did not accumulate in the Zymbal gland; within 24 hours after administration, radiolabel derived from ^{14}C-benzene in the Zymbal gland constituted less than 0.0001% of the administered dose. Thus, preferential accumulation of benzene or metabolites in the Zymbal gland does not account for tissue-specific tumor induction in this organ.

3.2.2. Inhalation Exposure

Results from test animal studies indicate that absorbed benzene is distributed throughout several body 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 hours 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 in blood, with phenol being eliminated more rapidly than catechol or hydroquinone after exposure. Ghantous and Danielsson (1986) exposed pregnant mice to a benzene concentration of 6400 mg/m^3 (2000 ppm) for 10 minutes 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-fold the concentration of benzene in blood; benzene levels in muscles and organs were onefold to threefold that in blood, and erythrocytes contained about twice the amount of benzene found in plasma.

Studies in pregnant mice demonstrated that after inhalation exposure, ^{14}C-benzene crossed 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 the peak in maternal tissues. No firmly tissue-bound metabolites of benzene were detected in the fetal tissues in late gestation, indicating that the mouse fetus did not have the ability to form the reactive metabolites.

Sato et al. (1975) compared elimination kinetics of benzene in men and women of similar ages. Exposure was for 2 hours at 25 ppm. The level of benzene in the blood and the end-tidal air was different for males and females. The shape of the decay curve was significantly steeper in the males. The authors attributed these results to the higher fat content of females.

3.2.3. Dermal Exposure

No relevant studies were found regarding distribution in humans following dermal exposure to benzene, and only one study in animals was identified, providing minimal information on the distribution of benzene via the dermal route.

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 gram 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

Despite extensive research, the metabolism of benzene is still not thoroughly understood. It is generally accepted that benzene itself is not directly responsible for causing the toxic effects; however, the metabolic product or products responsible for the noncancer and carcinogenic effects of benzene exposure have not been clearly defined. The evidence suggests that several metabolites, as well as interactions between these metabolites, may be necessary to explain the toxic effects of benzene. A complete review of the metabolism of benzene is beyond the scope of this toxicological review. The reader is referred to several excellent reviews for more complete coverage (ATSDR, 1997; Snyder et al., 1993b; Snyder and Hedli, 1996; Ross,

1996; Witz et al.,1996). This section provides an overview of benzene metabolism, focusing on the issues that are relevant to the interpretation of the noncancer effects due to benzene exposure.

3.3.1. Metabolic Pathways

The first step in benzene metabolism is the formation of the epoxide, benzene oxide, catalyzed by cytochrome P450 2E1 (CYP2E1) (Figure 1). After formation of benzene oxide, the metabolism of benzene branches into several alternative metabolic pathways (Jerina et al., 1968; Lovern et al., 1997). However, although Jerina et al. (1968) provided a kinetically plausible argument for the existence of benzene oxide, they did not show that it was formed from benzene. Benzene oxide rearranges nonenzymatically to form phenol, the major product of initial benzene metabolism. Alternatively, benzene oxide may react with glutathione (GSH) to form phenyl-mercapturic acid; undergo enzymatic conversion by epoxide hydrolase to benzene dihydrodiol with subsequent formation of catechol; or undergo an iron-catalyzed, ring-opening reaction to form *trans,trans*-muconaldehyde (MUC) with subsequent metabolism to *trans,trans*-muconic acid (MA). Phenol is further oxidized by CYP2E1 catalysis to hydroquinone. Further oxidation of hydroquinone to *p*-benzoquinone is catalyzed by myeloperoxidase (MPO) (Smith et al., 1989). All of the phenolic products may be conjugated with sulfate or glucuronic acid, and the conjugates of phenol and hydroquinone are the major benzene metabolites excreted in urine (Sabourin et al., 1989; Wells and Nerland, 1991).

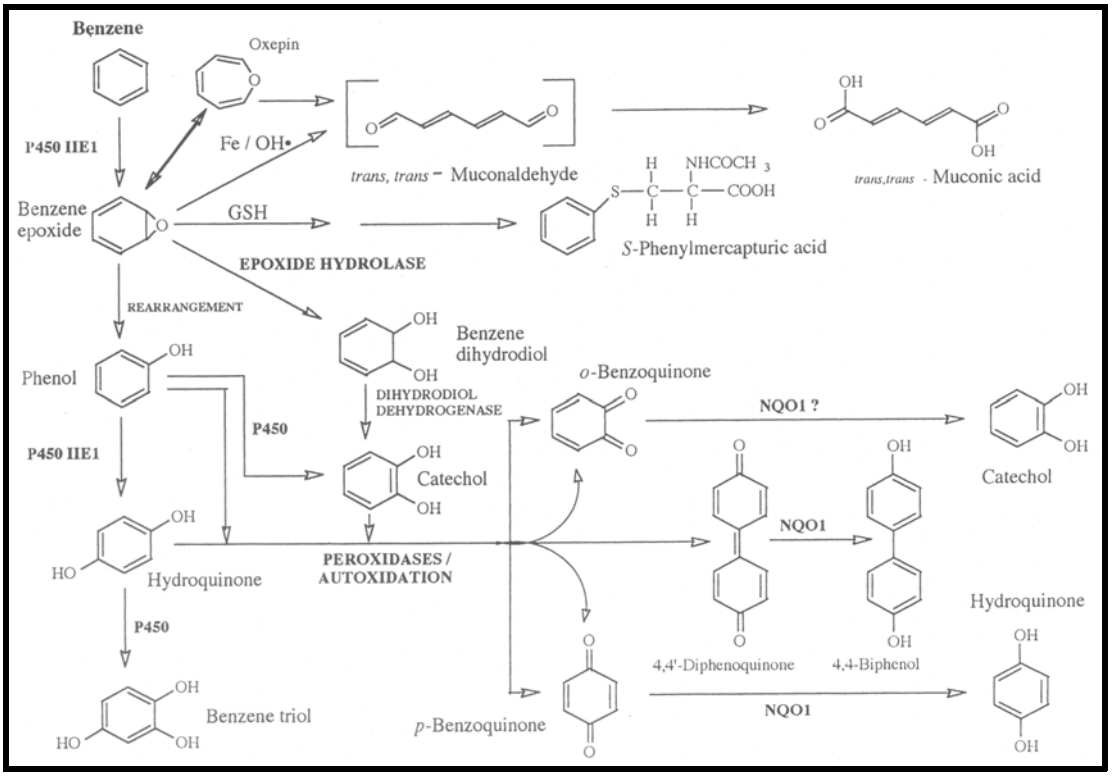
Mathews et al. (1998) studied the metabolism of ^{14}C-benzene in male F344 rats over a wide range of oral (gavage) doses, 0.02, 0.1, 0.5, or 100 mg benzene/kg body weight, in male B6C3F1 mice at oral doses of 0.1 and 100 mg/kg and in male hamsters at 0.02, 0.1 and 100 mg/kg. In F344 rats, at lower doses (0.02, 0.1, and 0.5 mg/kg), greater than 95% of the dose was recovered in the urine within 48 hours, and a small percentage (about 3%) was recovered in the breath. At higher doses, 10 and 100 mg/kg, the percentage eliminated in the breath increased to about 9 and 50%, respectively. Excretion in the feces was a minor route at all doses. A similar pattern of disposition of the radiolabel dose was also observed in mice and hamsters. Mathews et al. (1998) also examined the profile of urinary metabolites formed. Interestingly, the percentage of prephenylmercapturic acid and phenylmercapturic acid, indicators of benzene oxide production, was relatively constant across all doses for rats (~13%), mice (~5%), and hamsters (~7%). However, the percentage of hydroquinone and related conjugates ranged from about 3% at the highest dose to as much as 7% at the lowest doses. A higher percentage of hydroquinone metabolites was seen in mice (~30%) and in hamsters (~30%), but it did not appear to be dose dependent.

Figure 1. Metabolic pathways for benzene.

Source: Ross, 2000.

3.3.2. Requirement for CYP2E1

Oxidation of benzene by the CYP2E1 isoenzyme has been demonstrated to be required for the expression of hematotoxicity and genotoxicity. Administration of toluene, a competitive inhibitor of benzene metabolism, causes a decrease in benzene metabolite formation and a reduction in toxicity (Andrews et al., 1977). The primary oxidation of benzene by CYP2E1



occurs in the liver, and further metabolism to the final toxic compound occurs in the target tissues. Sammett et al. (1979) showed 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 isoenzyme involved in benzene metabolism (Johansson and Ingelman-Sundberg, 1988; Koop and Laethem, 1992). Convincing evidence that CYP2E1 activity is required for expression of benzene toxicity was provided using transgenic CYP2E1 knockout mice that do not express hepatic CYP2E1 activity (Valentine et al., 1996). Benzene exposure at 200 ppm (640 mg/m^3) for 6 hours/day for 5 days resulted in severe genotoxicity and cytotoxicity in wild-type mice, but no toxicity was observed in CYP2E1 knockout mice. Thus, the requirement for CYP2E1 metabolism has been clearly established, but the identity of the toxic metabolites remains uncertain.

Recently, Bernauer et al. (2000) investigated a role of CYP2E1 expression in bone marrow and its intra- and interspecies variability in rats, rabbits, and humans, because it is a target organ for several chemicals, including benzene. The data demonstrated a presence of CYP2E1 in the bone marrow of all species investigated, thus supporting the hypothesis of CYP2E1-dependent local metabolism of several chemicals that includes benzene possibly contributing to myelotoxicity and hematotoxicity.

3.3.3. Toxicity of Benzene Metabolites

The products of the phenol pathway (catechol, hydroquinone, and *p*-benzoquinone), MA, and benzene epoxide have been proposed as benzene metabolites that may cause the toxic effects of benzene exposure.

3.3.3.1. *Phenolic Products*

The production of the initial benzene metabolites occurs primarily in the liver; however, the effects of benzene toxicity are primarily expressed as hematotoxicity and myelotoxicity in the bone marrow (Snyder and Hedli, 1996). The evidence suggests that secondary metabolism in bone marrow is required for expression of the toxicity of benzene (Schlosser and Kalf, 1989; Subrahmanyam et al., 1990, 1991). An alternative hypothesis is that metabolism of benzene to toxic metabolites can occur in the bone marrow itself. Metabolism of benzene to hydroquinone in the bone marrow of rats has been demonstrated, but the amount of metabolites produced was small (Irons et al., 1980). The presence of CYP2E1 has been detected in rabbit bone marrow (Schnier et al., 1989); however, CYP2E1 could not be detected in the bone marrow of mice, the species demonstrated to be most sensitive to benzene hematotoxicity (Genter and Reico, 1994). Rickert et al. (1979) observed that catechol and hydroquinone concentrations persisted in the bone marrow long after blood levels had declined following inhalation exposure.

Most research, therefore, has focused on the possibility that phenol, catechol, and hydroquinone, generated by reactions in the liver, are transported to the bone marrow and other target tissues and subsequently activated by the action of peroxidase (Smith et al., 1989; Rushmore et al., 1984; Low et al., 1995). A potential role for conversion of hydroquinone to *p*-benzoquinone by the peroxidase component of prostaglandin H synthase has also been suggested (Schlosser and Kalf, 1989). Subsequent research, however, suggested that the prostaglandin H synthase is not involved in benzene toxicity (Ganousis et al., 1992).

A major problem with the hypothesis that phenolic metabolites are responsible for bone marrow toxicity is that administration of phenol fails to duplicate the effects of benzene (Tunek et al., 1981; NCI, 1980). Kenyon et al. (1995) suggested that the distribution of phenol-conjugating enzymes and benzene-oxidizing enzymes within the liver might have accounted for this result. The authors observed that phenol-conjugating enzymes were more concentrated in the periportal area of the liver, the first region to absorb orally administered phenol, whereas oxidizing enzymes were more concentrated in the pericentral region of the liver. This could have lead to rapid excretion of orally administered phenol before it was further metabolized to hydroquinone by CYP2E1 in the pericentral region of the liver. A combination of phenol and hydroquinone is needed to cause bone marrow toxicity (Eastmond et al., 1987). Intraperitoneal (i.p.) injection of either phenol or hydroquinone alone in B6C3F1 mice failed to cause significant bone marrow toxicity; however, co-administration of these metabolites caused a reduction in bone marrow cellularity with a clear dose-response curve. The presence of phenol apparently stimulated peroxidase-dependent metabolism of hydroquinone to *p*-benzoquinone. Increased covalent binding of ^{14}C-hydroquinone was observed in bone marrow when a combination of phenol and hydroquinone was administered to mice (Subrahmanyam et al., 1990, 1991).

In contrast to the observations of Eastmond et al. (1987), catechol was reported to markedly stimulate peroxidase activation of hydroquinone in murine stroma (Ganousis et al., 1992). In addition to the effects of phenol on the activity of MPO, co-administration of phenol was shown to increase the concentration of phenol and hydroquinone in the blood in B6C3F1 mice. Legathe et al. (1994) measured the area under the curve (AUC) blood concentration-time curve for phenol and hydroquinone administered alone or in combination at the same dose levels used by Eastmond et al. (1987). Co-administration increased the phenol AUC by 1.4-fold and the hydroquinone AUC by 2.6-fold in comparison to each compound administered alone. Legathe et al. (1994) suggested that this resulted from saturation of the enzymes that form sulfate and glucuronide conjugates of phenolics in the liver. These results suggest that interactions of two or more phenolic metabolites of benzene may be necessary to cause the observed bone marrow toxicity.

3.3.3.2. *Trans,trans-Muconaldehyde*

Muconaldehyde (MUC), a highly reactive six-carbon diene dialdehyde, has also been proposed to be a benzene metabolite responsible for bone marrow toxicity. Evidence supporting this hypothesis has been summarized by Witz et al. (1996). MUC has been shown to cause hematotoxicity following short-term exposures. Administration of 2 mg/kg/day MUC to CD-1 mice for 16 days caused significant decreases in bone marrow cellularity, lymphocytes, red blood cell (RBC) counts, hematocrit (HCT) (volume percentage of erythrocytes in whole blood), and hemoglobin (Hgb) and significant increases in white blood cell (WBC) count and spleen weight (Witz et al., 1985). Snyder et al. (1989) also found that administration of MUC caused bone marrow toxicity in mice and that co-administration of MUC and hydroquinone resulted in a dramatic decrease in ^{59}Fe incorporation into red cell Hgb.

Production of MUC is expected as a step in the ring-opening pathway leading to MA. Excretion of MA in the urine was demonstrated in rabbits in the early work of Parke and Williams (1953), and excretion of MA was also demonstrated in mice, rats, cynomolgus monkeys, chimpanzees, and humans (Gad-El Karim et al., 1985; Sabourin et al., 1988a, 1989, 1992). Metabolism of MUC to MA has been shown in vivo in mice (Witz et al., 1990a,b). Urinary excretion of MA has been used as a sensitive biomarker of benzene exposure in humans. At low doses, urinary MA concentration was found to be linearly correlated with time-weighted average (TWA) benzene exposure concentrations (Bechtold and Henderson, 1993; Bechtold et al., 1991). Thus, the ring-opening pathway is active in several animal species. There is no direct evidence, however, for the in vivo formation of MUC from benzene.

Although MUC formation has not been demonstrated in animals in vivo, formation of MUC from benzene has been demonstrated in a mouse hepatic microsomal system (Latriano et al., 1986; Zhang et al., 1995a). MUC may be too reactive to be isolated from in vivo systems. Using isolated rat livers perfused with 0.7 mM benzene solutions through the portal vein, Grotz et al. (1994) demonstrated that the complete pathway for formation of MA from benzene was active in the liver, but MUC was not detected in the perfusate collected via the hepatic vein. When MUC was added to the perfusion solution, it was rapidly and efficiently metabolized to MA, and only traces of MUC were detected in the perfusate collected after a single pass through the isolated rat livers. Thus, it seems unlikely that sufficient quantities of MUC could reach the target tissues by circulation in the blood. The acid-alcohol 6-hydroxy-*trans,trans*-2,4-

hexadienoic acid (COOH-M-OH) and MA were the major MUC metabolites detected in the rat liver perfusate, but both the acid-aldehyde 6-oxo-*trans,trans*-2,4-hexadienoic acid (COOH-M-CHO) and the aldehyde-alcohol 6-hydroxy-*trans,trans*-2,4-hexadienal (CHO-M-OH) were detected as minor peaks (Grotz et al., 1994). The CHO-M-OH has been demonstrated to react with GSH, to be cytotoxic to isolated rat hepatocytes, and to be hematotoxic in mice (Goon et al., 1993; Zhang et al., 1995b). Thus, CHO-M-OH may be the ring-opened metabolite that causes the hematoxic effects of administered MUC, and it may play a role in causing the toxic effects of benzene exposure.

3.3.3.3. *Benzene Oxide*

Benzene oxide was believed to be too reactive to escape the liver and to cause toxicity in the bone marrow. However, Lovern et al. (1997) recently showed that benzene oxide constituted 7% of the benzene metabolites after 18 minutes' incubation with liver microsomes. Lindstrom et al. (1997) demonstrated the presence of benzene oxide in the blood and estimated its half-life to be about 8 minutes. Using a PBPK model, Lindstrom et al. (1997) predicted that the dose to the body from benzene oxide would be about 22-fold greater than from 1,4-benzoquinone. Thus, circulating benzene oxide can contribute to observed DNA and protein adduct formation. Further research is needed to establish the role of benzene oxide in causing bone marrow toxicity.

3.3.4. Species, Route, and Rate Differences

Differences in the rates of benzene metabolism and the metabolites formed correlate with observed differences in sensitivity to benzene toxicity. Mice are more sensitive than rats to the toxic effects of benzene (Huff et al., 1989; Snyder et al., 1978; Ward et al., 1985). Sabourin et al. (1987) found that metabolism of benzene, determined by excretion of water-soluble metabolites in the urine, became saturated at lower doses in B6C3F1 mice than in F344/N rats. The amount of metabolites per kilogram body weight was similar in the two species at gavage doses up to 50 mg/kg, but above this level, total metabolites in rats continued to increase but total metabolites in mice did not increase further. Following inhalation exposures, total metabolites excreted were higher for mice than for rats at all benzene concentrations due to a higher amount inhaled by mice. Total metabolite excretion was exponentially related to benzene concentration, but the concentration needed to reach half of the maximal metabolite formation was lower in mice (220 mg/m^3 [69 ppm]) than in rats (260 mg/m^3 [81.5 ppm]). By both oral and inhalation routes, the metabolism of benzene was saturated at lower concentrations in mice than in rats. Thus, differences in total metabolite formation between rats and mice did not explain the greater sensitivity of mice to benzene.

Sabourin et al. (1988a) compared the metabolites of benzene in the blood, liver, lung, and bone marrow of male B6C3F1 mice and F344/N rats following 6 hours of inhalation exposure to benzene at 50 ppm (160 mg/m^3). Hydroquinone glucuronide, hydroquinone, and MA were present in much higher concentrations in the mouse than in the rat tissues. Thus, metabolism in mice leads to much greater exposures to potentially toxic benzene metabolites, which may explain the greater sensitivity of mice to benzene toxicity.

Exposure concentration and routes also affect the distribution of metabolites formed in mice and rats (Sabourin et al., 1989). Male B6C3F1 mice and F344/N rats were given gavage doses of 1, 10, or 200 mg/kg benzene or they were exposed to 5, 50, or 600 ppm (16, 160, or 1916 mg/m^3) for 6 hours. Water-soluble metabolites were determined in blood, urine, liver, lung, and bone marrow. A shift in the distribution from potentially toxic metabolites, hydroquinone glucuronide, and MA to the detoxification metabolites, phenylglucuronide and prephenylmercapturic acid, was observed from low concentrations to high concentrations following both oral and inhalation exposures in mice. A similar shift was observed in rats, except that hydroquinone glucuronide was a minor metabolite in rats at all concentrations. Thus, extrapolation from toxicological studies conducted at high exposure concentrations may significantly underestimate risks at lower exposure concentrations. There was also no simple correlation between the dose of metabolites to tissues resulting from oral and inhalation exposures. This may be due to saturation of benzene metabolism following bolus doses of benzene administered by gavage.

Urinary excretion of benzene metabolites has also been examined in cynomolgus monkeys and chimpanzees (Sabourin et al., 1992). The proportion of an oral gavage dose excreted in the urine decreased from 50 to 15% as the dose rate increased from 5 to 500 mg/kg in cynomolgus monkeys. Phenyl sulfate was the primary metabolite excreted, and the proportion excreted as hydroquinone conjugates and MA decreased as the dose increased, as was observed previously in mice and rats. The proportion excreted as MA decreased from 4.4% at the low dose to 1.3% at the high dose. At all levels the proportion of MA excreted as MA was much lower in the monkey than in either mice or rats. Chimpanzees were exposed at only one low dose rate of 1 mg/kg by intravenous injection. Less than 15% of the administered dose was recovered as hydroquinone conjugates and MA, and 79% was recovered as phenyl conjugates. On the basis of the urinary metabolite profiles, the mouse appears to metabolize the largest fraction of benzene via pathways leading to hydroquinone conjugates and MA (67% at low doses) followed by monkeys (31%), rats (17%), and chimpanzees (14%). Thus, there are apparently large quantitative differences among species in the metabolism of benzene to potentially toxic metabolites. Because few data are available on the proportion of benzene metabolized to potentially toxic metabolites in humans, considerable uncertainty exists in determining which animal model best represents human metabolism.

Henderson (1996) reviewed the species differences in benzene metabolism. The pathways of benzene metabolism appear to be similar in all species studied; however, there are quantitative differences in the fraction of benzene metabolized by different pathways. Monkeys and mice metabolize more of the benzene dose to hydroquinone metabolites than do rats or chimpanzees, especially at low doses. Mice appear to have a greater overall capacity to metabolize benzene than do rats and primates. This finding may explain why mice are more sensitive than rats to benzene. In all species, a greater proportion of benzene is converted to hydroquinone and ring-open metabolites at low doses than at high doses.

3.3.5. Induction of CYP2E1

Benzene exposure has been found to induce CYP2E1 activity, thereby increasing the rate of toxic metabolite formation. Pretreatment of mice, rats, and rabbits subcutaneously with benzene increased benzene metabolism in vitro without increasing total cytochrome P450

concentrations (Arinc et al., 1991; Gonasun et al., 1973; Saito et al., 1973). In contrast, Sabourin et al. (1990) found no significant effect on the metabolism of benzene when F344 rats and B6C3F1 mice were pretreated by inhalation exposure to 600 ppm (316 mg/m^3) of benzene. The rate of benzene metabolism can be altered by pretreatment with various compounds. CYP2E1 also metabolizes alcohol and aniline, and CYP2E1 can be induced by these substrates (Chepiga et al., 1990; Parke, 1989; Snyder et al., 1993a). Phenol, hydroquinone, benzoquinone, and catechol have also been shown to induce P450 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 GSH 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.

Exposure to benzene was found to interfere with the disappearance of ethanol from the body in rats (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). The possibility therefore exists of a synergism between alcohol and benzene.

3.3.6. Mechanism of Toxicity

Just as several benzene metabolites have been implicated in benzene toxicity, several different mechanisms also may contribute to the overall toxic effects of benzene, as discussed below.

3.3.6.1. *Formation of Covalent Adducts*

Benzene metabolites form covalent adducts with both cell proteins and DNA; however, the role of adduct formation in toxicity is unclear. Treatments that reduce benzene toxicity also reduce adduct formation. Partial hepatectomy of rats resulted in reduced hematotoxicity and also in reduced covalent binding in bone marrow (Sammett et al., 1979). Levels of adduct formation in the hematopoietic tissues of mice strains have been shown to correlate with the relative sensitivity of the strains (Longacre et al., 1981).

Covalent binding of benzene metabolites to DNA was first demonstrated by Lutz and Schlatter (1977). Mazzullo et al. (1989) found that formation of DNA adducts of benzene metabolites was linear at low benzene concentrations, but saturation of adduct formation occurred at high benzene concentrations. Hedli et al. (1996) investigated DNA adduct formation from 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 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. 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.

Although the mechanism by which adduct formation results in bone marrow toxicity is not well established, a few experiments have suggested that binding of hydroquinone or *p*-benzoquinone to sulfhydryl groups at the active sites of proteins could be responsible for the genotoxic effects of benzene. Hydroquinone and *p*-benzoquinone interfere with binding of guanosine triphosphate (GTP) to tubulin by alkylating nucleophilic sulfhydryl groups (Irons and Neptun, 1980; Pfeifer and Irons, 1983). Binding of GTP is required for stabilization of tubulin polymerization during microtubule formation and, therefore, interferes with spindle formation during mitosis. Several metabolites of benzene have also been shown to inhibit the activity to human topoisomerase II (Frantz et al., 1996). Topoisomerase II enzymes relieve torsional strain on DNA during replication and transcription and are also believed to function during recombination and chromosome condensation. Both *p*-benzoquinone and MUC directly inhibited topoisomerase at concentrations as low as 10 μM. With the addition of horseradish peroxidase and peroxide to the assay mixture, the benzene metabolites phenol, 4,4'-biphenol, 2,2'-biphenol, hydroquinone, catechol, and 1,2,4-benzenetriol all inhibited topoisomerase II. Topoisomerase inhibitors tend to be strong clastogens in mammalian cells. Thus, inhibition of topoisomerase II could lead to the clastogenic effects observed following benzene exposure. Addition of GSH to the assay mixture also protects against topoisomerase II inhibition, thus suggesting that interaction of benzene metabolites with an essential sufhydryl residue occurs. Hutt and Kalf (1996) also reported that *p*-benzoquinone inhibits the activity of topoisomerase II.

Protein adduct formation by benzene metabolites differs greatly between mice and rats. McDonald et al. (1994) used uniform ^{14}C-benzene to determine total protein binding and C_6-^{13}C-benzene to determine formation of adducts due to the reactions of benzene oxide, 1,4-benzoquinone, or 1,2-benzoquinone. Total binding to protein and specific binding to cysteine residues were estimated for blood Hgb and bone marrow total protein. Formation of adducts of benzene oxide, 1,4-benzoquinone, and 1,2-benzoquinone accounted for 74% of the binding to cysteine residues in rat Hgb but for only approximately 25% of the cysteine binding in mouse Hgb or bone marrow proteins of either species. Thus, other benzene metabolites must also bind to cysteine. Adducts of benzene oxide were highest in rat Hgb but accounted for only a small proportion of adduct formation in mouse Hgb. Benzene oxide adducts accounted for less than 3% of total adduct formation in the bone marrow of either mice or rats, and benzoquinone adduct formation accounted for a much greater proportion of total protein binding in bone marrow. However, 1,2-benzoquinone adducts were more prevalent in rat bone marrow proteins and 1,4-benzoquinone adducts were more prevalent in mouse bone marrow proteins. This suggests that there are significant differences in benzene metabolism between mice and rats. A background level of both 1,2- and 1,4-benzoquinone protein adducts that greatly exceeded the level of

labeled adduct formation was also observed in bone marrow proteins of control mice and rats. This result suggests that low doses of benzene may not cause toxicity through protein adduct formation.

3.3.6.2. *Genotoxicity*

The association between benzene exposure and the appearance of structural and numerical chromosomal aberrations in human lymphocytes suggests that benzene may be considered as a human clastogen. In in vivo animal studies, benzene induced cytogenetic effects, including chromosome and chromatid aberrations, sister chromatid exchanges, and micronuclei (Anderson and Richardson, 1981; Au et al., 1991; Erexson et al., 1986; Fujie et al., 1992; Kolachana et al., 1993; Siou et al., 1981; Toft et al., 1982; Ward et al., 1992). Several lines of evidence also indicate that benzene is genotoxic in humans under occupational exposure conditions (Ding et al., 1983; Sasiadek et al., 1989; Yardley-Jones et al., 1990; Major et al., 1992; Eastmond, 1993; Tompa et al., 1994). However, these studies lacked good exposure monitoring data, involved multiple chemical exposures, and were often poorly designed, with inappropriate control groups.

Benzene has been shown to produce DNA breaks in Chinese hamster ovary cells independent of metabolic activators; however, in vitro assays indicate that genotoxicity of benzene is primarily due to its metabolites (Zhang et al., 1993; Eastmond et al., 1994). Benzene is known to affect cell cycle progression, RNA and DNA synthesis, and DNA binding (Forni and Moreo, 1967). Chen and Eastmond (1995) showed that benzene metabolites can adversely affect human topoisomerases; however, some DNA repairs may occur in human cells (Chenna et al., 1995).

Even with these studies, no data exist on the quantitative relationship between measured benzene exposures and clastogenic effects.

Benzene itself is not mutagenic in short-term assays in either bacterial or animal systems (Dean, 1985). Mutagenicity of benzene metabolites, however, is well established. Hydroquinone, catechol, 1,2,4-trihydroxybenzene, and *trans*-1,2-dihydrodiol were active in inducing elevated resistance to 6-thioguanine in Chinese hamster V79 cells. MUC is an active mutagen in V79 cells, but it is only weakly active in Salmonella (Witz et al., 1990a).

3.3.6.3. *Oxidative Stress*

Benzene may also produce oxidative stress in the target tissues. The benzene metabolite *p*-benzoquinone is highly reactive and can deplete cellular levels of GSH (Brunmark and Cadenas, 1988). Benzene metabolites can also be involved in redox cycling, resulting in the production of reactive oxygen species that can also react with macromolecular components (Rao and Snyder, 1995).

3.3.6.4. *Inhibition of Cytokine Formation*

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 benzene for 2 days. The stromal macrophages removed from these mice and cultured with lipopolysaccharide produced the IL-1 precursor 34-kilodalton (Kd) pre-interleukin-1alpha (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-IL-1α 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-IL-1α 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

The information base on the clearance of benzene from the body after oral exposure to the compound is limited to reports of studies in experimental animals. For example, in one of a series of toxicological reports on the metabolism and excretion of radiolabeled xenobiotics, Parke and Williams (1953) recovered a substantial proportion of the administered dose on the breath as unmetabolized product (43%). This amount compared with 33% urinary excretion in which the label partitioned into such components as conjugated phenols (23% of the dose), hydroquinone (4.8%), and catechol (2.2%), among others. Residual amounts of radioactivity remained deposited in the tissues or were excreted in the feces.

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, MA, phenyl glucuronide, phenyl sulfate, catechol glucuronide, hydroquinone sulfate, and pre-phenylmercapturic 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. 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 (10 mg/kg), urinary excretion was the major route of elimination. Hydroquinone glucuronide, phenyl sulfate, and MA 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, whereas 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 physiological function occurring with age; the significance of toxicity versus aging could not be ascertained.

Fluctuation in the proportion of radiolabeled benzene excreted in urine was observed with increasing dose by Sabourin et al. (1987), who exposed B6C3F1 mice, F344 rats, and Sprague-Dawley rats via gavage to doses ranging from 0.5 to 300 mg/kg ^{14}C-benzene. At the lower doses (< 15 mg/kg), near quantitative amounts of label were recovered in various urinary metabolites, whereas at the higher doses, unchanged ^{14}C-benzene began to appear in expired air in increasing proportions. For example, at doses of 150 mg/kg and above, greater than 50% of the load was cleared on the breath as unchanged starting compound. These data point to the saturation of the metabolic processes with increasing dose, although even at lower doses a degree of fluctuation in the metabolic pattern was implied by dose-dependent changes in the profile of metabolic products detected in the urine.

3.4.2. Inhalation Exposure

In a key report of studies that addressed the metabolic fate of inhaled benzene issue in humans, Nomiyama and Nomiyama (1974) showed that at least a proportion of the absorbed compound can be excreted in the urine as sulfate- or glucuronide-conjugated phenols or MAs. At the benzene concentrations employed (52–62 ppm [166–198 mg/m^3]), respiratory uptake was considered to be in the region of 47% and respiratory excretion for the 4-hour exposure period was approximately 17%. These values were in reasonably good agreement to those obtained in an earlier study by Srbova et al. (1950), who showed that the respiratory excretion of retained benzene could vary between 16.4 and 41.6% across a 7-hour exposure period.

In general, insufficient data exist to unequivocally assign one elimination route or another as being of primary importance when human beings are exposed to benzene via inhalation. For example, Sherwood (1988), using a complex experimental protocol, employed a single human subject who was alternately exposed to either 6.4 ppm (20 mg/m^3) benzene for 8 hours or 99 ppm (316 mg/m^3) for 1 hour to monitor the kinetics of benzene elimination via the various routes. Sherwood separated the release of benzene on the breath into several distinct phases and was able to show that a greater proportion of the total dose was excreted in urine rather than via expiration. These results also showed that urinary excretion of phenol conjugate

was biphasic, with an initial rapid excretion phase followed by a slower excretion phase. The importance of urinary excretion was also emphasized by the work of Inoue et al. (1986), who showed a good correlation between urinary phenol levels and benzene exposure across a relatively wide concentration range (1–200 ppm [3.2–640 mg/m^3]).

Occupational exposure and cross-sectional studies have also pointed to the appearance of benzene metabolites in urine as a consequence of exposure to such agents as sidestream tobacco smoke (Bartczak et al., 1994) and gasoline vapors (Lagorio et al., 1994) or when the urine content of smokers versus nonsmokers was compared (Kok and Ong, 1994; Melikian et al., 1994). In general, one cigarette delivers an average of 55 μg of benzene, and an average smoker takes in about 1.2–1.8 mg of benzene per day from smoking. Popp et al. (1994) detected muconic acid and S-phenyl-N-acetyl cysteine levels in the urine of car mechanics at levels that correlated with levels of the compound in the bloodstream and the breathing zone. These observations support the earlier suggestion by Ghittori et al. (1993) that benzene and its metabolites in the urine may be an important biomarker of occupational exposure to the compound.

An accumulation of experimental data in laboratory animals has shown an essentially similar pattern of benzene elimination and excretion as in human beings. In broad terms, this is characterized by the release of unchanged compound on the breath and the appearance of metabolites in the urine. For example, at high incidental concentrations of benzene (500 ppm [1597 mg/m^3] for 6 hours), an initial rapid phase of elimination was followed by a slower phase of much longer duration (up to 13 hours or more) (Rickert et al., 1979).

Perhaps the most rigorous experiments that have examined the dose-response effects of benzene via inhalation were those carried out by Sabourin et al. (1987) in parallel to those already described for the oral route. The experimental protocol featured a 6-hour, nose-only exposure to concentrations of ^{14}C-benzene ranging from 10 to 1000 ppm (32 to 3195 mg/m^3). At the lower concentrations, less than 6% of the radioactivity was expired on the breath. However, at the higher concentrations of benzene (> 850 ppm [2718 mg/m^3]), both rats and mice exhaled considerable proportions of the unchanged compound, amounting to 48% of the load in rats and 14% in mice. In a similar experiment, Dow Chemical Co. (1992a) reported that Duroc-Jersey pigs exposed to 0, 20, 100, or 500 ppm (0, 64, 319, or 1597 mg/m^3) benzene for 6 hours/day, 5 days/week for 3 weeks excreted levels of phenol in the urine that increased linearly according to dose.

3.4.3. Dermal Exposure

Only limited data on excretion of benzene after dermal exposure in humans were found. Franz (1984) reported that four volunteers exposed to ^{14}C-benzene (0.0024 mg/cm^2) on the skin excreted trace amounts of label in the urine over a 36-hour period, suggesting the ability of the compound to penetrate the dermal barrier. Similar results were obtained in monkeys and minipigs, with excretion of the compound in monkeys approaching 0.065% (range 0.033–0.135%) of the applied dose, compared with 0.042% (range 0.030–0.054%) in minipigs (Franz, 1984).

3.4.4. Other Routes of Exposure

This section discusses very limited data available from three studies in which benzene was either given intraperitoneally or subcutaneously. In these studies, the exposure was limited to either a single injection or daily injections for up to 3 days.

Experiments by Cornish and Ryan (1965) used the i.p. route of administration to examine the influence of the fed or fasted state on the rates of production of urinary metabolites such as conjugated or unconjugated phenols, components that appeared to be increased markedly in the fasted state versus fed. In line with their findings of the dose-response effects of benzene when administered via gavage or inhalation, Sabourin et al. (1987) found that the proportion of radioactivity (mainly as phenyl sulfate) excreted in the urine decreased with increasing dose when monkeys were injected intraperitoneally with concentrations of ^{14}C-benzene ranging from 5 to 500 mg/kg, again suggesting that benzene metabolism can be saturated at the higher dose levels. Finally, Longacre et al. (1981) exposed C57BL/6N and DBA/2 mice to benzene subcutaneously and monitored the appearance of conjugated phenol, catechol, and hydroquinone in the urine, although with varying proportions when one strain was compared with another. Amounts of phenyl mercapturic acid were similar, however.

3.5. PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELS

PBPK models can be used to address the uncertainty of extrapolating from data of an experimental study to a hypothetical administered dose for the same response in other animals or humans. The approach seeks to predict the dose-response characteristics of a chemical's potential toxicity through an understanding of the underlying mechanisms by which a substance is absorbed, transported to, and metabolized in the primary target organs, on the assumption that the biochemical processes involved in the toxicological response will be the same for both humans and the animal model(s) in which the harmful effects of the compound had been demonstrated. Necessary inputs to the model include a physiologically realistic discrimination of a chemical's movement between metabolically and functionally linked compartments of the body and the information necessary to describe this movement mathematically in terms of (1) the kinetics of metabolism, (2) rates of movement to and from different target organ groupings, and (3) the partitioning of a compound between physiological media. Thus, by modeling a target organ-specific internal dose surrogate (parent compound or metabolite, as applicable) from the NOAEL or LOAEL of an experimental study, an equivalent allometrically scaled internal dose surrogate in human beings can be used to back-extrapolate to a hypothetical "effective" dose for the same toxicological response in human beings.

Potential advantages of the PBPK approach reside in its ability to quantitatively address interspecies differences and to take into account the nonlinearity of biological processes when extrapolating outside the range of available experimental data. Limitations reside in the accuracy/validity of the estimates that constitute the inputs to the model and in the oversimplification that is inherent in seeking to define the movement and metabolism of a xenobiotic or its metabolites in terms of a small number of functionally defined subcompartments.

Several attempts have been made to develop PBPK models to define the understanding of interactions between benzene metabolism and toxicity. The first model for benzene was developed by Sato (Sato and Nakajima, 1979; Sato, 1988), who exposed three men to 25 and 100

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

A model (Medinsky et al., 1989a, b, c) was developed to describe and predict the fate of benzene and to determine whether differences in the metabolic rates between rats and mice could explain the differences in toxicity between these species. The model indicated that for inhalation concentrations up to 1000 ppm (3195 mg/m^3), mice metabolize at least two to three 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 MA metabolites linked to toxic effects; on the other hand, 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 (1) the liver, presumed to be the only organ where benzene metabolism takes place; (2) a group of poorly perfused tissues, including muscle and skin; (3) a group of richly perfused tissues, including bone marrow, kidney, and intestines; and (4) a fat compartment. All metabolism of benzene, consisting of initial metabolism to benzene oxide that 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). However, Bois et al. (1991b) found that the Medinsky model did not simulate the data of Rickert et al. (1979) very well, thereby indicating a need for the model to be further refined.

Since the original Medinsky model was published, additional compartments have been added to reflect an 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 a 13-fold variability in CYP2E1 activity in human hepatic microsomes and compared this to 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 conjugation reactions, phenol sulfonation and hydroquinone glucuronidation, in the hepatic microsome preparations of humans, 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 in rats (2.28 vs. 0.83 nM), and

predicted hydroquinone concentrations were also higher in mice than in rats (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. The authors also suggested that the mouse might be more sensitive than the human and that in vitro metabolism data must always be placed within the context of the whole animal's physiology.

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. Although current PBPK models may provide insights about putative toxic metabolites and potential biochemical mechanisms, they are insufficiently developed to be able to reduce scientific uncertainty (Medinsky et al., 1995, 1996; Medinsky, 1995).

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

The model developed by Bois and Paxman (1992) provided evidence that exposure rate had a strong influence on the rate of formation of several important metabolites of benzene. This model has three components describing the pharmacokinetics of benzene and the formation of metabolites in the rat. Distribution and elimination of benzene from a five-compartment system composed of liver, bone marrow, fat, poorly perfused tissues, and well-perfused tissues make up the first component of the model. The bone marrow compartment is 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 describes the metabolic transformations of benzene and its by-products in the liver and bone marrow. The reactions are assumed to follow Michaelis-Menten kinetics, except for the transformation of benzene oxide into phenol, which occurs spontaneously and may be described as a first-order reaction. The third component is the distribution of phenol. In addition to the compartments described for benzene, phenol is also assumed to distribute to the lung and gastrointestinal tract.

The model was validated against the data of Cassidy and Houston (1984), Sabourin et al. (1987, 1988a, 1989), and Sawahata and Neal (1983). It was also used to predict metabolite production for male rats exposed to benzene (nose only) at three different concentrations and for

three different exposure durations in comparison with the experimental data of Sabourin et al. (1989). The three exposure regimens were established to maintain a constant concentration/time product. Simulation results indicated that the model may over- or underestimate the level of urinary metabolites.

More recent efforts on development of the Bois and Paxman model have focused on defining the physiological pharmacokinetic parameter distributions needed to develop models useful in risk assessment (Spear and Bois 1994; Spear et al., 1991; Watanabe and Bois, 1996; Bois et al., 1991a, 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, a National Cancer Institute chronic study (NCI, 1980) 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. On the basis of the researchers' modeling effort, hydroquinone was rejected as the ultimate toxic agent, and the pathway through benzene glycol to catechol and MUC appeared to provide a better fit to the data.

Kenyon et al. (1995) investigated the metabolism of phenol. Even though phenol is thought to be a key intermediate in benzene metabolism leading to toxicity, orally administered phenol is neither carcinogenic or genotoxic (NCI, 1980). The authors found markedly higher excretion of hydroquinone glucuronide after oral benzene exposure as compared with 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.

Watanabe and Bois (1996) examined three methods (multiplicative, additive, and allometric) to extrapolate physiological parameter distributions across species, specifically from rats to humans. The results indicated that the multiplicative and allometric techniques were able to extrapolate the model parameter distributions.

Bois et al. (1996) applied techniques from population pharmacokinetics, Bayesian statistical inference, and physiological modeling to model distribution and metabolism in humans. Statistical distributions for the parameters of a physiological model of benzene were derived on the basis of existing data. The relationship between the fraction of benzene metabolized in bone marrow and benzene exposure was 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 [CI] 47–67%). At levels approaching occupational inhalation exposure (continuous 1 ppm [3.2 mg/m^3]), the estimated amount metabolized in bone marrow ranged from 2 to 40 mg/day. However, this model has not been tested for its ability to predict data from other studies (Smith and Fanning, 1997).

In summary, PBPK models continue to improve as additional data become available and are incorporated and as additional techniques from other scientific fields are applied to modeling benzene dosimetry. However, the current published models are insufficiently refined to allow them to predict human metabolism accurately (Smith and Fanning, 1997). The key areas for refinements appear to be the inclusion of the kinetics of the putative toxic metabolites of benzene or their stable precursors. If benzene metabolites such as hydroquinone/ benzoquinone, MUC, and/or benzene oxide are the toxic species, then PBPK models need to include descriptions of their kinetics if they are to be useful in improving uncertainties in risk assessment.

3.6. TOXICOKINETICS SUMMARY

Benzene is almost completely absorbed from the gastrointestinal tract over a wide range of dose levels in rats and mice. In contrast, several human studies have indicated that the respiratory absorption of benzene is approximately 50%. At a concentration of 10 ppm (31.9 mg/m^3), retention of benzene during a 6-hour exposure was 33% in rats and 50% in mice. Retention decreased at higher concentrations (Sabourin et al., 1987). At high concentrations, benzene metabolism is saturated, and respiratory excretion of unmetabolized benzene increases. Dermal absorption is less than 1% of the applied dose due to rapid volatilization from nonoccluded skin.

Benzene is rapidly distributed throughout the body regardless of the exposure route. Following oral exposure, Low et al. (1989) found the highest concentrations in liver and kidneys, intermediate concentrations in blood, and the lowest concentrations in the Zymbal gland, nasal cavity, oral cavity, mammary gland, and bone marrow. Benzene is preferentially stored in fat (Rickert et al., 1979). Metabolites of benzene are also found throughout the body, with phenol being eliminated from bone marrow more rapidly than catechol or hydroquinone (Rickert et al., 1979).

Despite extensive research, the metabolism of benzene to toxic metabolites is still not completely understood. Metabolism of benzene by CYP2E1 is necessary for the expression of hematotoxicity (Sammett et al., 1979; Valentine et al., 1996). Following initial oxidation by CYP2E1 to benzene oxide, however, the benzene metabolic pathway branches to produce several putative toxic metabolites. Most research has focused on the hypothesis that phenol, catechol, and hydroquinone are produced in the liver and transported to the bone marrow, where hydroquinone is activated to *p*-benzoquinone by the action of MPO (Smith et al., 1989; Rushmore et al., 1984; Low et al., 1995). However, the fact that administration of phenol does not produce the same effects as benzene is a problem in the phenolic metabolite hypothesis. A combination of phenol and hydroquinone is required to reproduce the hematotoxic effects of benzene (Eastmond et al., 1987).

A ring-opening pathway of benzene metabolism leads to excretion of MA in the urine. The production of the highly reactive ring-opened dialdehyde MUC as an intermediate in the pathway to MA has been demonstrated in isolated hepatic microsomes; however, circulation of MUC in the blood has not been demonstrated (Witz et al., 1996). Administration of MUC reproduces the hematotoxic effects in mice; however, MUC is so reactive that it is not likely to reach the target tissues. The less reactive ring-opened metabolite CHO-M-OH has been detected in rat liver perfusate (Grotz et al., 1994) and has been shown to be hematotoxic in mice (Zhang

et al., 1995b). Thus, CHO-M-OH is another potentially toxic benzene metabolite, but there has been little research to define whether CHO-M-OH is quantitatively important.

Benzene oxide was believed to be too reactive to escape the liver, but it has recently been measured in the bloodstream of mice, and its dose to the body may exceed that of *p*-benzoquinone (Lovern et al., 1997; Lindstrom et al., 1997). Thus, several metabolites of benzene may play a role in inducing the toxic effects of benzene, and a combination of several metabolites may be required to cause the full range of benzene-induced toxic responses. Large quantitative differences in the production of putative toxic benzene metabolites have been observed among different animal species (Sabourin et al., 1989, 1992). Because few data exist on the proportion of different benzene metabolites produced in humans, there is considerable uncertainty in selecting the most appropriate animal model for humans.

There is evidence to support several different mechanisms by which benzene metabolites may cause hematotoxicity. Benzene metabolites form adducts with both proteins and DNA (McDonald et al., 1994; Lutz and Schlatter, 1977). Adduct formation with protein sulfhydryl groups inhibits tubulin polymerization during spindle formation and the activity of topoisomerase II in DNA replication and transcription (Irons and Neptun, 1980; Pfeifer and Irons, 1983; Frantz et al., 1996). These effects may explain the clastogenic activity of benzene. Benzene itself is not mutagenic; however, mutagenicity of several of its metabolites is well established (Dean, 1985). Benzene metabolites may also induce oxidative stress by depleting levels of GSH and by production of reactive oxygen species that react with cellular macromolecules (Snyder et al. 1987; Brunmark and Cadenas, 1988; Rao and Snyder, 1995). Benzene also disrupts production of the cytokine IL-1, which is essential for hematopoiesis. Niculescu et al. (1995, 1996) found that *p*-benzoquinone inhibits the activity of calpain, an enzyme that catalyzes the processing of pre-IL-1α to the mature cytokine. All of these effects may be involved in causing the toxic effects of benzene, but it is not possible to determine which of these effects is the primary mechanism for benzene toxicity.

At low exposure levels, benzene is excreted primarily in the urine as sulfate or glucuronide conjugatges of phenolic metabolites or as MA (McMahon and Birnbaum, 1991; Sabourin et al., 1987). At oral doses of less than 15 mg/kg, ^{14}C from benzene is nearly quantitatively recovered as metabolites in urine in rats and mice, but at a dose of 150 mg/kg, greater than 50% was cleared unmetabolized in the breath (Sabourin et al., 1987). Similarly, less than 6% of ^{14}C from benzene was exhaled in the breath from inhalation exposure to 10 ppm (32 mg/m^3) in rats or mice, but at concentrations greater than 850 ppm (2718 mg/m^3) rats exhaled 48% and mice exhaled 14%. Urinary phenol and MA concentrations are correlated with benzene exposure level and can be used to monitor benzene occupational exposure (Ghittori et al., 1993). However, the database for evaluating the relative importance of different excretion pathways in humans is limited. Thus, there is a limited database for comparing the importance of human excretion pathways at varying dose levels with the results of experimental animal studies.

Several different PBPK models have been developed to mathematically describe the uptake, distribution, metabolism, and excretion of benzene (Sato, 1988; Medinsky et al., 1989a, b, c; Travis et al., 1990a, b; Bois and Paxman, 1992). Each of these PBPK models has advantages, and some successfully simulate uptake, metabolism, and excretion in mice, rats, and humans. However, the utility of PBPK modeling to predict the dose of toxic metabolites to

target organs in humans is limited by the incomplete knowledge of the toxic metabolites and difficulties in identifying a suitable experimental animal model for humans.

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). 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). Lethality in humans has been attributed to respiratory arrest, central nervous system depression, or cardiac collapse (Greenburg, 1926). 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*

The recognition of benzene as etiologically significant 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, dates back to the 19th century (Goldstein, 1988). Exposure to benzene has also been associated with acute myelogenous leukemia (AML) and lymphoid malignancies in humans (Goldstein, 1988; Snyder, 1987), although the precise relationship between frank pancytopenia and the benzene-induced malignancies remains unclear (Snyder, 1987). That the hematopoietic abnormalities observed in humans exposed to benzene in an occupational setting have been reproduced in numerous animal experiments points to their universality. The comparatively low doses associated with their onset suggest that they may represent a sensitive effect of benzene toxicity.

Human exposure to benzene occurs primarily via inhalation in the workplace, from gasoline vapors, tobacco smoke, and automotive emissions. Individuals exposed to benzene exhibit bone marrow depression, as evidenced by anemia (decreased RBC count), leukopenia (decreased WBC count), and/or thrombocytopenia (decreased platelet count). A depression of all three elements is called pancytopenia, and the simultaneous depression of RBCs, WBCs, and platelets, accompanied by necrosis of the bone marrow, is diagnostic of aplastic anemia. 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 profile may vary from aplasia to hyperplasia, and these symptoms may vary in frequency and severity in different patients. Other hematologic changes observed in humans chronically exposed to benzene include decreased

osmotic resistance in leukocytes, decreased phagocytic function of neutrophils, reduced glycogen content, and decreased activity of peroxidase of neutrophils and increased delta-aminolevulinic acid activity in erythrocytes and increased coproporphyrins in the urine (Aksoy, 1991).

As summarized in Table 1, the large number of epidemiologic studies that have explored the health consequences of benzene exposure in the workplace provide a considerable body of inferential evidence for the compound-related onset of hematopoietic effects in chronically exposed subjects. It should be recognized that for most of the studies there are uncertainties and/or variabilities in precisely delineating the concentrations of benzene to which subjects were exposed. For example, uncertainty surrounding the past or present workplace concentrations of the chemical may not permit the dose to which subjects were exposed to be estimated accurately. Even when an exposure term can be defined within an adequately cohesive range, its overall contribution to the understanding of the compound's dose-response characteristics will probably be limited to a single data point from any given study. Thus, the utility of such a study might only arise through its consideration with others as a group, for example, in a "meta-analysis." In such a survey, the establishment of a combined range for estimated exposures and their toxicological consequences can permit (1) the emergence of a unifying concept of the compound's toxicity to humans and (2) the delineation of a range of concentrations or doses within which the compound's toxicological threshold may reside.

The paragraphs that follow describe the many epidemiologic studies that have helped to establish the onset of benzene's hematologic effects.

In general, it is assumed that for the occupational exposure studies under consideration, the levels of exposure to benzene were greater in earlier studies than in more recent studies, a reflection of times when occupational exposure standards and guidelines were less rigorous. For example, studies that have examined occupational exposure to benzene at relatively high concentrations include several that evaluated hematologic surveillance records of 459 rubber hydrochloride workers from the Pliofilm production departments of a rubber products

Table 1. Hematotoxicity of benzene—occupational exposure

Population/Industry	No. of Subjects	Exposure	Effects	Reference
Rotogravure printers	6	77–3386 mg/m^3 benzene	Pancytopenia	Erf and Rhoads, 1939
Rotogravure printers, New York	332	35–3386 mg/m^3 benzene	23 cases of significant cytopenias	Goldwater, 1941; Goldwater and Tewksbury, 1941; Greenburg et al., 1939
Shoe factory, Finland	147	≤1278 mg/m^3 benzene for ~10 yr	107 cases of hematologic abnormalities	Savilahti, 1956
Shoe factory, Finland	147	Followup of Savilahti (1956) study, 9 years 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) benzene	83 cases of mild hematologic 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 years after cessation of exposure to benzene	Four 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 benzene (prior to installation of control measures)	Follow-up suggested mild persistent anemia	Pagnotto et al., 1961
Shoe manufacturing, Turkey Controls (healthy hospital workers)	217 100	96–671 mg/m^3 benzene for 3 months–17 years	51 cases of hematologic abnormalities, including 6 cases of pancytopenia	Aksoy et al., 1971
Various industries using benzene-containing adhesives, Turkey	32 diagnosed with blood dyscrasias	479–1597 mg/m^3 benzene for 4 months–15 years	32 cases of significant aplastic anemia; eight deaths from thrombocytopenic hemorrhage and infection	Aksoy et al., 1972
Various industries using benzene-containing adhesives, Turkey	44 diagnosed with blood dyscrasias	Follow-up study over 2–17 years after last exposure to 479–2077 mg/m^3 benzene for 4 months–15 years	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 1. Hematotoxicity of benzene—occupational exposure (continued)

Population/Industry	No. of Subjects	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: two 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; one 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 years 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 TWA exposures: Benzene—105 and 188 mg/m^3 (55 and 65 months) for men and women, respectively; Toluene—173 and 154 mg/m^3 (95 and 73 months); 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 months)	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., 1987b

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

Population/Industry	No. of Subjects	Exposure	Effects	Reference
Rubber industry	459	Mean estimated exposure from 1948 to 1975, 48–64 mg/m^3	Significant increases in WBCs, RBCs, and Hgb during the 1940's (Pearson correlations: r = 0.50 for WBCs, r = 0.44 for RBCs, 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., 1988, 1989
Workers Unexposed controls	200 268	0.03–4.5 mg/m^3 8-hour TWA over a 10-year 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 years; doses were measured by personal dosimetry for 2 weeks	Significant increase in mean corpuscular volume and significant decreases in ALC, WBCs, RBCs, HCT, and platelets in above-median group; significant reductions in ALC, RBCs, and platelet count in below-median group; but only reduction in ALC was significant in low-exposure group	Rothman et al., 1996a
Rubber industry	657	Estimates of benzene exposure (≤109 mg/m^3) for last 30, 90, or 180 days before blood tests were correlated with hematologic 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

manufacturer in Ohio (e.g., Kipen et al., 1988, 1989; Ward et al., 1996). The data indicate that during the time period 1940–1948, significant increases in erythrocyte, Hgb, and leukocyte levels were observed among the workers, with the responses displaying a positive time-related trend (1940–1948). Thus, overall cell numbers and values appear to have been higher at the later time points, the prevailing levels of exposure to benzene becoming reduced as industrial hygiene practices improved. Kipen et al. (1988, 1989) compared the utility of two exposure assessment models for correlating the observed hematologic consequences with plausible estimates of benzene exposure in the absence of definitive monitoring data at the earlier time points. Thus, between 1948 and 1975, when the workers were exposed to mean 8-hour TWA concentrations of only 15–20 ppm (48–64 mg/m^3), no significant relationships between benzene levels and any hematologic parameters were observed. By contrast, significantly lower cell counts and hemoglobin concentrations were observed in exposed subjects, where the levels of benzene exposure were thought to be as high as 75 ppm (240 mg/m^3) at the earlier time points (pre-1948) (Kipen et al., 1989).

Cody et al. (1993) observed significant hematologic effects, including reduced RBC and WBC counts in 161 male rubber workers exposed to median peak concentrations (i.e., only the peak concentrations for any given exposure time were reported) of 30–54 ppm or more for a 12-month period during 1948. The 30 ppm value was considered a 1-year LOAEL for hematologic effects. In this rubber plant, workers who had blood dyscrasias were excluded from working in the high-benzene units. Furthermore, individual workers who had more than a 25% decrease in WBC counts from their pre-employment background count were removed from the high-benzene units and placed in other units with lower benzene concentrations. Sensitive individuals therefore could have been excluded from the analysis. The 30 ppm value is the low end of the range of median values (30–54 ppm) reported by Crump and Allen (1984) and used in the Kipen et al. (1988) and Cody et al. (1993) studies. These are the same Pliofilm workers studied by Rinsky et al (1981, 1987).

The analysis of Ward et al. (1996) differs from the earlier investigations of the same rubber worker cohort by Kipen et al. (1988, 1989) 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 data set, evaluated the exposure-response relationship on the basis of individual dose metrics, and controlled for the temporal trends in pre-employment blood count screening.

The Ward et al. (1996) analysis used hematologic screening data 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 used a case-control design and estimated benzene exposures using the job exposure matrix developed by Rinsky et al. (1981). The effects of benzene exposure in the 30, 90, and 180 days before the blood test date, as well as cumulative exposure until the blood test date, were examined using conditional logistic regression. For WBC count there was a strong exposure response, and all of the exposure metrics selected showed a significant relationship with low blood count. For RBC count there was a weak positive exposure response that 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 hematologic effects of benzene exposure, suggesting that even exposure to relatively low levels (e.g., < 5 ppm [16 mg/m^3]) could result in hematologic 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; therefore, if one assumes that Ward's Pliofilm workers exposure estimates are correct, they suggest that hematologic effects of benzene occur even at 5 ppm. Because of controversy surrounding the exposure estimates used by Ward et al., this finding could be biased.

The nature of the problem is that the estimates of early exposure to benzene (prior to 1946) are not based on any measured ambient air inhalation data for any of the more than 1200 Pliofilm workers who begn work in the plants prior to 1946. Estimates of exposure to benzene in the set of exposure indices chosen by Ward and her associates (1996) were based on the exposure scenario described by Rinsky et al. (1981), which basically assumed that the exposures were the same as the local and State standards established for benzene at the time (in the years prior to 1946). This assumption has been challenged by others (Crump, 1992; Paustenbach et al., 1992), who claim that the exposures during this time were much higher. Thus, the assumption that the exposures could have been as low as < 5 ppm may not be correct. The weaknesses in the Ward et al. study are discussed further in U.S. EPA (1998a). No LOAEL or NOAEL could be established for this study.

In another occupational study that examined the health effects of benzene, Fishbeck et al. (1978) studied the hematologic characteristics of 10 Dow Chemical employees who had been exposed to high concentrations of the chemical in an ethyl cellulose sheeting operation for various durations between 1937 and 1965. Duration of exposure was from fewer than 4 to nearly 30 years, with average 8-hour TWA benzene concentrations exceeding 25 ppm (80 mg/m^3) for at least a part of the period of employment. As described by Fishbeck et al. (1978), in a 1953 industrial hygiene survey, workers who had an "operator" job classification had the highest exposure to the compound, with an 8-hour TWA of 30–35 ppm (96–112 mg/m^3) and occasional "spikes" of up to 937 ppm (2990 mg/m^3). However, a 1963 study described by Fishbeck et al. (1992) revealed 8-hour TWAs ranging from 37 to 132 ppm (118 to 422 mg/m^3), with four employees' exposure levels exceeding 100 ppm TWA (319 mg/m^3). Concurrent hematologic evaluations undergone by these employees included Hgb, HCT, RBC and WBC counts, sedimentation rates, platelet counts, differential blood counts, clot retention determinations, and blood indices.

Key findings included enlarged RBCs, transient anemia, reduced hemoglobin concentrations, and mean corpuscular volumes (MCVs) exceeding medically accepted values. However, the authors noted that since 1963, the employees' MCVs had shown a reduction from the highest values obtained, such that 5 of the 10 employees had MCVs within the normal range. From these data, the authors concluded that at least some of benzene's effects on the hematopoietic system may be reversible. This conclusion may be premature. This small group of 10 employees was a survivor population and therefore may have introduced a potential bias. They were selected because they were listed on current employment rolls of the company when the study was in progress. Employees who had left employment earlier, perhaps with a benzene-

related nonreversible condition, could not be selected for inclusion in this study. Therefore, persons with an irreversible condition due to exposure to benzene could not have been represented in this group of 10. This study cannot be used to establish a NOAEL or a LOAEL.

Aksoy et al. (1971) assessed hematologic abnormalities in 217 apparently healthy men exposed to benzene in the shoe manufacturing industry. The test battery included RBC, WBC, platelet, and differential cell count; 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 (671 mg/m^3) during the use of adhesives containing benzene; exposure duration ranged from 3 months to 17 years. A total of 217 exposed workers were involved in the study, with 51 (23.5%) displaying hematologic abnormalities. Of these, 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 (1) normocellularity with no abnormal histology (two patients), (2) slight hypocellularity (three patients), (3) hypercellularity (one patient), or (4) 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 follow-up study, Aksoy et al. (1972) reported that 8 of 32 workers diagnosed with pancytopenia died, mainly from infection and bleeding.

In an industrial hygiene survey of 282 Dow Chemical workers who were categorized by job classification into four groups on the basis of their exposure to benzene, exposed individuals were monitored for a number of clinical chemistry and hematologic parameters and compared with an age- and sex-matched cohort of unexposed Dow employees serving as controls (Townsend et al., 1978). Ranges of benzene concentrations within the groups were < 2, 2–9, 10–24, and > 24 ppm (< 6.4, 6.4–29, 32–77, and > 77 mg/m^3). However, as noted by the authors, neither the RBC counts nor any other hematologic parameter correlated with exposure intensity, duration of exposure, or estimated career dosage (Townsend et al., 1978). In fact, no consistent relationship between any monitored parameter and benzene exposure was apparent from their data.

A report by Yardley-Jones et al. (1988, 1990) described a survey in which blood samples were obtained from 66 refinery workers exposed to benzene at comparative low concentrations and 33 unexposed employees serving as controls. Exposure levels of < 10 ppm (32 mg/m^3) were estimated through consideration of the exposed employee's workstation, supplemented by a small amount of personal monitoring data. Exposed subjects were assessed for the appearance of sister chromatid exchange in peripheral lymphocytes and the ability of a subject's β-glucuronidase/sulfatase-treated urine to promote gene reversion in the Ames test. In addition, some hematologic parameters were measured in whole blood, although the largely negative

findings gave no indication of a cause-effect relationship for benzene within the estimated exposure range.

Bogadi-Sare et al. (2000), in a medical surveilance study, examined 49 female workers in the shoemaking industry who were exposed to solvent mixtures and 27 nonexposed controls. Exposures were as high as 15 ppm benzene and 50 ppm toluene. Significant differences in the levels of benzene and toluene in blood and phenols in postshift urine between the exposed and the control group confirmed solvent exposure. The number of B-lymphocytes was lower in the shoe workers than in the controls (p=0.01). Significant correlations were found between the level of immunoglobulin G and benzene in the work atmosphere. Confounding factors appeared to play no role in the immunological findings. The authors found that exposure to benzene concentrations lower than 15 ppm can induce depression of the circulating B-lymphocytes.

Khuder et al. (1999) reported on a group of 105 petroleum workers exposed to 0.14–2.08 ppm (0.45–6.6 mg/m^3) benzene who, over time, had small but statistically significant falls in certain blood counts. However, there were problems with this study, including a decrease in the red cell MCV, a finding contrary to what is observed in benzene toxicity (Goldstein and Cody, 2000).

Collins et al. (1991) used a cross-sectional study design to compare five hematologic parameters in 200 benzene-exposed workers with those of 268 unexposed employees in the same plant. The type of work is not identified in the study. Estimates of the 8-hour TWA benzene concentration were obtained from monitoring information coupled with knowledge of the applicable work assignment and professional judgment. Benzene concentrations ranged from 0.01 to 1.4 ppm (0.03–4.5 mg/m^3) for exposed subjects. Parameters under investigation included RBCs, WBCs, Hgb concentration, platelets, and MCV. The authors noted statistically significant differences in some of the hematologic parameters, based on demographic and lifestyle factors. Thus, when multiple regression analyses were applied using the confounding factors and current exposure as independent variables, no significant correlations between benzene exposure and any hematologic parameter was evident. Again, this was a study of currently employed workers and, as such, represented a healthy group of individuals, who, having survived potentially adverse effects from exposure to benzene and other environmental hazards, were able to continue to work and be included in this cross-sectional study. Hence, this study cannot be used to establish a LOAEL.

Tsai et al. (1983) examined mortality patterns in a cohort of 454 former and current male employees who were selected by industrial hygienists from a larger group of 20,000 present and former refinery employees. The cohort members were chosen because they had worked directly in the benzene, ethylene, aromatic distillate hydrogenation, or caiman units, the principal petrochemical units in the refinery, for 1 to 21 years beginning in 1952. Average length of "exposure" was 7.4 years. Maintenance workers who were assigned on a regular basis to these same units were also included. Irregularly assigned workers to these units were not included, although they may have been exposed to benzene. Personal exposures to benzene were determined from 1979 to 1982. The median air concentration was 0.53 ppm in the "benzene-related units," while in the refinery as a whole, the median was 0.14 ppm. In nonbenzene exposure areas, the median was 0.07 ppm. This one-time "snapshot" of benzene exposure levels says little about what the exposures may have been in the past or about study duration, nor do

they tell us whether these personal samples relate to the 303 medically surveyed workers discussed below, as they are a different group from the 454 who were part of the mortality cohort. From this study it is impossible to determine the concentrations of benzene to which the medically surveyed workers were exposed.

The analysis of overall mortality in the mortality cohort of 454 revealed no excesses. In fact, mortality from all causes and from diseases of the circulatory system was significantly below expected values, based on comparable groups of U.S. males. The authors concluded that the presence of a healthy-worker effect produced significantly lower risk estimates, all causes (standard mortality ratio = 0.53, $p<0.05$); only 34 deaths occurred, whereas 58.7 were expected. The authors noted the relative youthfulness of the cohort. An internal but separate analysis of a comparison group of 823 people consisting of 10% of the workers who were employed in the same plant in operations not related to benzene produced higher relative risks of 0.90 and 1.31 for all causes and cancer at all sites, respectively ($p<0.28$ and $p<0.23$), higher than those of the so-called "benzene-exposed" workers. Because of the appearance of significantly reduced risks in this preselected cohort, the possibility of bias in the selection process should not be ruled out.

In addition, hematologic parameters were analyzed in a separate group of 303 male "benzene workers" (according to the supervisors who selected them) beginning in 1959 and ending in 1979. During this time, 11 deaths occurred in this group, whereas 21.5 were expected. Up to four hematologic tests per year were conducted on just these workers. Total and differential WBC counts, Hgb, HCT, RBC counts, platelets, and clotting times were calculated, and a 95% range and standard deviation were derived for each hematologic test. Unfortunately, as the authors pointed out, the data could not be compared with any "healthy population" standard, because existing "normal" ranges are derived largely from hospital experiences. The authors suggested that, based on the experience of this program, there is a need to establish different interpretation criteria for screening for adverse effects of benzene exposure.

Thus, the Tsai et al. (1983) study is similar to the studies by Collins et al. (1991), Fishbeck et al. (1978), and Yardley-Jones et al. (1988, 1990) in that the study population was a currently employed group of workers (except for the 11 who died after the medical surveillance was begun) and, as such, a survivor population in which few or no adverse health effects would have been found. In addition, these medically surveyed employees were healthy and young. They had been exposed for a short time to minimal levels of benzene that generally did not exceed 0.53 ppm and probably were a lot less, based on survey results. Therefore, the selection of a NOAEL is not considered on the basis of this study.

Considering data from the studies that have addressed the possible toxicological consequences of exposure to benzene, a continuum of hematologic responses that are etiologically linked to exposure to benzene is evident, although the precise threshold level for these effects remains uncertain. However, some clarification of this issue has been provided by an ongoing collaboration between the National Cancer Institute and the Chinese Academy of Preventive Medicine that has generated a series of reports on both large and small cohorts of subjects who were occupationally exposed to benzene.

Data from China describe clinical aplastic anemia in factories with exposure levels said to range from 93 to 1156 mg/m^3. Yin et al. (1987b) found 2676 cases of benzene

poisoning—defined as a WBC count less than 4000 cells /mm^3 and a history of benzene exposure—from a review of more than 500,000 benzene-exposed workers in China. The geometric mean concentration in 50,255 workplaces was 18.1 mg/m^3. It was reported that 64.6% of the workplaces had benzene exposure levels less than 40 mg/m^3. From their review, the authors concluded that cases of benzene poisoning can occur even in factories with less than 40 mg/m^3 concentration of benzene.

Dosemeci et al. (1996) discussed the findings of a large-scale epidemiologic study involving subjects employed between 1949 and 1987 in 672 factories in 12 cities in China. The authors sought to establish plausible estimates of the levels of benzene exposure through an industrial hygiene-based survey of all factory/work unit/job title/calender period data, providing assessments that were subsequently correlated with one of six hypothetical exposure ranges between < 1 and > 50 ppm. Thirty-eight percent of a total of 18,435 exposure estimates were confirmed by workstation monitoring. The authors set hematologic criteria that defined benzene poisoning to include a WBC count of < 4000 cells/mm^3, platelets < 80,000/mm^3, etc., thereby allowing the identification of a case-defined subset of individuals who could be matched with estimated levels of benzene exposure as described above. Relative risks of benzene poisoning by intensity of exposure during the most recent 1.5-year exposure period compared with those exposed to < 5 ppm (16 mg/m^3) yielded estimated risks of 2.2 (95% CI = 1.7–2.9), 4.7 (95% CI = 3.4–6.5), and 7.2 (95% CI = 5.3–9.8) for exposure levels of 5–19 ppm (16–61 mg/m^3), 20–39 ppm (64–125 mg/m^3), and > 40 ppm (> 128 mg/m^3), respectively. Clearly, if a significantly elevated risk of benzene poisoning is an indication of hematotoxicity, then certainly exposures to benzene at 5–19 ppm are hematotoxic. The decrease in ALC contributed substantially to the decrease in WBC count, based on the results of the Rothman et al. (1996a) study discussed in the next review. In general, recent exposure history appeared to be more closely related to elevated risk than exposure duration, suggesting the comparatively rapid onset of benzene hematotoxicity. These results support a threshold of benzene hematotoxicity in humans in the 5–19 ppm range, in broad agreement with the emerging exposure-response range that is apparent from the epidemiologic studies described in this section.

Some data that have shed further light on this issue have been provided by the same group (Rothman et al., 1996a) that conducted a small cross-sectional study that compared 44 workers exposed to a range of benzene concentrations similar to those of Dosemeci et al. (1996) with 44 age- and gender-matched unexposed controls. The purpose of this study was to show that exposure to benzene affects all the major peripheral blood elements, with the ALC being the most sensitive indicator of benzene-induced hematotoxicity. From public health district records, workers from three workplaces in Shanghai, China, 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 test subjects and controls, respectively. The three workplaces using benzene included a factory that manufactured rubber padding for printing presses, a factory that manufactured adhesive tape, and a factory that used benzene-based paint. The control workplaces included a factory that manufactured sewing machines and an administrative facility. Subjects who had a 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 bone marrow-toxic chemicals or ionizing radiation. Controls who had

no history of occupational exposure to benzene or other bone 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- to 2-week period prior to collection of blood samples. Benzene exposures of controls in the sewing machine factory were monitored for 1 day, but no exposure monitoring was performed in the administrative facility. Compound 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. Median levels of the urine metabolites phenol, catechol, MA, and hydroquinone correlated positively with the 8-hour TWA personal air level measurements of benzene by exposure category (Table 2).

It is possible that air exposure to benzene may have been overestimated among workers wearing marginally effective respirators. However, a comparison of urinary phenol levels to the air benzene levels can be made. Urinary phenol is thought to increase in a linear fashion up to 100 ppm benzene. Roughly, 50 μg/mg creatinine is thought to be equivalent to an 8-hour TWA of 10 ppm benzene. For 19 workers exposed to more than 31 ppm who had an air and urine sample taken on the same day, the median benzene exposure was 73 ppm and the median urine phenol level was 351 μg/mg creatinine, about what one would predict (letter from Nathaniel Rothman, National Institutes of Health, Bethesda, MD, to David Bayliss, NCEA-W, July 28, 1999). This is a test of the validity of the benzene air sampling effort. Dosemeci et al. (1996) estimated that only about 5% of more than 1000 workers who were exposed to 100 ppm benzene or higher in their factories were diagnosed with benzene poisoning. It is not known whether severe hematotoxicity occurred to that 1 individual in 19 who was reported to have a TWA of 328.5 ppm. It is not always possible to obtain a perfect correlation between a surrogate measure of exposure and an effect.

Historical benzene exposure of the subjects was evaluated by examining employment histories. However, this information was not used to classify subjects by category of current exposure. Data on age, gender, current and lifelong tobacco use, alcohol consumption, medical history, and occupational history were collected by interview.

Six hematologic parameters were evaluated: total WBC count, ALC, HCT, RBC count, platelet count, and MCV. Total WBC counts and ALCs were performed using a Coulter T540 blood counter. Abnormal counts were confirmed by hand. The Coulter company estimated that the machine used in this study counts about 10,000 WBCs to generate a total WBC count and the percent lymphocytes, which are multiplied to provide the ALC. The hand count involves counting a standard 100 cells. The manual count also used to generate the percent lymphocytes in this study was the standard 100 cells. Much higher-quality data would be expected for the former and less precision for the latter, due to sampling variation (as well as human scoring error). However, the Coulter counting machine actually generated larger and more significant

Table 2. Median benzene urine metabolites, by exposure category, in a study of workers exposed to benzene in Shanghai, China, 1992[a]

Exposure Category	Phenol	Catechol	MA	Hydroquinone
Control (N = 44)	17.3	3.2	0.18	1.6
Exposed (< 31 ppm, N = 22)	38.9	7.0	8.15	12.8
Exposed (> 31 ppm, N = 22)	348.7	65.7	46.8	64.3

[a]Units µg/mg creatinine for all metabolites.

Source: Adapted from Rothman et al., 1996a.

differences in the ALC between the exposure groups than did manual counts. The authors reported that previous studies that reported ALC and percent lymphocytes based on manual counts may have underestimated group differences due to nondifferential measurement error. Benzene metabolites in urine were measured by an isotope dilution gas chromatography/mass spectrometry assay. Correlation analyses were completed using the Spearman rank order correlation method. The Wilcoxon rank sum test was used to test for hematologic differences. Twenty-one of the 44 subjects in both the exposed and control groups were female.

Mean standard deviation years of occupational exposure to benzene was 6.3 (standard deviation = 4.4 years) with a range of 0.7–16 years. The median 8-hour TWA benzene exposure concentration for the 44 exposed workers was 31 ppm (99 mg/m^3). The 8-hour TWA for each subject was derived from the geometric mean of the five air measurements that were gathered on each of the exposed workers on the five different days during which the samples were taken. Exposure to toluene and xylene was less than 0.2 ppm (0.6 mg/m^3) in all exposure categories. The exposed group was then subdivided into two equal groups of 22 workers consisting of those exposed to greater than the median concentration of 31 ppm versus those exposed to less than the median concentration according to the 8-hour TWA. 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, none of whose five separate measurements exceeded 31 ppm (100 mg/m^3) at any time during the monitoring period, was also analyzed for ALC. The median (range) 8-hour TWA exposure of these individuals was 7.6 (1–20) ppm (24 [3.2–64] mg/m^3). All five air measurements were completed within 1 to 2 weeks of the phlebotomies on all subjects.

All six blood elements measured were significantly different in the highest benzene exposure group when compared with controls (Table 3). ALC, WBCs, RBCs, HCT, and platelets were all significantly decreased, and MCV was significantly increased with increasing benzene exposure. These effects are consistent with the hematotoxic effects of benzene shown in Aksoy (1989) and Goldstein (1988). ALC was reduced from $1.9 \times 10^3/\mu L$ blood in controls to

Table 3. Comparison of mean peripheral blood counts (with standard deviations), by exposure status, in a study of workers exposed to benzene in Shanghai, China, 1992

Exposure Category	WBC Count ($\times 10^3$/μL blood)	ALC ($\times 10^3$/μL blood)	RBC Count ($\times 10^6$/μL blood)	HCT (%)	MCV (μm^3)	Platelets ($\times 10^3$/μL blood)
Control (median = 0.02 ppm, N = 44)	6.8 (1.7)	1.9 (0.4)	4.7 (0.6)	42.0 (5.6)	88.9 (4.9)	166 (59)
Exposed (≤ 31 ppm, N = 22)	6.4 (1.8)	1.6 (0.3)[a]	4.6 (0.6)[b]	41.2 (5.7)	89.8 (3.9)	132 (45)[b]
Exposed (> 31 ppm, N = 22)	5.6 (1.9)[a]	1.3 (0.3)[c]	4.2 (0.6)[c]	38.8 (5.3)[b]	92.9 (3.4)[c]	121 (43)[a]

[a] $p<0.01$
[b] $p<0.05$
[c] $p<0.001$

Source: Adapted from Rothman et al., 1996a.

1.6×10^3/μL ($p<0.01$) in the < 31 ppm (99 mg/m^3) group and to 1.3×10^3/μL ($p<0.001$) in the group exposed to > 31 ppm (99 mg/m^3) benzene. A dose-response relationship is evident in five measures of hematotoxicity. 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 lowest-exposure subgroup (median = 7.6 ppm, 24 mg/m^3). In this subgroup of 11 workers, whose median 8-hour TWA exposure was 7.6 ppm (24 mg/m^3) benzene, ALC (1.6×10^3/μL) was similar to that of the larger group of 22 who were exposed to < 31 ppm benzene. Thus, ALC is the most sensitive measure of benzene hematotoxicity, representing a sentinel of a continuum of the likely hematotoxic consequences of prolonged or enhanced exposure to benzene below 31 ppm.

One feature of the Rothman et al. (1996b) study was its attempt to probe the mechanisms by which benzene may bring about the hematotoxicological symptoms that were evident in the blood samples of exposed subjects. For example, although most peripheral blood cell levels were decreased in exposed workers as compared with controls, peripheral cytokine levels (IL-3, IL-6, erythropoietin, granulocyte colony-stimulating factor, and tissue necrosis factor-alpha) were similar in exposed workers and controls, suggesting that benzene may not affect these growth factor levels in peripheral blood. By contrast, when the rates of mutations were measured at the glycophorin A (GPA) locus, an increased frequency of somatic cells with the NN variant was interpreted as an indication of the compound's capacity to produce gene-duplicating but not gene-inactivating mutations at the GPA locus in bone marrow cells (Rothman et al., 1995).

The study by Rothman et al. (1996a) is one of few occupational studies in which individual- and workstation-monitored benzene concentrations provide a range of values that encompass the exposure-response area into which, as judged by the data from other larger-scale epidemiologic studies, the point-of-departure for the compound's hematologic effects appears likely to fall. Further marking the study's relevance as a basis for developing quantitative reference values for the compound is the exposure-response relationship that emerged between ALCs and benzene concentrations. This observation is based on the inverse correlations between these parameters that were reported for benzene-exposed workers and on the exposure response that was observed when the data for exposed subjects were grouped on either side of the overall median benzene concentration of 31 ppm (99 mg/m^3).

Further assurance that the study met sufficient criteria for its use in developing an inhalation RfC for benzene is provided by the facts that worker exposure to toluene and other potentially harmful solvents was minimal in the exposed group and that the study protocol fulfilled adequate criteria for age- and sex-matching of exposed and control subjects.

As discussed above, a number of subgroups of benzene-exposed workers have been defined in the Rothman et al. (1996a) study, including (1) a high-dose subset of 22 workers, the means of whose 5-day passive dosimetry measurements for benzene were greater than the median (31 ppm [99 mg/m^3]) of the 5-day mean benzene exposure concentration values for the benzene-exposed group as a whole; (2) a low-dose group whose 5-day mean TWA benzene exposure values were lower than 31 ppm (99 mg/m^3) (median benzene concentration = 13.6 ppm [43.4 mg/m^3]); and (3) a subset of the low-dose group whose exposure levels in all 5-day consecutive breathing zone measurements were lower than 31 ppm (99 mg/m^3) (median benzene concentration = 7.6 ppm [24.3 mg/m^3]). Because a reduction in ALC was the only significant hematological effect obtained for all of the benzene groups, reduction in ALC was designated as the critical effect, and the lowest exposure median of 7.6 ppm was defined as the LOAEL. There is some concern in selecting the 7.6 ppm (24.3 mg/m^3) concentration as the point of departure for RfC development, because the subjects in question (N = 11) may provide an inadequate group-wise comparison to the entire control group (N = 44). Nonetheless, a significant reduction in the sensitive endpoint of ALC was observed in the subgroup exposed to a median benzene level of 7.6 ppm. Thus, this value is used as the LOAEL in Section 5.1.4. to derive a chronic inhalation RfC for benzene and in Section 5.2.4. to derive a chronic oral RfD.

The primary quantitative analysis for developing the RfC and the RfD, however, uses BMD modeling approaches to model the Rothman et al. (1996a) ALC data and derive a BMD at the low end of the range of observation (Section 5.1.2). The BMDL is then used as the point of departure for the application of UFs in the calculation of the RfC or RfD (Sections 5.1.3 and 5.2.3, respectively). Clearly, this is a preferable methodology because more of the exposure-response data can be included in the analysis, and one is not restricted to selecting one of the exposure group medians as a LOAEL. The LOAEL-based RfC and RfD estimates are provided for comparison with the BMD-based estimates. RfC and RfD estimates are also derived from applicable experimental animal studies for comparison with the human-based estimates (Sections 5.1.5 and 5.2.5, respectively).

4.1.2.2. *Neurotoxicity*

Humans have displayed symptoms of neurotoxicity following acute inhalation of relatively high concentrations of benzene (Snyder, 1987). 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 700–3000 ppm (2236–9584 mg/m^3) (ATSDR, 1997). Death may result from exposure to 20,000 ppm (63,894 mg/m^3) benzene for 5–10 minutes (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).

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 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 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) conducted neurological examinations on eight patients who had a history of chronic exposure to benzene and 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). 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 who had preleukemia (cases 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 cases 1, 5, 6, 7, and 8.

Four cases (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 (cases 4, 5, 6, and 7) had normal neurological test results. EMG tests revealed neurogenic involvement in cases 1 and 2 but were normal in cases 3–7. Five of the cases (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 cases 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.

The investigators 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, cases 3 and 4 had moderate to severe hematologic findings and aplastic anemia, but no significant neurological findings. For these two cases, 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; 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.

4.1.2.3. *Reproductive Toxicity*

The available studies on the reproductive toxicity of benzene in humans are summarized in Table 4.

Vara and Kinnunen (1946) examined 12 female workers who had 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; 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

Table 4. 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 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 (a major source of benzene) and chlorinated hydrocarbons via skin and inhalation; benzene levels, < 5 mg/m^3; 40% of chlorinated hydrocarbon 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., 1987a
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%); spontaneous abortion (exposed, 5.7%; control, 2.4%); gestosis (exposed 22.6%; control, 10.5%); all differences were statistically significant	Huang., 1991

ND = no data.

subjects. The investigators tentatively attributed the sparseness in menstruation to the ovarian hypoplasia rather than to the benzene.

Pushkina et al. (1968) evaluated a group of workers who had 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 who had 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. The justification for the changes in the ascorbic acid as an index of toxicity was not well desribed.

A Russian study evaluated 360 women exposed to gasoline (a source of exposure to 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 theplacental membrane rupture during parturition was impeded, and the number of cases of intrauterine asphyxia of the fetus. This study, although demonstrating exposure-related reproductive effects and fetal toxicity, fails to distinguish between the effects of benzene and chlorinated hydrocarbons.

Yin et al. (1987a) 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 hematologic 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 and 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 similar observations in other studies; however, the significance of subjective symptoms is debatable.

A Chinese report (Huang, 1991) described reproductive dysfunction in female workers exposed to benzene and toluene in the leather shoemaking industry. The exposed group (223 women) exhibited increased incidence in the rate of menstrual disorders as compared with the 327 controls. The incidence rates of "mense-blood anomaly" 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.

The data presented above provide limited evidence regarding reproductive effects of benzene exposure in humans and involve concomitant exposure to other chemicals. These studies did not provide good exposure monitoring data or quantitative dose-response information.

4.1.2.4. *Developmental Toxicity*

The following discussion reviews the developmental toxicity of benzene in humans, focusing on more recent reports. Available studies on the developmental toxicity of benzene in humans are summarized in Table 5. 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 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).

Benzene crosses the human placenta and is present in cord blood in quantities equal to or greater than those 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; Pushkina et al., 1968; Mukhametova and Vozovaya, 1972; Funes-Cravioto et al., 1977; Axelsson et al., 1984; Yin et al., 1987a; Savitz et al., 1989; Huang, 1991). Exposures were either to benzene alone or, as is characteristic of occupational exposure, to multiple 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).

Axelsson et al. (1984) evaluated by questionnaire the outcome of pregnancy among personnel employed in laboratory work at the University of Gothenburg between 1968 and 1979. 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% 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 the woman had stopped working at the laboratory, thereby altering the miscarriage rate in the

Table 5. 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 = 1.31, 95% CI = 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 (a major source of benzene) and chlorinated hydrocarbons via skin and inhalation; benzene levels, < 5 mg/m^3; 40% of chlorinated hydrocarbon 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 5. Developmental toxicity of benzene—humans (continued)

Population	No.	Exposure	Effects	Reference
23-year-old female	1	21 intramuscular injections of benzene in an unsuccessful attempt 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 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

RR = relative risk.
CT = computerized transverse tomography.

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 (Funes-Cravioto et al., 1977), and stillbirth (Holmberg, 1979).

Savitz 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, which was 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 on the basis of 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 (ORs) relating exposure to effect were adjusted for selected potential confounders. Elevated ORs were found for maternal exposure to benzene and stillbirth (OR = 1.3, 95% CI = 1.0–1.8). Increased risks across low-, medium-, and high-linkage levels (crude ORs 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, and black and who had parity four or greater, little or no prenatal care, and low education. However, in spite of these limitations, the authors concluded that the results of the study encourage further evaluation of the developmental effects of paternal exposure to benzene.

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

4.2. ACUTE AND CHRONIC STUDIES IN EXPERIMENTAL ANIMALS

4.2.1. Hematotoxicity

4.2.1.1. *Oral Exposure—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 4.2.4.1.

Hsieh et al. (1988b) exposed male CD-1 mice to benzene in drinking water at concentration levels 0, 31, 166, or 790 mg/L (0, 8, 40, or 180 mg/kg/day) for 28 days. Dose-related hematologic effects (erythrocytopenia, leukocytopenia, lymphocytopenia, increased MCV) were observed at all exposure levels. It can be estimated that these dose levels in drinking water are roughly equivalent to inhaled doses of 6.4, 32, and 154 ppm. A major drawback of this study is that there were only five animals per group. Details of the study are discussed in Section 4.2.4.1.

In the National Toxicology Program (NTP) study (NTP, 1986), F344 rats and B6C3F1 mice (10/species/group/sex; 6–8 weeks of age) were treated 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. Hematologic 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.

No compound-related deaths were observed for rats. Final body weight depression of $\geq$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 four of five male and three of five 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 hematologic 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 hematologic 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 hematologic 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 (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. Leukopenia was reported for 10 mg/kg; at higher dose levels erythrocytopenia and leukopenia were also observed. No quantitative data or statistical analysis were 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 hematologic effects in female rats treated by gavage for 6 months.

Aoyama (1986) showed that a 14-day exposure of mice to 50 ppm (162 mg/m^3) benzene resulted in a significantly reduced blood leukocyte count.

4.2.1.2. *Oral Exposure—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 males 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 leukopenia was significant in female rats treated with ≥25 mg/kg for 3, 6, 9, and 12 months; leukocyte levels were comparable with controls after 15, 18, 21, and 24 months of treatment. In male rats, dose-related leukopenia 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, leukopenia 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). 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 and then observed them 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 and 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 it 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 RBCs (only at 92 weeks), WBCs, and lymphocytes were observed. Insufficient information was provided to establish LOAEL or NOAEL levels from these studies.

4.2.1.3. *Inhalation Exposure—Subchronic Studies*

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 hematotoxicity of inhaled benzene to test animals are summarized in Table 6. These studies form a database that supports the understanding that exposure to benzene at high concentrations causes hematotoxic effects, including bone marrow suppression and the greater sensitivity of mice over that of rats. Benzene-induced hematotoxicity has been observed from short-term as well as long-term exposure to the chemical.

Table 6. 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 weeks	Increased spleen weight, total nucleated cells per spleen, and nucleated cells per spleen	LOAEL: 32	Green et al., 1981a, b
CD-1 mice (30 males and 30 females/group)	HCT; Hgb; WBC, RBC, platelet, reticulocyte, and differential counts; MCV; mean corpuscular Hgb; 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, 96, 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 MCV, mean cell Hgb, glycerol lysis time, and incidence and severity of RBC morphological 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: 96	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, RBC acetylcholinesterase, reduced GSH, 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 weeks for test animals, 39 weeks for controls), severe lymphocytopenia, bone marrow hypoplasia, granulocytosis, and reticulocytosis	LOAEL: ND NOAEL: ND	Snyder et al., 1978

Table 6. 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 standard error of mean at 9/19 monitoring periods); increased neutrophils (± 2 standard error of mean 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 weeks, test animals; 75 weeks, controls); lymphocytopenia (WBC counts, ~3000/mm^3 or 15% of normal); anemia (RBC counts, $8 \times 10^6/mm^3$ or ~80% of normal); neutrophilia appearing at 17 weeks; 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

Table 6. Peripheral blood and hematopoietic effects of benzene in animals—inhalation exposure (continued)

Strain/Species	Endpoints	Exposure	Effects	LOAEL/NOAEL (mg/m^3)	Reference
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 weeks for benzene-exposed animals, 52.7 weeks for controls); depressed peripheral RBC and lymphocyte counts (± 2 standard error of mean); 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
Sprague-Dawley rats (10 males and 10 females/group)	HCT; Hgb; WBC, RBC, platelet, reticulocyte, and differential counts; MCV; mean corpuscular hemoglobin; 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, 96, 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: 96	Ward et al., 1985

Table 6. 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 (45 males, test; 27 males, control)	WBC and RBC counts (Coulter), reticulocyte counts, differential counts, Hgb, HCT, lactic acid dehydrogenase, RBC acetylcholinesterase, and reduced GSH, 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-C	0, 319, 958, or 2875 mg/m^3, 6 hr/day, 5 days/wk for 16 weeks	Reduced lymphocyte count at ≥319 mg/m^3, decreased CFU-E, BFU-E, CFU-S, and GM-CFU-C	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 (5–10/group)	WBC, RBC, differential counts, HCT, 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 weeks	Reduced lymphocyte counts after 2 weeks of exposure at 80 mg/m^3 but no effect at 32 mg/m^3	LOAEL: 80 NOAEL: 32	Cronkite et al., 1985
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

Table 6. Peripheral blood and hematopoietic effects of benzene in animals—inhalation exposure (continued)

Strain/Species	Endpoints	Exposure	Effects	LOAEL/NOAEL (mg/m^3)	Reference
Male B6C3F1 mice (24/group exposed, bone marrow tests on 3–16/group)	Bone marrow; cell counts, CFU-E, GM-CFU-C, 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 weeks	Reduced bone marrow cellularity, progenitor cells, and differentiating hematopoietic cells, at ≥319 mg/m^3, but no effects observed at ≤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 weeks	Persistent and rapid reductions in splenic and femoral lymphocytes at ≥319 mg/m^3, transient reduction of thymic cell count and splenic B-lymphocytes at 32 mg/m^3 after 2 weeks, but comparable with controls by 4 weeks	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 weeks	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

ND = not determined because only one concentration was used.
NA = not applicable, adverse effects were observed at the lowest dose tested.
CFU-S = colony-forming unit–spleen.
BFU-E = burst-forming unit–erythroid.
CFU-E = colony-forming unit–erythroid.
GM-CFU-C = granulocyte/macrophage colony-forming unit–culture

Early general toxicity studies reported leukopenia in dogs and fatal anemia in mice exposed to 600 ppm (1917 mg/m^3) 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) benzene for 23–187 exposures (Wolf et al., 1956); and leukopenia in rats exposed to 61 ppm (195 mg/m^3) benzene for 2–4 weeks or to 44 ppm (141 mg/m^3) for 5–8 weeks (Deichmann et al., 1963).

Male CD-1 mice (11–12/group) were exposed for 6 hours/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 hematologic 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 RBCs 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 RBCs 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 hours/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 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. Hematologic 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 HCT 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 morphological changes. The values for RBC, motor conduction velocity, mean corpuscular Hgb, 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 hematologic 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 than the rat to the effects of benzene, and male mice appear to be more sensitive than female mice. The experiment provided a LOAEL of 300 ppm (958 mg/m^3) and a NOAEL of 30 ppm (96 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.

4.2.1.4. *Inhalation Exposure—Chronic Studies*

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 this concentration 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 hours/day, 5 days/week for life. Benzene concentrations, monitored every 30 minutes by ultraviolet absorbance at 255 nm, were read from a calibration curve. Tail bleeding of test and control animals took place at least every 3–5 weeks during exposure and at about the same time of day for analysis of the following endpoints: WBC and RBC counts (Coulter), reticulocyte counts, differential counts, Hgb, and HCT. Evaluations of lactic acid dehydrogenase levels, RBC acetylcholinesterase levels, and reduced GSH 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) benzene. The rats had mild body weight depression that started at 30 weeks and persisted throughout life.

The only significant hematologic findings in the rats (± 2 standard error [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 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) 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) 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) 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.

No statistically significant differences were seen between the AKR/J 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 of 30 monitoring periods (WBC counts were about 3000/mm^3 or ~15% of control values), and of anemia in 30 of 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 RBCs 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 than the Sprague-Dawley rat to the hematologic effects of benzene 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) 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 and included decreased median survival time (25.5 weeks for benzene-exposed animals, 52.7 weeks for controls), statistically significantly depressed peripheral RBC and lymphocyte counts after the first week of exposure (± 2 SE), and statistically significant neutrophilia (± 2 SE) after 29 weeks (~209 days) of exposure and the appearance of Howell-Jolly bodies (remnants of nuclear chromatin seen in erythrocytes in certain anemic conditions) at 7 days, anisocytosis (erythrocytes showing excessive variation in size) at 22 days, and poikilocytosis (erythrocytes showing excessive variation in shape) 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 hours/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 the 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 they were not sufficient for the derivation of LOAEL or NOAEL.

4.2.1.5. *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 units–erythroid (BFU-E), which responds to a factor called "burst-promoting activity" or to IL-2, and the more differentiated erythroid progenitor, the colony-forming units–erythroid (CFU-E), which responds to erythropoietin. The granulocyte/macrophage colony-forming units–culture (GM-CFU-C) respond 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) benzene 6 hours/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 CF-U-S, BF-U-E, CFU-E, and GM/(CFU-C) using standard procedures. The assays, conducted about every 2 weeks, usually took place about 6 hours after the end of the exposure period of the preceding week.

There was a dose-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 dose 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 (colony-forming unit–culture) 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 dose tested after 8 weeks of exposure. Recovery studies demonstrated a gradual regeneration of progenitor cell numbers, and animals exposed to 300 ppm (958 mg/m^3) for 16 weeks had completely recovered by 73–185 days postexposure. The investigators concluded that the erythroid system is more sensitive than the myeloid cell system to the effects of benzene, 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; Baarson and Snyder, 1991). 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) benzene vapor 6 hours/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 RBCs 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 effect would be warranted.

In the 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.

Vacha and coworkers (1990) exposed female C57BL/6×DBA/2F1 hybrid mice to 0 or 300 ppm (0 or 958 mg/m^3) benzene 6 hours/day, 5 days/week for 6–7 weeks. Indices of hematopoiesis were measured in peripheral blood (RBC and WBC count, Hgb, HCT, reticulocyte, and leukocyte count) in addition to ^{59}Fe accumulation in the erythropoiesis organs (spleen and bone marrow) and in the peripheral RBCs. 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 to 200 ppm (3.2 to 639 mg/m^3). Exposures were either continuous (24 hours/day for 4 to 10 days) or intermittent (8 hours/day, 5 days/week for 2 weeks) using five mice per group. Endpoints 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 ≥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) were cells/tibia, ~24% of control; CFU-C/tibia, ~32% of control; micronuclei/500 PCE, 6 (control value, 0.41/500).

Furthermore, intermittent inhalation 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 ≥21 ppm (67 mg/m^3), and concentration-dependent increases in cellularity, significant at ≥50 ppm (160 mg/m^3). Animals exposed intermittently to 95 or 201 ppm (303 or 642 mg/m^3) benzene 2–8 hours/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 hours/day) and increases in the frequency of micronuclei (95 ppm [303 mg/m^3] for 4 hours). At 201 ppm (642 mg/m^3), all exposure protocols, except for the 2 hours/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. The investigators further concluded that intermittent exposure is less effective than 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 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 hours/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. Five days after exposure, BFU-E and CFU-F were the same as in controls, 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, which resulted in a growth advantage for granulocytic cells in both the bone marrow and the spleen of exposed mice. This observed shift toward granulopoiesis occurred even in mice treated with the erythropoiesis 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) benzene or ambient air 6 hours/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-hour 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 the three parameters were statistically analyzed using temporal averages and 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 by 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 for 6 hours/day, 5 days/week to benzene concentrations of 10, 25, 100, or 400 ppm (32, 80, 319, or 1278 mg/m^3) for 2 weeks or to 300 ppm (958 mg/m^3) for durations of exposure ranging from 2 to 16 weeks. The endpoints of toxicity included WBC, RBC (Coulter), and differential counts; HCT; bone marrow cellularity; CFU-S in the bone marrow and spleen; and tumor development. Assays were performed 18–22 hours 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) benzene for 2 weeks did not affect granulocyte counts but did result 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). HCT 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 about 20 to 65% of control for the three highest exposure groups. For mice exposed to 300 ppm for 2 weeks, lymphocyte levels were similar to control levels by 4 weeks after the end of exposure, and recovery of the remaining exposure duration 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). These results differed from those of Ward et al. (1985), which 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 CFU-S. Cellularity was determined in animals inhaling 0, 10, 25, or 100 ppm (0, 32, 128, or 319 mg/m^3) for 2 weeks (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) significantly depressed bone marrow cellularity and stem cell CFU-S numbers (p<0.003 and p<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 p<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 values 4 weeks after the last exposure, but they 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) 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 nonthymic lymphomata and solid tumors, beginning at 330 days of age. In a continuation of this study, Cronkite et al. (1989) (1) evaluated the effects of benzene on erythrocyte progenitors (BFU-E, CFU-E) and on neutrophil and macrophage progenitors (GM-CFU-C), (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) benzene 6 hours/day, 5 days/week for 9.5 weeks and from 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 conditioned medium. The clots were scored for erythrocytic colonies (CFU-E) or megakaryocytes (BFU-E). The cells used in the CFU-G and GM-CFU assays were cultured in agar for subsequent scoring. Progenitor cell assays resulted in the following: CFU-E numbers did not change significantly after 4 days of exposures, but the numbers had decreased significantly (to approximately 45–70% 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 just a few percentage points of control values) at 29, 48, and 65 days, but they 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 values by day 29 and had recovered to about 85% of control values by 12 days after exposure. The GM-CFU cells in an agar assay decreased to approximately 40% of control values after 30 days of exposure, increased to about 75% by termination of exposure at day 65, decreased to approximately 60% by 72 days, and then recovered to almost 80% 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 to either 316 ppm (1010 mg/m^3) benzene administered 6 hours/day, 5 days/week for 19 exposures or to 3000 ppm (9584 mg/m^3) benzene administered 6 hours/day for 2 successive days (Cronkite et al., 1989). After 19 exposures, 316 ppm (1010 mg/m^3) 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 1 day after the end of the exposure. The differences in the effects of

the two regimens still persisted at 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, whereas those in the group treated with 300 ppm (958 mg/m^3) had not. All leukocyte cell types were decreased in number as compared with those in sham-exposed mice on day 1, and all absolute counts except for the large unstained cells (mostly large lymphocytes) 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 exposures (depression to ~33% of control value) than in those exposed to 3000 ppm (9584 mg/m^3) for 2 days (depression to ~59% 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 that by day 214 after exposure recovery was incomplete for the group exposed to 316 ppm (958 mg/m^3).

In another experiment, exposure to 300 ppm (958 mg/m^3) benzene for 80 weeks produced a severe depression in CFU-S (to 38% of control) that had not completely recovered 178 days after exposure ended. Exposure to 3000 ppm (9584 mg/m^3) for 8 weeks produced a milder depression in CFU-S (to 62% of control) that had recovered by 30 days. Decreases in bone marrow cellularity were nearly the same for the two exposure scenarios.

Rescue assay. The injection of normal bone marrow cells into lethally irradiated rodents enhances their survival (Cronkite et al., 1989). 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) benzene 6 hours/day, 5 days/week for 16 weeks 752 days earlier. 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 that received cells from control mice was 93%, and survival of the mice that received cells from benzene-exposed mice was 64%.

In the secondary transplant, no mice died by 30 days, but the mice that received control cells started dying at day 171 and mice that received cells from donors exposed to benzene started to die at day 70. In the tertiary rescue, 50% of the recipients of bone marrow from benzene-exposed donors were dead by day 30 and 95% were dead by day 245. Among the animals that received 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 (0, 319, 958, or 2875 mg/m^3) benzene 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 in 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 (0 or 319 mg/m^3) benzene 6 hours/day, 5 days/week for life (American Petroleum Institute, 1983). Blood samples were obtained at 2- to 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 rarely statistically significant. Nonetheless, the frequencies of depression were highly significant for each cell type (p<0.002, Wilcoxon Rank Sum Test), and the overall results were interpreted as evidence of hematotoxicity. Significantly increased incidences of splenic hyperplasia (p<0.005) and hemosiderin pigments (p<0.001) were observed in benzene-exposed rats. The incidences of normally rare tumors in treated rats were 4/40 in liver, 2/40 in Zymbal gland, and 1/40 chronic myelogenous leukemias. The authors considered these tumors to be related to the benzene exposure. This study identifies a LOAEL of 100 ppm (319 mg/m^3) for hematologic effects in rats.

Decreases in bone marrow cellularity in the femur, HCT, and leukocytes were seen in DBA/2 mice (20/group) exposed to 300 ppm (958 mg/m^3) benzene in air 6 hours/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, HCT, 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 C57B1/6J mice to inhaled benzene with the in vitro sensitivity of culture bone marrow cells to benzene metabolites. Mice were exposed to 300 ppm (958 mg/m^3) benzene 6 hours/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 than CFU-E from C57B1/6J mice to 1,4-benzoquinone or hydroquinone. Thus, both in vitro and in vivo data indicated that Swiss-Webster mice were more sensitive to benzene toxicity than were C57B1/6J mice.

Female BDF1 mice (C57BL/6 × DBA/2F1 hybrids) were exposed to 0, 100, 300, or 900 ppm (0, 319, 958, or 2875 mg/m^3) benzene 6 hours/day, 5 days/week for up to 8 weeks (Plappert et al., 1994). Hematologic studies included peripheral blood data, T4 and T8 lymphocyte counts in the blood and spleen, and hematopoietic stem and progenitor cell assays in the marrow CFU-S, CFU-C, and BFU-E, CFU-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 hours/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, high proliferative potential colony-forming unit (CFU-HPP), percentage of CFU-HPP cells in S-phase, bone marrow CFU-E, bone marrow GM-CFU, 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 (32 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 (320 mg/m^3) exposure and within 11 days following the 200 ppm (640 mg/m^3) exposure. 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 (320 and 640 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 (320 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 hours/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 hours 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 $p<0.05$.

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 with 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.

Robinson and coworkers (1997) exposed male Sprague-Dawley rats (16/group) to 0, 30, 200, or 400 ppm (0, 96, 639, or 1278 mg/m^3) benzene 6 hours/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 (SRBCs) at 4 days before the end of the exposure to allow an assessment of humoral immunocompetence using the enzyme-linked immunosorbent assay technique. Thymus and spleen weights were determined, as were total cell counts 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 with 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 SRBCs 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 (Robinson et al., 1997). 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.

Taken as a group, the results demonstrate that the reduction in bone marrow function following benzene exposure is related to decreases in the growth and maturation of pluripotential stem cells (CFU-S), lineage-restricted stem cells (e.g., GM-CFU), progenitor cells in various stages of maturation, and the stromal cells that provide growth factors necessary for bone marrow function. These studies tend to demonstrate the sensitivity of erythropoiesis and lymphopoiesis to benzene and the relative resistance of granulopoiesis to benzene.

Snyder and Kalf (1994) reviewed the effects of benzene on bone marrow cells. Bone marrow stromal cells, which include leukocytes, erythrocytes, endothelial cells, reticular cells, and fat cells, constitute the hematopoietic connective tissue in which stem cells undergo maturation and amplification to form the cells of the circulation. Garnett et al. (1983) reported that the marrow adherent layer from benzene-treated animals had an altered morphology and failed to support the differentiation of stem cells. In a related study, Kalf et al. (1996) demonstrated that *p*-benzoquinone, an important reactive metabolite of benzene, prevents the conversion of pre-interleukins-1α and -1β to the active cytokines by inhibiting calpain and the

interleukin-1β converting enzyme, respectively. Thus, a key benzene metabolite inhibits the formation and release of an important cytokine, IL-1, from stromal macrophages.

Irons et al. (1992) isolated human $CD34^+$ human bone marrow myeloid progenitor cells, which proliferate in response to IL-3 and GM-CSF. Addition of hydroquinone, within a limited range of concentrations, resulted in synergistic enhancement of colony formation. Hazel et al. (1996) made similar observations using mouse bone marrow CD32 cells. The mechanism is thought to include the up-regulation of the 5-lipoxygenase pathway in these cells, with increases in the formation of LTD4, which in turn reacts with the LTD4 receptor to increase cell replication. Hydroquinone independently reacts with the LTD4 receptor to also enhance cell replication. Morphological evaluation suggests that maturation of the myeloblast stage to the myelocyte stage has been enhanced. These cells remain in their immature state in the presence of hydroquinone and do not advance to mature neutrophils, but proliferation continues.

The implications of these observations are significant in terms of a mechanism by which benzene can cause expansion of the pool of myelocytes, which do not then undergo either further differentiation to neutrophils or apoptosis, which is the usual method used by the bone marrow to control the size of the myelocyte pool. This might provide a pathway for a benzene-induced leukemogenic response. It may be hypothesized that if benzene, acting via DNA adduct formation or oxidative damage to DNA, causes cell transformation at an earlier stage of differentiation, the creation of an expanding pool of myelocytes by benzene and hydroquinone provides a mechanism for promotion. Thus, the criteria for a two-stage mechanism of carcinogenesis (i.e., initiation followed by promotion) are satisfied. However, the specific issue of progression has not been addressed by studies of this type.

The studies by Tennant et al. (1995) have demonstrated the production of a granulocytic leukemia in TgAC mice when treated with benzene dermally but not when given benzene by gavage. This is the first animal model that we have had for a benzene-induced granulocytic leukemia. The TgAC mouse is derived from the FVB mouse in which the viral Ha-ras gene has been inserted into chromosome 8. The granulocytic leukemia develops within 6 months. TgAC mice may represent initiated animals in which benzene and hydroquinone act as promoting agents leading to proliferation of the leukemia cells, and thus could support the argument for the two-stage model for benzene-induced leukemogenesis. It is significant that in the course of these studies bone marrow depression was observed, indicating the close relationship between noncancer and cancer endpoints in benzene exposure.

The following discussion offers some thoughts about the attempt to understand the mechanism by which DNA damage occurs as a result of benzene exposure. DNA damage is not extensively dealt with here because this document concentrates on noncancer endpoints. It is not clear, however, that there is no impact on noncancer endpoints by DNA damage. Some discussion of DNA damage leading to translocation and gross breaks as well as a discussion of DNA repair and the impact of benzene metabolites is warranted. The study by Snyder and Kalf (1994) and the more recent study by Smith et al. (1998) relate to benzene-induced chromosome damage. On the issue of DNA damage induced by benzene, there is ample evidence to show that benzene metabolites covalently bind to protein and DNA. Also, the structures of putative DNA adducts are known. An alternative mechanism for DNA damage is via the generation of reactive oxygen species. For example, *p*-benzoquinone and hydroquinone increase superoxide, nitric

oxide, and hydrogen peroxide in HL-60 cells activated with phorbol ester. Boersma et al. (1994) suggested that hydroquinone itself is unlikely to generate reactive oxygen species.

An interesting complimentary alternative mechanism, proposed by Singh et al. (1994), suggests that benzene- induced DNA damage is mediated by (1) release of free iron in the bone marrow of benzene-treated animals (probably by polyphenolic metabolites of benzene), followed by (2) the chelation of iron by hydroquinone or benzenetriol to yield (3) a reactive oxygen-generating species such as superoxide, which in turn (4) causes oxidative damage to DNA. Their suggestion that glutathionyl-hydroquinone may be a key intermediate resonates well with the suggestion of Brunmark and Cadenas (1988). In a radical-rich system, glutathionyl-hydroquinone may well be converted to glutathionyl-benzenetriol. Singh et al. (1994) suggest a chemical structure for the iron-benzenetriol chelate in which the iron is bound between the hydroxyl group at positions 1 and 2 on 1,2,4-benzenetriol with the release of two protons. Indeed, it may well be that the auto-oxidation of glutathionyl-benzenetriol is enhanced with an iron chelated between positions 1 and 2. These hypotheses can be reconciled by assuming that the superoxide produced by the auto-oxidation of gluthionyl-benzenetriol is converted to hydroxyl radical and singlet oxygen by the benzenetriol-iron chelate. The resulting reactive oxygen species can hydroxylate guanine and other DNA bases, resulting in a mutagenic effect.

The discussion of the mechanisms of benzene toxicity provides a framework for understanding the total disease process. It has become clear that benzene toxicity/leukemogenesis is a continuity of effects. Although this is not intended to be a discussion of the carcinogenic effects of benzene, all of the effects on the bone marrow caused by benzene appear to be time- and dose-related in a sequential manner. The literature suggests that chronic low doses lead to cytopenias, which may be reversible if exposure ceases. At intermediate chronic doses cytopenias persist and may eventually lead to myelodysplastic syndrome (MDS), which is a preleukemic state terminating in AML. If the cytopenias are sufficiently severe the patient may succumb to an infection due to failure of the immune system. Higher doses may lead directly to bone marrow aplasia and death without the characteristic appearance of MDS. Death is usually the result of immune deficiency. Thus, the cytopenias (the ultimate form of which is aplastic anemia) leading to decreases in circulating cell numbers and immune impairment are not separable from MDS and, eventually, AML.

The following studies of exposure to 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 benzene to body weight were injected subcutaneously for 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 4, 5, and 6. 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 to 440 mg/kg/day benzene on 6 consecutive days and were sacrificed on day 7. Increased levels of micronuclei

were observed at doses above 28 mg/kg/day, and their frequency increased dramatically at doses above 55 mg/kg/day. At ~100 mg/kg/day, micronuclei levels were about 50/2000 PCE, whereas at the highest dose of 440 mg/kg/day, they were 218/2000 PCE (vs. 3 micronuclei/2000 PCE for controls). CFU-C/tibia and bone marrow cellularity were suppressed by about 40% at doses as low as 3.5 mg/kg/day. At 100–440 mg/kg/day, both values dropped to 5–10% of control values.

Six injected doses of hydroquinone ranging from 20 to 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 to 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 benzene would yield an excretion of ~20 mg/kg hydroquinone. However, 20 mg/kg hydroquinone was virtually nontoxic, whereas 440 mg/kg benzene produced severe hematologic effects. On the basis of these differences and the differences in the sequence of the responses to the two chemicals, Tunek et al. (1981) 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.

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

MacEachern et al. (1992) evaluated the effects of benzene on the morphology and function of bone marrow phagocytes. Male Balb/c mice received i.p. 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 sacrificed 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 when 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.

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.

Eastmond et al. (1987) demonstrated that coadministration of 75 mg/kg phenol and 25–75 mg/kg 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, RBCs, and Hgb (Rao et al., 1988). *Trans,trans*-muconaldehyde administered to CD-1 mice i.p. daily for 10 and 16 days produced hematotoxicity similar to that of benzene (Witz et al., 1985). Benzene metabolites added to macrophage cultures significantly and selectively inhibited macrophage function (Lewis et al., 1988).

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 MUC 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 AML.

4.2.1.6. *Summary of Principal Hematotoxic Effects*

A considerable number of studies in experimental animals have pointed to the changes in peripheral blood and bone marrow induced by benzene as being among the most sensitive effects of the compound's toxicity. That these effects were seen in most, if not all, of the species tested, as well as in humans occupationally exposed to benzene (see Section 4.1.2.1.), suggests that their threshold dosimetry may be important in identifying a point of departure for the derivation of

quantitative risk estimates. Principal among the adverse effects described are leukopenia, lymphocytopenia, granulocytosis, anemia, and reticulocytosis. Comparing the incidence of one response with another, lymphocyte counts appear to be depressed sooner and more severely than other cell types, and granulocytes may be the most resistant (Snyder et al., 1978, 1980). Differences in responsiveness to benzene have been observed among species, with mice being more sensitive than rats to the hematotoxicity of benzene (Ward et al., 1985; Snyder et al., 1978, 1980). Intraspecies variability was demonstrated by Snyder et al. (1978, 1980), who reported that AKR/J mice were more sensitive than C57BL/6 mice to the compound. Ward et al. (1985) showed that in CD-1 mice, males were more sensitive than females.

A striking feature of the studies of Ward et al. (1985) and Cronkite et al. (1985) was that hematologic abnormalities were evident after only 2 weeks of exposure, with comparatively little progression during the rest of the exposure period. However, the results of Cronkite et al. (1985, 1989) point to the possibility that peripheral blood elements may have the capacity to recover from benzene-induced toxicity, depending on the duration of exposure and concentration. For example, lymphocyte counts, which were depressed in C57BL/6 mice exposed to benzene at 300 ppm (32–1279 mg/m^3) for 2–16 weeks, tended toward normal values during a 4- to 8-week recovery period.

Dose-response studies such as that of Ward et al. (1985) have served as a basis for the identification of NOAELs and LOAELs. For example, a NOAEL of 30 ppm (96 mg/m^3) and a LOAEL of 300 ppm (958 mg/m^3) have emerged for CD-1 mice from the Ward et al. study. The data from this study have also served as a basis for BMD modeling. Using the BMDL derived from this modeling as a point of departure for RfC determination yields a value that is in good agreement with the RfC obtained from the hematotoxicity data from the occupational exposure study of Rothman et al. (1996a), as described in Section 5.1.

Benzene-induced peripheral blood abnormalities reflect a disruption of all levels of hematopoiesis in the bone marrow. For example, several studies have demonstrated concentration-dependent depression of bone marrow cellularity in mice treated with benzene, the lowest concentration producing this effect being 21 ppm administered for 2 weeks (Toft et al., 1982). Similarly, the stem cell compartment (CFU-S) appears to be sensitive to the adverse effects of benzene, particularly when drawn into the cell cycle in response to benzene-induced damage to other cells (Snyder, 1987). Thus, concentrations of 100 ppm (320 mg/m^3) benzene have produced significant depressions in the CFU-S population in the bone marrow of mice exposed for 2–4 weeks (Cronkite et al., 1985; Seidel et al., 1989).

The granulocytic and erythropoiesis progenitor cells are also sensitive to the effects of the compound, exhibiting significant depression at 21 ppm (67 mg/m^3) benzene for 2 weeks and 10 ppm (32 mg/m^3) for 4 weeks, respectively (Toft et al., 1982; Seidel et al., 1989). In addition, both the stem cells and the progenitor cells can recover from benzene-induced depression, at least partially, depending on the concentration and duration of exposure. Cronkite et al. (1989) observed that depression of CFU-E and BFU-E were paralleled by depressions in peripheral RBC counts.

Bone marrow macrophages also appear to be a target of benzene toxicity (MacEachern et al., 1992). Thus, stromal macrophages from benzene- or hydroquinone-treated mice failed to

convert IL-1 to the mature cytokine 17-Kd, a component essential for hematopoiesis (Renz and Kalf, 1991). This allows the suggestion that the inhibitory effect of benzene on lymphocyte proliferation can be mediated through the suppression of cytokine production.

Another important consideration is whether the benzene-induced perturbation of the hematopoietic system is mediated through the effects of the parent compound or via one or more metabolites. The fact that both in vivo and in vitro studies have demonstrated the ability of some metabolites to induce qualitatively similar toxicological effects to those of benzene provides evidence consistent with the concept that the hematotoxicity of the compound may be mediated through its metabolites.

4.2.2. Reproductive/Developmental Effects

4.2.2.1. *Reproductive Toxicity*

Multiple-generation reproductive toxicity studies of benzene were not found in the literature. Relevant information pertinent to the reproductive toxicity of benzene in animals is described below.

4.2.2.1.1. ***Oral exposure.*** NTP conducted the 90-day subchronic toxicity and 2-year bioassay studies of benzene in mice and rats (NTP, 1986). Other than ovarian effects, no signs of reproductive toxicity were reported. 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/week, ranged from 25 to 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 the 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. However, the incidence of nonneoplastic lesions was 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 benzene were ovarian atrophy (32, 79, 65, and 46%, respectively) and epithelial hyperplasia (26, 89, 63, and 60%, respectively).

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 benzene to body weight. The effects were measured in three animals per group 7, 14, 21, 28, and 70 days after treatment. Testicular cells were 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.

Benzene treatment did not affect body weight or testes weight, but it 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 in animals exposed to 6 and 7 mL/kg benzene was depressed to ~80% of control values. The percentage of round spermatids in animals exposed to 7 mL/kg benzene was also decreased to ~80% of control values; 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 they were still incomplete by 70 days. Recovery of the round spermatids began by day 28 posttreatment 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 concluded from this study that benzene can induce acute cytotoxicity in mouse germ cells. Both the NTP (1986) and the Spano et al. (1989) studies provide valuable descriptive information regarding the effects of benzene on the reproductive system. It should be noted that longer term and more relevant studies are needed to confirm the conclusion of Spano et al. (1989).

4.2.2.1.2. ***Inhalation exposure.*** The available studies on the reproductive toxicity of inhaled benzene to test animal species is summarized in Table 7.

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) benzene for 13 weeks but not in rats exposed to 88 ppm (281 mg/m^3) for 30 weeks, to 2200 ppm (7028 mg/m^3) for 30 weeks, to 4400 ppm (14,056 mg/m^3) for 5 weeks, or to 9400 ppm (30,030 mg/m^3) for more than 1–19 days. Groups of 5–10 male guinea pigs exposed to 88 ppm (281 mg/m^3) benzene for ~9.6 months had a slight increase in testicular weights, but guinea pigs

Table 7. 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 hrs/day, 5 days/wk for 13 weeks	Moderate changes in testes weight (no quantitative data)	LOAEL: NA NOAEL: NA	Wolf et al., 1956
Rabbit	1–2	256 mg/m^3, 7 hrs/day, 5 day/wk for ~8.5 months	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 hrs/day, 5 days/wk for ~9.6 months	Slight change in weight of testes (no quantitative data)	LOAEL: NA NOAEL: NA	Wolf et al., 1956
Male and female Sprague-Dawley rats	50/sex	0, 3.2, 32, 96, 958 mg/m^3 6 hrs/day, 5 days/wk for 13 weeks; 10/sex/group sacrificed after 7, 14, 28, 56, and 91 days of treatment	No microscopic lesions of ovaries and testes	LOAEL: 958[a] NOAEL: 96	Ward et al., 1985

Species	No./ group	Treatment	Reproductive effects	LOAEL/ NOAEL (mg/m^3)	Reference
CD-1 mouse	150/sex	0, 3.2, 32, 96, 958 mg/m^3 6 hrs/day, 5 days/wk for 13 weeks; 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 hrs/day, 5 days/wk for a 10-week 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]Effects on hematologic parameters place the LOAEL for the overall study in rats at 958 mg/m^3 and the NOAEL at 96 mg/m^3.

GD = gestation day.
NA = not applicable (only one concentration tested).

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 histopathologic 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 hours/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 specifically designed to detect reproductive effects, the gonadal effects noted stimulated 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 hours/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. The mice exposed to 300 ppm (958 mg/m^3) 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 with 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) benzene. These testicular and ovarian lesions were not observed at earlier sacrifices, but similar lesions did appear at lower concentrations; however, biological significance is questionable. 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.

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 hours/day, 5 days/week for a 10-week premating and mating period, and daily from gestation days (GDs) 0 to 20 and lactation days 5 to 20. Daily vaginal smears were examined to determine whether estrus was affected by treatment.

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. 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 a 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.

4.2.2.2. *Developmental Toxicity*

4.2.2.2.1. ***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 GDs 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. Benzene had no effect on maternal body weight, but it 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 was a screening study that used only one dose, and therefore it does not provide dose-response information. However, the study does demonstrate evidence for fetal toxicity in the absence of observable maternal toxicity.

In the Exxon Chemical Company (1986) study, pregnant female Sprague-Dawley rats (20–22/group) were treated by gavage with 0, 50, 250, 500, or 1000 mg/kg/day benzene on GDs 6–15. No dose-related mortality was observed. Significant 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 ($p<0.05$). Developmental toxicity was limited to decreased fetal body weights at ≥500 mg/kg ($p<0.05$). 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 a NOAEL of 250 mg/kg/day and a LOAEL of 500 mg/kg/day for developmental toxicity in Sprague-Dawley rats.

4.2.2.2.2. ***Inhalation exposure.*** 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 hours/day) and continuous (24 hours/day) exposures. Some studies tested more than one concentration of benzene and demonstrated a concentration response and a LOAEL and/or a 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 hematologic 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 8.

Table 8. Developmental toxicity of inhaled benzene in test animals

Strain/species	No. of dams/group	Exposure[a]	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 hrs/day on GDs 6–15; sacrificed on day 20	Decreased body weight and body weight gain at 160 and 1597 mg/m^3, days 5–15; dose-related[b]	Decreased mean live body weight at 160 and 1597 mg/m^3, day 20[c]; decreased crown-rump distance, 1597 mg/m^{3c}; skeletal and visceral (brain) abnormalities at 160 and 1597 mg/m3; 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 hrs/day on GDs 6–15; sacrificed 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 hrs/day on GDs 6–15; sacrificed 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: 7028/319 NOAEL: 958/NA	Green et al., 1978
Rat	5–12	1.0–670 mg/m^3 24 hrs/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 8. Developmental toxicity of inhaled benzene in test animals (continued)

Strain/species	No. of dams/group	Exposure[a]	Effects		Effect levels maternal/developmental (mg/m^3)	Reference
			Maternal	Developmental		
CFY rat	19 exposed, 28 controls	0 or 1000 mg/m^3, 24 hrs/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 hrs/day on GDs 7–14; sacrificed on day 21	Decreased body weight gain at ≥150 mg/m^3 ($p<0.001$), somewhat dose related; liver/body weight 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 hrs/day on GDs 7–15	Decreased body weight gain ($p<0.001$); increased relative liver weight ($p<0.05$)	Retarded weight gain ($p<0.01$); skeletal growth retardation ($p<0.001$)	LOAEL: NA NOAEL: NA	Ungvary, 1985

Table 8. Developmental toxicity of inhaled benzene in test animals (continued)

Strain/species	No. of dams/group	Exposure[a]	Effects		Effect levels maternal/developmental (mg/m^3)	Reference
			Maternal	Developmental		
Swiss-Webster mouse	5	16, 32, or 64 mg/m^3, 6 hrs/day on GDs 6–15	None observed	16-day-old fetus: no effect on hematologic parameters 2-day-old neonates: reduced circulating erythroid precursor cells (all concentrations) ($p<0.05$ at 64 mg/m^3); increased hepatic hemato-poietic blast cells, lympho-cytes, and granulopoietic precursor cells and decreased hepatic erythropoiesis 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 GDs 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[d]	LOAEL: NA NOAEL: NA	Corti and Snyder, 1996

Table 8. Developmental toxicity of inhaled benzene in test animals (continued)

Strain/species	No. of dams/group	Exposure[a]	Effects		Effect levels maternal/developmental (mg/m^3)	Reference
			Maternal	Developmental		
CF-1 mouse	35–37	0 or 1597 mg/m^3 ppm, 7 hr/day on GDs 6–15; sacrificed 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
CFLP mouse	15 exposed, 115 controls	0, 500 or 1000 mg/m^3, 24 hrs/day on GDs 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 GDs 6–18; sacrificed 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 hrs/day on GDs 7–20	Decreased weight gain and increased relative liver weight 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]Statistically different from control as determined by pairwise multiple comparison procedures
[c]Statistically different from control as determined by Cochran's approximation to *t (t')*
[d]Data from abstract; no other details available

GD = gestation day
NA = not applicable
ND = not determined

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). 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).

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 hours/day on GDs 6–15 and sacrificed the dams on day 20 to evaluate maternal and developmental effects. Hematologic evaluation of the dams, performed on GDs 5 and 20, included RBC, WBC, and differential counts.

There were no deaths, observable illness, or hematologic changes for dams in any exposure group. During GDs 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 values in the 500 ppm (1597 mg/m^3) group during GDs 5–15 (p<0.05 for both groups), whereas during GDs 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) 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 and 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 skeletal 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 at concentrations of 50 and 500 ppm (160 and 1597 mg/m^3). The study appears to have been conducted according to standard protocols and recommendations (U.S. EPA, 1991) for developmental toxicity. The study establishes a LOAEL for the developmental toxicity of inhaled benzene of 50 ppm (160 mg/m^3) and a NOAEL of 10 ppm (32 mg/m^3).

Coate et al. (1984) exposed pregnant Sprague-Dawley rats by inhalation to 0, 1, 10, 40, or 100 ppm (0, 3.2, 32, 128, or 319 mg/m^3) benzene 7 hours/day on GDs 6–15. 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.

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 hematologic 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 they were not dose related. Delays in ossification of the skull, vertebral centra, and extremities occurred at 100 ppm (319 mg/m^3). 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 establishes 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.

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 hours/day on GDs 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. 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 GDs 8 to 20 to ~44%–83% ($p<0.01$ for all days) of control values. 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 with 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 for females over males ($p<0.05$). 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 over males ($p<0.05$). 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-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.

In an early study, Gofmekler (1968) exposed female rats to benzene concentrations ranging from 0 to 210 ppm (670 mg/m^3) 24 hours/day for 10–15 days prior to mating and all during pregnancy. The rats exhibited a dose-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 hours/day on GDs 6–15). This suggests 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 decreased, but the effect was not dose related. 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 a NOAEL for the study.

Hudak and Ungvary (1978) conducted an experiment with CFY rats exposed to 313 ppm (1000 mg/m^3) benzene 24 hours/day on GDs 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 dose precluded the derivation of a LOAEL and/or a NOAEL for the study.

A later study (Tatrai et al., 1980) examined the developmental toxicity of benzene using several doses. Pregnant CFY rats inhaled 0, 47, 140, 465, or 930 ppm (0, 150, 450, 1500, or 3000 mg/m^3) 24 hours/day on GDs 7–14. Animals were sacrificed on day 21 to evaluate developmental effects. The dams exhibited significantly decreased body weight gain at all concentrations (p<0.001, ANOVA). The effect was dose 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) benzene was 28.5% of the starting body weight compared with the control value of 62.8%, whereas the body weight gain for dams exposed to 930 ppm (3000 mg/m^3) benzene was 37.0%. 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 attributable 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 toxicant. Groups of pregnant CFY rats inhaled combinations of 123 ppm (400 mg/m^3) benzene and 307 ppm (1000 mg/m^3) toluene or 189 ppm (600 mg/m^3) xylene 24 hours/day on GDs 7–15. Additional groups of 7–14 animals were exposed to 798 ppm (2600 mg/m^3) benzene on GDs 10–12 and given oral doses of either 250 or 500 mg/kg of ASA on GD 12.

At 400 mg/m^3, benzene produced maternal toxicity, as evidenced by decreased weight gain (47% gain in the benzene-treated dams vs. 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 or 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 administered by inhalation in combination with orally administered ASA, it 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; 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 (0, 16, 32, or 64 mg/m^3) benzene 6 hours/day on GDs 6–15 (Keller and Snyder, 1986). Two fetuses/litter/sex were sacrificed on GD 16, two neonates/litter/sex were sacrificed 2 days after birth, and one adult/litter/sex was sacrificed 6 weeks after birth to measure hematopoietic progenitor cells (CFU-E, BFU-E, and GM-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 to measure hematopoietic progenitor cells from the bone marrow and spleen.

There was no evidence of maternal or nonhematopoietic developmental toxicity in benzene-exposed mice. There was a significant increase (p<0.05) in the numbers of BFU-E from livers of male and female fetuses exposed to the low- and mid-exposure levels. The following significant changes (p<0.05) 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; and 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 decreased ($p<0.05$) 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 they 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 that persisted 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). However, the responses observed 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 of animals (two fetuses, two neonates, or one adult/litter/sex) 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) further examined the effects of in utero benzene on the developing recognizable hematopoietic precursor cells and 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 hours/day on GDs 6–15. Maternal toxicity was evaluated on the basis of 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 (two neonates/sex/litter) were assayed at 2 days. In experiment 3, progeny (one/sex/litter) were examined at 6 weeks 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, and blood cell differentials; Hgb levels; and the number of liver cells in the hematopoietic differentiating, proliferating pool. The responses of 6-week-old progeny were evaluated on the basis of peripheral WBC and RBC counts, Hgb, and smears made from femur bone marrow or spleen to determine the number of cells in the differentiating, proliferating pool. Although not stated, 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.

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 or 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 with those of the controls. The 2-day-old neonates

exposed in utero to 5 ppm (16 mg/m^3) 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) benzene had increased RBC counts. However, these effects 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) 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 the control value, 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 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) 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 nondividing 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.

The authors 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 by 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. However, the toxicological significance of these results 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, were 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 are questionable. Thus, a NOAEL or a LOAEL cannot be derived from the results of the Keller and Snyder (1988) report.

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 hours/day for 10 consecutive days (GDs 6–15 for the pregnant females). One-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 6-week-old males and females exposed in utero. 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 with the control value as compared with 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 reductions of as much as 50% in comparison with controls for males, but there were no significant reductions for females. In fact females exposed to benzene and ethanol in utero showed a 40% elevation of CFU-E in comparison with unexposed controls. For both ethanol and benzene, only a single concentration was used in these experiments. Thus, it is not possible to evaluate whether 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 hematologic parameters in the offspring. CF-1 mice were exposed to 500 ppm (1597 mg/m^3) benzene 7 hours/day on GDs 6–15 and were sacrificed on GD 18. There were no significant signs of maternal toxicity, and values for PCV, RBCs, Hgb, and WBCs were comparable with 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. The investigators concluded that benzene did not induce malformations in mice at 500 ppm (1597 mg/m^3), but it was toxic to the fetuses. This study used only one dose, and statistical analysis for the variations were not clearly stated; however, the study did demonstrate fetal toxicity at a dose 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 156 or 313 ppm (500 or 1000 mg/m^3) benzene 24 hours/day during GDs 6–15. Unexposed and air-exposed (chamber) animals served as controls. The mice were sacrificed on GD 18. The authors mentioned in the abstract of their 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%, respectively, 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 it does induce fetal toxicity in mice exposed to 156 and 313 ppm (498 and 1000 mg/m^3), a maternally toxic dose. 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 hematologic parameters in the offspring. New Zealand rabbits were

exposed to 500 ppm (1597 mg/m^3) benzene 7 hours/day on GDs 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 with control values. 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 malformations were not significant. Values for PCV, RBCs, Hgb, and WBCs 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 dose, and statistical analyses for the variations observed were not clearly stated.

Ungvary and Tatrai (1985) also examined the developmental toxicity of benzene and other solvents in New Zealand rabbits. Groups of 11–15 pregnant animals inhaled 156 or 313 ppm (500 or 1000 mg/m^3) benzene 24 hours/day on GDs 7–20. Unexposed and air-exposed (chamber) animals served as controls. The rabbits were sacrificed 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 times 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 times 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 times 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 it induces fetal toxicity in rabbits at 313 ppm (1000 mg/m^3), a maternally toxic concentration. The evidence supporting the LOAEL and the NOAEL in rabbits is weakened by a lack of experimental details in the report, the small numbers of pregnant rabbits employed, and the use of the fetus rather than the litter as the experimental unit.

4.2.2.3. *Summary of Principal Reproductive/Developmental Toxicity Effects*

As shown in the studies summarized in the previous subsections and in Tables 7 and 8, administration of benzene has been associated with the onset of reproductive/developmental effects in a wide range of experimental animals.

The susceptibility of reproductive organs to the toxic effects of benzene was shown in studies on the longer-term administration of benzene in which, among other organs, the rat testes (Wolf et al., 1956) and the ovaries of female CD-1 mice (Ward et al., 1985; NTP, 1986) were reduced in weight compared with controls and histopathologically altered as a result of benzene administration. The potential for these changes to affect reproductive function in males is suggested by reduced sperm count and increased numbers of abnormal sperm when CD-1 mice inhaled up to 300 ppm (958 mg/m^3) benzene for 13 weeks.

The experiments of Kuna et al. (1992) provided evidence that the inhalation of benzene by female Sprague-Dawley rats before and during pregnancy resulted in reduced body and liver weights in weanlings, a valid reproductive/developmental response because it occurred in the absence of any overt effects of the compound on maternal toxicity. In addition, a considerable number of experiments have addressed the potential of inhaled benzene to induce reproductive, developmental, or teratogenic effects in the offspring of pregnant rodents exposed to the compound during the period of principal organogenesis. For example, the studies by Kuna and Kapp (1981) of Sprague-Dawley rats point to the possibility of benzene-induced skeletal variations and abnormalities in the fetuses of exposed dams, although the quantitative extent of the effect was marginal at best. However, the weight of evidence for benzene as a developmental toxicant arises from the extensiveness of the overall information base. Several studies that employed essentially similar experimental protocols (see, e.g., Green et al., 1978) contribute to an overall picture of the compound as inducing reduced fetal growth and delayed ossification. In addition, other studies reported reductions in fetal body weights, for example, in Sprague-Dawley rats (Coate et al., 1984) and New Zealand white rabbits (Ungvary and Tatrai, 1985).

The developing hematopoietic system in mice is affected by maternal exposure to benzene (Keller and Snyder, 1986, 1988; Corti and Snyder, 1996), which is consistent with benzene-induced hematologic abnormalities in humans and adult animals. The offspring of dams exposed during gestation exhibited increased granulopoiesis and changes in the numbers of hematopoietic progenitor and precursor cells, particularly during postnatal development.

Multigenerational animal studies on the reproductive/developmental toxicity of benzene were not found in the literature, an information gap that contributes to uncertainty about the overall context of the compound's reproductive and developmental toxicity. As discussed in Sections 4.1.2.3 and 4.1.2.4, the evidence regarding reproductive and developmental effects from human studies is limited. The human studies suffer from major limitations, including small numbers of subjects, exposures to a mixture of chemicals, and poorly defined benzene exposure levels and durations of exposure. In many cases data on controls are lacking.

4.2.2.4. *Mechanisms of Developmental and Reproductive Toxicity*

The mechanisms 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 in 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 it decreased DNA content in the maternal liver. The authors 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, and it can be translocated from liver to blood and bone marrow. The enzymes responsible for its production appear in the rat fetus late in pregnancy. However, in the human fetus, the enzymes are present during weeks 9–13 of pregnancy. The authors suggested 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).

4.2.3. Neurotoxicity

4.2.3.1. *Oral Exposure*

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

Hsieh et al. (1988a) evaluated the effects of benzene on neurotransmitters of the brain. CD-1 adult male mice (five/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. However, based on water consumption, the actual daily estimated doses of benzene were 0, 8, 40, and 180 mg/kg/day.

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 values. 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 from this study did not establish 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 the peripheral nervous system could be of particular concern for the developing central nervous system, resulting in permanent effects.

4.2.3.2. *Inhalation Exposure*

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).

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) benzene 6 hours/day for 3 days were divided into two groups of four or six animals. In the first group (–inhibitor), rats that served as controls were sacrificed immediately following exposure. In the second group (+inhibitor), the injection of tyrosine hydroxylase inhibitor immediately followed exposure, and the animals were sacrificed 2 hours 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 dopamine turnover in certain areas of the hypothalamus. The study included only one concentration of benzene and a small number of animals. 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 465 (1500 mg/m^3) benzene 8

hours/day on GDs 8–10 ppm. The abundance of fluorescent noradrenergic fibers normally found in the ovaries and uterus of the pregnant rats decreased, whereas background fluorescence increased (interpreted by the investigators to indicate an increased release of noradrenalin). To confirm that the effect was selective to nerve fiber, the investigators 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 hours 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 hematologic effects of inhaled benzene. Mice (30–45/group) were exposed to benzene concentrations of 100–3000 ppm (319–9584 mg/m^3) 6 hours/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 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 and then it was 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 five exposures to 100 ppm (319 mg/m^3) benzene, to 50% after two exposures to 300 ppm (958 mg/m^3), and to 50% after one 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 were 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. 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 hematologic effects. Short-term exposures to high concentrations did not increase milk licking, but they 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 one exposure 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 hours/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). 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 was observed after exposure to benzene. Mice exposed to 300 ppm (958 mg/m^3) 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 whether benzene induces behavioral changes by direct action on the central nervous system.

Frantik et al. (1994) found that male albino SPF rats from a Wistar-derived strain exposed to benzene for 4 hours 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 hours, the 30% effect level for depression of evoked electrical activity in the brain was 856 ppm (2735 mg/m^3).

Adult male Kunming mice (five/group) were exposed to 0, 0.78, 3.13, or 12.52 ppm (0, 2.5, 10, 40 mg/m^3) benzene 2 hours/day, 6 days/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 apparently it was 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 a Y maze in less than 3 seconds) followed a pattern similar to forelimb grip strength. The frequency increased significantly ($p<0.05$) from 33.7% in controls to 43.8% in the 0.78 ppm 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 histologic 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 reported in this study—forelimb grip strength and frequency of rapid response—are unique and interesting. However, several limitations in this study prevent its use in establishing an RfC. The number of animals used in each group (five) 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 with the findings of most other studies, which found minimal or no response in bone marrow parameters at similar exposure concentrations. Most other studies on the hematotoxicity of inhaled benzene used exposure durations of 6 hours/day, in contrast to the 2 hours/day in this study. Thus, the dramatic effects on bone marrow parameters in this study suggest that actual benzene exposure may have been higher than reported.

4.2.3.3. *Summary of Neurotoxic Effects*

The database for establishing threshold dose levels for neurotoxicity effects is limited. Neurological effects of benzene have been observed in animals and humans, and no clear NOAEL could be identified in any species. No complete neurological testing has been conducted in animals or humans. The exposure levels used in the neurotoxicity studies summarized here were typically high and the exposure durations were short. The longest exposure period was 4 weeks (Hsieh et al., 1988a). It should be noted that neurological concerns have not been raised in occupational studies where exposure was at lower levels. Kahn and Muzyka (1973) reported neurological complaints in workers exposed to 6–16 ppm (20–52mg/m^3), but no objective evaluation was conducted, exposure was poorly characterized, and there was confounding exposure to low levels of toluene (1.6 ppm [5 mg/m^3]). Other human neurotoxicity studies reported much higher exposure levels. No complete functional observational battery has been conducted in animals. Dempster et al. (1984) reported reduced milk licking in mice exposed to 100 ppm (319 mg/m^3 for 1 or 2 days, but grip strength was not affected until exposure reached 1000 ppm (3195 mg/m^3). Li et al. (1992) reported effects on grip strength, locomotor activity, and Y-maze performance (rapid response) at 0.78 ppm (2.5 mg/m^3), but poor exposure characterization and marked inconsistencies between this and other studies' hematologic effects mean that this value is unreliable.

4.2.4. Immunotoxicity

4.2.4.1. *Oral Exposure*

Male Charles River CD-1 mice (five/group, 6–7 weeks of age) were exposed to 0, 31, 166, or 790 mg/L (0, 8, 40, or 180 mg/kg/day) benzene in drinking water 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 control values. Dose-related hematologic effects (erythrocytopenia, leukocytopenia, lymphocytopenia, increased MCV) were observed at all exposure levels. The authors indicated that the increased MCV and decreased HCT and numbers of RBCs were indicative of severe macrocytic anemia.

Biphasic responses were observed in immunological tests, including mitogen-stimulated (lipopolysaccharide [LPS], pokeweed mitogen, concanavalin 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 SRBC, 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 at the low-exposure level. This study identified a LOAEL of 8 mg/kg/day (the lowest dose tested) for hematologic 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 sacrificed after 7, 14, 21, or 28 days of exposure. An unspecified number of mice were exposed to 152 mg/L for 28 days and sacrificed 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 IL-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 IL-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 that of controls. Splenic IL-2 production was significantly depressed after 28 days in both groups. Spleen cell numbers and IL-2 production were also depressed in the mice exposed to 152 mg/kg/day for 28 days and sacrificed 7 and 14 days (IL-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 decreased at the high-exposure level ($p<0.05$). A dose-related decrease in absolute and relative spleen weight was observed ($p<0.01$). In one test, spleen cellularity was reported to be significantly decreased at all exposure levels ($p<0.05$), and in a separate test at only the mid- and high-exposure levels. Dose-related leukopenia and lymphocytopenia were observed ($p<0.05$). A dose-related decrease in eosinophils was observed ($p<0.01$). At the high-exposure level, significant decreases in levels of erythrocytes and Hgb were observed ($p<0.05$). 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 changes were observed in immunological tests on spleen cells and in assays of bone marrow ($p<0.05$); decreases were observed with respect to IgM antibody forming cells/spleen in response to SRBC, lymphocyte proliferation response to the T cell mitogen Con A and the B cell mitogen LPS, number of T lymphocytes, and femoral GM-CFU; and 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 Con A) was significantly decreased at 12 mg/kg/day ($p<0.05$). 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 of 12 mg/kg/day and a LOAEL of 195 mg/kg/day for hematologic effects in mice exposed to benzene in drinking water for 30 days, and a LOAEL of 12 mg/kg/day for immunological effects.

4.2.4.2. *Inhalation Exposure*

Male C57BL/6J mice (7–8/group) were exposed to benzene at a concentration of 0, 10.2, 31, 100, or 301 ppm (0, 32.6, 99, 319, or 962 mg/m^3) in whole-body dynamic inhalation chambers 6 hours/day for 6 days (Rozen et al., 1984). Mice were bled within 30–90 minutes of the last exposure to determine 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 levels) 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 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 the number was reduced to less than 10% of the control value at 100 or 300 ppm (319 and 962 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 962 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 (320 mg/m^3) or 300 ppm (960 mg/m^3) 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 hours/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 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 RBCs were scored.

None of the benzene exposure treatments affected *L. monocytogenes* counts in the spleen 1 day after infection. Preexposure 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 (96 mg/m^3) 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, as 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 ≥30 ppm, and counts did not return to control levels even after cessation of benzene exposure. This study identified a LOAEL of 30 (96 mg/m^3) ppm and a NOAEL of 10 (32 mg/m^3) ppm for effects on the immune system.

4.2.4.3. *Summary of Immunotoxic Effects*

As detailed above, dose-related adverse effects on spleen weight and cellularity and on various measures of immune function have been observed following both oral and inhalation exposures to benzene. Dose-related decreases in spleen weight and cellularity were consistently observed following oral benzene exposure (Hsieh et al., 1988b: Fan, 1992; White et al., 1984). Dose-related adverse effects on several measures of immune function have been observed, including lymphocyte count, mitogen-stimulated lymphocyte proliferation, primary antibody response to SRBCs, IL-2 production, femoral B lymphocyte colony-forming cells, number of T lymphocytes, and proliferation of *L. monocytogenes* bacteria in the spleen (Hsieh et al., 1988b; Fan, 1992; White et al., 1984; Rozen et al., 1984). The results indicate that exposure to benzene, whether oral or inhaled, adversely affects the immune response.

4.3. 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 endpoints 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.

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 (see detailed discussions and summaries in Sections 4.1.2.1 and 4.2.1 and Tables 1 and 6). In contrast to these blood cellularity depression effects, benzene is also known to induce bone marrow hyperplasia. AML 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, 1998a). Whether the hematotoxic/immunotoxic effects of benzene and its carcinogenic effects are caused by a common mechanism is not yet known, due in part 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 yet been developed. In addition, the hematotoxicity/immunotoxicity of benzene leads to significant health effects apart from potential induction of leukemia.

Although the decreased ALC and leukemias observed from benzene exposure both result from bone marrow toxicity, they do not necessarily result from the same mechanisms, and decreased ALC may not be a necessary precursor for leukemia. For example, decreased ALC may be the result of cytotoxicity independent of the interaction of benzene metabolites with DNA and/or DNA-associated proteins and tumor formation. Thus, integration of the cancer and

noncancer health assessments of benzene is not considered possible given the current state of knowledge regarding mode of action.

Leukocytopenia has been consistently shown to be a more sensitive indicator of benzene toxicity than anemia in experimental animal systems, and lymphocytopenia has been shown to be an even more sensitive indicator of benzene toxicity than is overall leukocytopenia (Snyder et al., 1980; Ward et al., 1985; Baarson et al., 1984). Rothman et al. (1996a) also found that a decrease in absolute lymphocyte count was the most sensitive indicator of benzene exposure in a group of workers. Similarly, Ward et al. (1996) demonstrated 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 on benzene and lymphocytopenia—except for the occupational study of Collins et al. (1991)—has been the absence of a clear threshold.

Human and experimental animal studies indicate that benzene affects both humoral and cellular immunity. Dose-related adverse effects on spleen weight and cellularity and various parameters of immune function have been observed following both oral and inhalation exposure to benzene.

Benzene toxicity to humans exposed in the workplace has been characterized as having either early hematotoxicity or, with prolonged exposure to high doses, irreversible bone marrow damage. Studies of worker populations in factories in which benzene was used as a solvent (Snyder and Kocsis, 1975) showed a range of hematotoxic effects, including anemia, leukopenia, and thrombocytopenia. In some cases, more than one cell type was decreased. A decrease in the levels of all the classes of blood cell types in circulation is termed pancytopenia and is usually associated with irreversible bone marrow aplasia. Aplastic anemia is fatal in most cases. In those who survive aplasia, the marrow appears to be dysplastic. Myelodysplastic syndrome, which has been called preleukemia, is likely an early stage of AML.

The evidence is strong that benzene metabolism plays a critical role in toxicity (Snyder and Hedli, 1996). Hepatic metabolism plays an important role in toxicity (Andrews et al., 1977). In addition to hepatic metabolism, it appears that secondary metabolism of benzene metabolites in bone marrow contributes to toxicity (Irons et al., 1980; Schlosser and Kalf, 1989). Thus, elucidation of the metabolic pathway for benzene biotransformation is essential for a full understanding of the mechanism of toxicity.

There are alternative routes by which the first step of benzene metabolism, namely, phenol formation, can occur. CYP2E1, and perhaps other cytochromes P450, can generate H_2O_2 when acting as oxidases of nicotinamide adenine dinucleotide phosphate (NADPH). The hydroxyl radical formed from H_2O_2 can hydroxylate benzene to yield phenol. An alternative mechanism for phenol formation reflects on the fate of the benzene oxide-oxepin system (see Figure 1). When benzene oxide is the first product, it can rearrange nonenzymatically to form phenol. Alternatively, benzene oxide can be further metabolized by epoxide hydrolase to yield 1,2-benzene dihydrodiol, which can in turn be oxidized via dihydrodiol dehydrogenase to form catechol. The reaction of benzene oxide with GSH catalyzed by GSHS transferase leads to the formation of the premercapturic acid. It is likely that benzene oxide or its oxepin (intermediate) are precursors to ring opening (Witz et al., 1996). Phenol can be further hydroxylated to form

hydroquinone or catechol. In theory, 1,2,4-benzenetriol may be formed by the hydroxylation of either hydroquinone or catechol, but Inoue et al. (1989) suggested that catechol is not a precursor of 1,2,4-benzenetriol in humans.

Any of the phenolic metabolites may be conjugated with either sulfate or glucuronide. In addition to L-phenylmercapturic acid reported by Parke and Williams (1953), other mercapturates include 6-N-acetylcysteinyl-S-2,3-cyclohexadienol (Sabourin et al., 1988a, b) and 2,5-dihydroxy-phenyl-mercapturic acid (Nerland and Pierce, 1990). The urine also contains two ring-opening products, MA (Drummond and Finar, 1938) and 6-hydroxy-*trans, trans*-2,4-hexadienoic acid (Kline et al., 1993), and the residue of a covalently bound DNA adduct, i.e., N^7-phenylguanine (Norpoth et al., 1988).

The production of benzene metabolites, largely in the liver, is followed by their transport to the bone marrow and other organs. There are many possible causes of bone marrow toxicity. Pfeifer and Irons (1983) suggested that covalent binding of hydroquinone to spindle fiber protein could explain inhibition of cell replication by benzene. Damage to DNA could result in bone marrow depression, leading to aplastic anemia, which in survivors leads to marrow dysplasia and ultimately to AML (Snyder and Kalf, 1994). Benzene metabolites could cause damage to DNA by two possible mechanisms. One pathway focuses on the metabolic activation of benzene to species that covalently bind to DNA to produce mutagenic events that are expressed as leukemia. The second mechanism involves the production of metabolites that cause oxidative stress, subsequent oxidative damage to DNA, and a mutagenic effect that has the same consequences.

Within the bone marrow, both hematopoietic progenitor cells and bone marrow stromal cells are potential targets of benzene toxicity (Aksoy, 1988; Gaido and Wierda, 1985). Progenitor cells are thought to be the cells of origin for leukemias (Greaves, 1993) and are attractive as potential targets of toxins such as benzene, whose toxic effects are not restricted to a single hematopoietic lineage. Stromal cells are critical in the regulation of normal hemopoiesis (Dorshkind, 1990) and have been considered as important targets of benzene toxicity (Gaido and Wierda, 1985). Stromal cells are intimately associated with developing blood cells and regulate hemopoiesis via direct cell-to-cell interactions, the production of extracellular matrix components, and the secretion of soluble mediators such as cytokines and eicosanoids (Billips et al., 1991).

Although metabolism is central to benzene toxicity, studies of the metabolic capability of human bone marrow are scarce, particularly in human stromal and $CD34^+$ cells.

Benzene hematotoxicity is a complex process that most likely involves interaction among several metabolites (Billips et al., 1991). Possible intermediates considered at present to be important in benzene hematotoxicity are polyhydroxylated benzene metabolites, for example, hydroquinone and 1,2,4-benzenetriol; their quinone oxidation products, for example, *p*-benzoquinone, formed via oxidation of hydroquinone, and semiquinone free radical intermediates formed during the oxidation of polyhydroxylated metabolites to quinones; reactive oxygen species formed during the oxidation of the polyhydroxylated metabolites; and ring-opened aldehydic benzene metabolites, for example, MUC and 6-hydroxy-*trans, trans*-2,4-hexadienal.

MUC and its metabolite 6-hydroxy-*trans, trans*-2,4-hexadienal are reactive ring-opened hematotoxic compounds. They exhibit a host of biological activities that could potentially be important in their mechanisms of toxicity in relation to benzene.

Benzene hematotoxicity occurs when its hepatic metabolites (Sammett et al., 1979)—phenol, catechol, and hydroquinone—are transported to the bone marrow (Greenlee et al., 1981) and further oxidized in a peroxidase-mediated (Smith et al., 1989) reaction to biologically reactive intermediates that can potentially affect hematopoiesis.

The ability to alter cytokine-dependent growth and differentiation in hematopoietic progenitor cells appears to be a property of many agents, with leukemogenic potential for humans (Irons and Stillman, 1993). Several studies have reported significant effects of benzene on hematopoietic stem and progenitor cells (Cronkite et al., 1989). In one study (Seidel et al., 1989), a dose-dependent depression of all stem cell compartments was observed in BDF1 mice exposed for 16 weeks to airborne concentrations of benzene as high as 99 ppm (317 mg/m^3) for 6 hours/day, 5 days/week. The results of this study indicated that the GM-CFU was much less sensitive than the erythroid CFUs at higher doses of benzene.

Another study (Dempster and Snyder, 1990) reported that short-term exposure of mice to benzene induced a shift toward granulocytic differentiation, a growth advantage for granulocytic progenitor cells in the bone marrow and spleen, and to a resultant increase in the total number of granulocytes. These results suggest that benzene, or hydroquinone, is acting on the myeloid stem or progenitor cells. The administration of benzene, or hydroquinone, specifically stimulates granulopoiesis in mice and induces granulocytic differentiation in myeloblasts of the human promyelocytic leukemia cell line, HL-60, as well as the normal murine IL-3 dependent myeloblastic cell line, 32D.3 (G). Benzene and hydroquinone do this by replacing the requirement for granylocyte colony-stimulating factor and leukotriene D_4, respectively, for induction of differentiation.

In conclusion, benzene-induced mechanisms of hematotoxicity are poorly understood. Most proposed mechanisms of benzene-induced effects such as cytotoxicity, apoptosis, mutagenesis, and cell replication can be demonstrated in vitro at relatively high concentrations of benzene metabolities (Snyder et al., 1993b). Benzene effects in vitro do not necessarily correlate with benzene-induced hematotoxicity following in vivo exposures to low concentrations, as in vitro assays of hematopoiesis lack the complex hematopoietic cell interrelationships, fine-tuned regulation, and compensation mechanisms that are essential in normal hematopoiesis. The most important aspects of benzene-induced hematotoxicity are the possible toxic effects caused by low concentrations of benzene. Concurrent assessment of toxicity elicited by high concentrations of benzene (100 [320 mg/m^3] and 200 [640 mg/m^3] ppm) established a reference for comparison.

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 700–3000 ppm (2236–9584 mg/m^3). 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 years 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 the dose and duration of exposure were unknown.

Few studies in test animals have examined the neurotoxicity of benzene, and those that have used short-term exposures, usually to concentrations that have been shown to induce significant hematotoxicity. One exception is the study by Li et al. (1992), which 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 2 hours/day, 6 days per week for 30 days. Both responses increased at the lower concentration and declined at the intermediate and high concentrations. There were, however, a number of uncertainties in the experimental protocol that need to be resolved before these observations can be used in establishing NOAEL or LOAEL values.

No complete neurological testing has been conducted in animals and humans. The toxicological significance of these responses is also not clear. A detailed discussion of neurotoxicity studies is presented in Section 4.2.3.

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 effects. In most cases, there was exposure to other chemicals as well, and the quantitative data were not sufficient to determine a NOAEL or a 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 used benzene concentrations that caused severe maternal toxicity, as indicated by large reductions in body weight gain. Studies that used lower benzene concentrations showed 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 benzene is fetotoxic and causes decreased fetal weight and/or minor skeletal variants at concentrations of greater than 47 ppm (150 mg/m^3) (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-old 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 (see detailed discussion of studies in Section 4.2.2).

Although benzene exposure has been shown to result in structural and numerical chromosomal abberations in human lymphocytes, the quantitative relationship between measured benzene exposures and clastogenic effects in humans is unknown. Associations between benzene exposure and genotoxic effects in humans under occupational conditions have been demonstrated. In animal studies benzene has been shown to induce cytogenic effects, including chromosome and chromatid aberrations, sister chromatid exchanges, and micronuclei

in vivo and in vitro. Mutagenicity of benzene metabolites is well established. However, benzene itself is not mutagenic in bacterial and animal systems (for a detailed discussion on benzene genotoxicity, see Section 3.3.6.2 and U.S. EPA, 1998a).

4.4. SUSCEPTIBLE POPULATIONS

4.4.1. Childhood Susceptibility

This section reviews information regarding whether infants and children may be more susceptible than adults to the hematotoxic effects of benzene. Developmental toxicity was described in Section 4.2.2. For example, inhalation exposure of benzene in pregnant rats on GDs 6–15 resulted in fetotoxicity at 100 ppm (319 mg/m^3). The study established 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. Benzene could be a potential risk factor for the development of childhood leukemia (see reviews, OEHHA, 1997; Smith and Zhang, 1998; U.S. EPA, 1998a). Evidence from human and animal studies (McKinney et al., 1991; Shaw et al., 1984; Shu et al., 1988; Buckley et al., 1989) suggests that increases in childhood leukemia may be associated with in utero exposures and maternal and paternal exposure prior to conception.

Evidence in animals suggests that exposure to benzene in utero alters maturation of lymphocytes, erythrocytes, and granulocytes (OEHHA, 1997). The consequences of in utero exposure to benzene can be detected as alterations in cell population numbers and functional properties into adulthood. Damage during the initial in utero stages of hematopoiesis could have lasting effects, as has been demonstrated for a number of other toxicants (OEHHA, 1997).

Exposure of mice to benzene in utero during development has been shown to cause changes in hematogenic progenitor cells in fetuses, 2-day-old neonates, and 6-week-old adults (Keller and Snyder, 1986, 1988; Corti and Snyder, 1996). 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 hematotoxicity 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. Corti and Snyder (1996) found effects on precursor cells but provided no information on peripheral blood effects. The absence of a clear NOAEL for fetal/neonatal exposure makes it difficult to directly compare fetal/neonatal and adult toxicity.

There are few data on the effects of direct exposure of children to benzene. However, some indirect evidence suggests that children may be susceptible to benzene-induced hematotoxicity. There is mounting evidence that key changes related to the development of childhood leukemia occur in the developing fetus. Several studies have reported that genetic changes related to eventual leukemia development occur before birth. For example, there is one study of genetic changes in sets of twins who developed T cell leukemia at 9 years of age (reviewed in Smith and Zhang, 1998). Because of their small size, increased activity, and increased ventilation rates, as compared with those of adults, children may have greater exposure to benzene in the air on a unit-body-weight basis (U.S. EPA, 1998a). Infants and children also

may be vulnerable to leukemia induction from benzene because their hematopoietic cell populations are undergoing maturation and differentiation (U.S. EPA, 1998a).

4.4.2. Gender Differences

Human exposure data regarding differential effects of benzene exposure on males and females are very limited. Some studies indicate that females may be at greater risk than males. Sato et al. (1975) exposed male and female workers to 25 ppm (80 mg/m^3) benzene for 2 hours and showed that benzene was retained longer in the female subjects. During 5 hours of exposure, the blood concentrations were higher in men, whereas at the end, exhalation breath levels of both men and women were essentially equivalent. However, 4 hours after the exposure had stopped, the female subjects' blood and exhaled air benzene levels were higher than those of male subjects. The shape of the decay curve was significantly steeper in the males. Recently, Brown et al. (1998), using the Sato et al. (1975) data, developed a PBPK model simulation for adult men and women. Results demonstrated that physiochemical gender differences result in women metabolizing 23–26% more benzene than do men when subjected to the same exposure conditions. Benzene blood levels were generally higher in men. These results suggest that women may be at significantly higher risk for certain effects of benzene exposure. In the occupational benzene exposure study by Rothman et al. (1996a), 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.

There are limited indications of gender differences in studies with test animals. The most frequently observed gender difference 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 did 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 hematologic effects in male B6C3F1 mice was 50 mg/kg, compared with 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 hematologic effects for female F344/N rats was 25 mg/kg, compared with 100 mg/kg for males.

Male mice are twofold 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. 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 do 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) benzene in comparison with 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 et al. (1981), 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.

4.4.3. Genetically Susceptible Populations

Significant sources of variability in the population stem from genetic polymorphisms in key enzymes involved in the metabolism of benzene, namely, CYP2E1, NADPH-dependent quinone oxidoreductase, MPO, GSH transferase, and others. Dietary and endogenous sources of phenol, hydroquinone, and other primary metabolites of benzene confer potentially large differences in susceptibility to benzene toxicity. These polymorphisms may increase or decrease an individual's susceptibility to the toxic effects of benzene, as described below.

Smart and Zannoni (1984, 1985) demonstrated the importance of reductase activity in bone marrow as a protective mechanism against benzene toxicity. Recent studies have also shown the importance of polymorphic forms of GSH transferase as a protective characteristic. By the same token it appears that the reason that the liver is not a target for benzene toxicity is its high level of reductase activity, compared with the relatively low level in the bone marrow, where oxidative reactions predominate. Furthermore, London et al. (1997) described a polymorphism in MPO, an enzyme important in activation of polyhydroxylated metabolites of benzene to quinones, suggesting that people who genetically display lower levels of this enzyme may display a lower risk to carcinogenesis. Although biomarkers for susceptibility to benzene have not been validated, it might be hypothesized that an individual who displays high CYP2E1 activity and low GSH transferases in liver and/or bone marrow and low bone marrow reductase and high MPO could be highly susceptible to benzene toxicity.

Ross et al. (1996) have suggested that NAD(P)H:quinone oxidoreductase (NQO1 NAD(P)H) may play a critical protective role in benzene toxicity. This enzyme detoxifies the reactive 1,4-benzoquinone generated by 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, because 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 who lack this enzyme activity could be especially susceptible to benzene toxicity. Rothman et al. (1997) carried out a case-control study in a population of benzene-exposed workers in Shanghai, China. CYP2E1 and NQO1 genotypes were determined by polymerase chain reaction-restriction fragment length polymorphism analysis, and CYP2E1 activity was estimated by the fractional excretion of chlorzoxanone. Investigators found that subjects who had both rapid chlorzoxanone excretion and two copies of the NQO1 ^{609}C→T mutation had a 7.6-fold increased risk of hematotoxicity when compared with subjects who had slow chlorzoxanone excretion and who carried one or two wild-type NQO1 alleles.

The joint effects of CYP2E1 and NQO1 status, shown in Table 9, indicate that rapid CYP2E1 activity or a nonfunctional NQO1 increase an individual's risk of benzene hematotoxicity. Those individuals who had rapid CYP2E1 activity and heterozygous or homozygous variant NQO1 genotypes were at greatest risk of developing benzene-induced hematotoxicty.

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.

Rossi et al. (1999) investigated the role of genetic polymorphisms in modulating urinary excretion of two benzene metabolites—MA and S-phenylmercapturic acid—in 59 nonsmoking city bus drivers, who were professionally exposed to benzene via vehicle exhausts. Genetic

Table 9. Joint effects of CYP2E1 activity and NQO1 genotype on benzene-induced hematotoxicity in Chinese Workers

CYP2E1 activity	NQO1 genotype	Cases	Odds ratio (95% CI) benzene hematotoxicity[a]
Slow	Wild type	8	1.0
Slow	Variant	6	2.4 (0.6–9.7)
Rapid	Wild type	21	2.9 (1.0–8.2)
Rapid	Variant	13	7.6 (1.8–31.2)

[a]Adjusted for the matching variables age and sex.

Source: Rothman et al., 1997.

polymorphisms at six loci encoding cytochrome P450-dependent monooxygenases (CYP2E1 and CYP2D6), GSH transferases (GSTT1, GSTP1, and GSTM1), and NQOR were determined by

polymerase chain reaction based methods. Metabolic variability was observed for CYP2D6, GSTT1, and NQOR, potentially contributing polymorphism as one of the risk factors for benzene-induced adverse health effects. However, no evidence emerged for a possible role of CYP2E1, GSTM1, and GSTP1 polymorphisms in determining the wide differences observed in the biotransformation.

4.5. HAZARD IDENTIFICATION SUMMARY

Studies in experimental animals and human occupational studies have pointed to the changes in peripheral blood and bone-marrow-induced exposure to benzene as being among the most sensitive indicators of the compound's toxicity. Principal among the adverse effects described are leukopenia, lymphocytopenia, granulocytosis, anemia, and reticulocytosis.

Human occupational exposure studies have revealed clear evidence of benzene-induced hematotoxicity (Table 1). Difficulties arise in directly applying most of these findings to quantitative estimation of potential human health impacts. This is due to uncertainty in defining the level of benzene exposure and to potential exposure to mixtures of other potentially harmful substances. One human occupational study, however, reported evidence that can be used to support the derivation of RfC and RfD values for benzene. In a small cross-sectional study of 44 age- and gender-matched controls, Rothman et al. (1996a) observed a dose-response relationship and inverse correlation between hematologic responses and exposure level. Six blood parameters (ALC, WBC count, RBC count, HCT, platelets, and MCV) were significantly different in the high-benzene exposure group (8-hour TWA of 91.9 ppm [294 mg/m^3]) in comparison with controls. However, in the lower-exposure group (median 8-hour TWA of 13.6 ppm [43.4 mg/m^3], only ALC, RBCs, and platelets were significantly different.

In a second case-control study of a rubber worker cohort employed between 1939 and 1976 that relied on controversial exposure estimates (Ward et al., 1996), a strong exposure-response relationship between WBC count and estimated benzene exposure concentration for 30, 90 and 180 days before the blood test date was evident; however, there was only a weak positive exposure response for RBC count. The maximum daily exposure estimate was 34 ppm (109 mg/m^3). There was no evidence for a threshold, suggesting that exposure to relatively low levels of benzene (e.g., < 5 ppm [16 mg/m^3]) could result in hematologic suppression, if the exposure estimates are correct.

Further support for the hypothesis that low exposures to benzene can reduce lymphocyte counts comes from a recent study (Bogadi-Sare et al., 2000) of female employees in the shoemaking industry. This study found that the number of circulating B lymphocytes was lower in 49 shoe workers exposed to benzene concentrations lower than 15 ppm (48 mg/m^3) as compared with 27 nonexposed controls. The authors concluded that benzene concentrations lower than 15 ppm (48 mg/m^3) can induce depression of circulating B lymphocytes. Additional evidence that low exposures to benzene induce reductions of blood parameters comes from a study of workers employed in factories in China (Dosemeci et al., 1996). Significant relative risks of "benzene poisoning" were recorded in workers exposed to as little as 5–19 ppm (16–61 mg/m^3) during the most recent 1.5 years as compared with workers exposed to < 5 ppm (16 mg/m^3). The authors defined benzene poisoning by two criteria: a WBC count of < 4000 cells/mm^3 and a platelet count of < 80,000/mm^3. These data suggest that a threshold of

hematotoxicity in humans is observable in the 5–19 ppm (16–48 mg/m^3) range of exposure to benzene.

Hematotoxicity has been observed in experimental animal studies following both oral and inhalation exposure (Table 6). Hematologic abnormalities developed rapidly and were evident after only 2 weeks of exposure, but there was comparatively little progression with longer exposure periods (Ward et al., 1985; Cronkite et al., 1985). The dose-response studies of Ward et al. (1985) identified a NOAEL of 30 ppm (96 mg/m^3) and a LOAEL of 300 ppm (958 mg/m^3) in CD-1 mice. The results of Cronkite et al. (1985, 1989) suggest that peripheral blood elements may have the capacity to recover from benzene-induced toxicity, depending on the duration of exposure and concentration. For example, lymphocyte counts, which were depressed in C57BL/6 mice exposed to benzene at 300 ppm (958 mg/m^3) for 2–16 weeks, tended towards normal values during a 4- to 8-week recovery period.

Benzene-induced peripheral blood abnormalities reflect a disruption at all levels of hematopoiesis in the bone marrow (Toft et al., 1982; Snyder, 1987; Cronkite et al., 1985; Seidel et al., 1989; MacEachern et al., 1992). Bone marrow cellularity, stem cell compartments, granulocytic and erythropoietic progenitor cells, and bone marrow macrophages have all been observed to be adversely affected following benzene exposure. Recent evidence has suggested that the inhibitory effect of benzene on lymphocyte proliferation can be mediated through the suppression of cytokine production (Renz and Kalf, 1991). The fact that both in vivo and in vitro studies have demonstrated the ability of some metabolites to induce toxicological effects qualitatively similar to those of benzene provides evidence consistent with the concept that the hematotoxicity of benzene may be mediated through its metabolites.

There is no convincing evidence that benzene produces either reproductive or developmental toxicity in humans. The available data are summarized in Tables 4 and 5. 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.

A number of reproductive and developmental studies have been conducted in a wide range of experimental animals (Tables 7 and 8). Changes in testicular weight in guinea pigs (with slight histopathologic alterations) and rats were observed by Wolf et al. (1956), and reductions in ovarian and testicular weights with histopathologic alterations were observed in mice by Ward et al. (1985) after prolonged exposure to benzene. However, the concentrations were higher than those observed to cause hematotoxicity. Several studies have suggested that benzene can cause developmental toxicity in the absence of maternal toxicity in rats. Effects observed include reduced body and liver weights in weanlings (Kuna et al., 1992), skeletal variations in fetuses (Kuna and Kapp, 1981), and reduced fetal weight and delayed ossification in fetuses (Green et al., 1978; Coate et al., 1984; Ungvary and Tatrai, 1985). Although benzene can cause reproductive and developmental toxicity, the LOAEL and NOAEL values associated with reproductive/developmental toxicity are higher than for hematotoxicity.

The hematologic effects in offspring of dams exposed to benzene during gestation were observed at exposure concentrations similar to those in adult animals (Keller and Snyder, 1986, 1988; Corti and Snyder, 1996). Changes in granulopoiesis and hematopoiesis in offspring at

some growth stages were observed. Thus, the results were not consistent. The results of these studies cannot be used for quantitative evaluation of human health risk because of the limited number of replicate animals.

Symptoms of neurotoxicity have been observed in humans following acute exposure to relatively high concentrations of benzene (Brief et al., 1980; Kraut et al., 1988). Kahn and Muzyka (1973) reported subjective symptoms—headache, fatigue, difficulty sleeping, and memory loss—in factory workers exposed to benzene. Baslo and Aksoy (1982) reported neurological abnormalities in patients diagnosed with aplastic anemia after prolonged exposure to benzene. These reports, however, have obvious deficiencies: lack of exposure data, small numbers of subjects, and unknown exposure to other chemicals. Thus, no reliable quantitative evaluation of neurotoxicity in humans is possible.

Neurotoxicity studies with experimental animals were also very limited. The exposure levels were generally high and exposure durations short. The longest exposure period was 4 weeks (Hsieh et al., 1988a). These investigators observed significant increases in monoamine neurotransmitters in the brain of rats at doses of 8 and 40 mg/kg/day, but further increases were not observed at 180 mg/kg/day. Benzene-induced behavioral and learning disorders in mice following inhalation exposures of 100 ppm (319 mg/m^3) or greater for 5 days (Evans et al., 1981; Dempster et al., 1984). Li et al. (1992) observed increased grip strength and enhanced rapid-response maze performance after exposure to 0.78 ppm (2.5 mg/m^3) for 30 days and decreases in these parameters at higher concentrations. There were, however, several limitations in the experimental procedures used in these experiments. There is a limited body of evidence indicating that benzene is neurotoxic; however, there are no human or animal studies that could be used for quantitative evaluation of potential human health risks.

No comprehensive immunotoxicity studies in human populations have been reported, but the study by Rothman et al. (1996a) indicates decreased ALC as the most sensitive indicator of benzene toxicity. This could be interpreted as an effect on immune function.

Immunotoxicity studies in experimental animals have demonstrated dose-related adverse effects on spleen weight and cellularity and on several measures of immune function following both oral and inhalation exposure to benzene (Hsieh et al., 1988b; Fan, 1992; White et al., 1984; Rosenthal and Snyder, 1985; Rozen et al., 1984). NOAEL and LOAEL values for adverse effects on immune function were similar to those established for hematotoxic effects. These studies indicate that even short-term exposures to benzene adversely affect the immune response in experimental animals.

There is no convincing evidence to indicate that children are more susceptible to the toxic effects of benzene; however, there is evidence that differences in gender and subpopulation susceptibility may exist. Differences in responsiveness to benzene have been observed among species. Mice were found to be more sensitive than rats (Ward et al., 1985; Snyder et al., 1978, 1980). Intraspecies variability has also been demonstrated. AKR/J mice were found to be more sensitive than C57BL/6 mice (Snyder et al., 1978, 1980). Absorption studies with humans suggest that absorption by females is higher than by males, and modeling results indicate that females metabolize 23–26% more benzene than men under the same exposure conditions (Sato et al., 1975; Brown et al., 1998). Differences in benzene metabolism could result in differences

in susceptibility, because conversion of benzene to metabolites is necessary for expression of toxicity. Several studies have indicated that male mice are more sensitive than females (Ward et al., 1985; NTP, 1986; Siou et al., 1981; Kenyon et al., 1996; Corti and Snyder, 1996). In contrast, other studies using mice, rats, and rabbits have indicated that females are more sensitive than males (Desoille et al., 1961; Ito, 1962a-d; Sato et al., 1975).

The balance of NQO1 NAD(P)H activity to peroxidase activity in cells may be important in determining benzene toxicity by modulating the concentration of 1,4-benzoquinone, a suspected toxic metabolite. A mutant allele for lack of NQO1 NAD(P)H activity has been reported in a human population at a frequency of 13% (Rosvold et al., 1995). Edwards et al. (1980) reported that 4% of the British population lack NQO1 NAD(P)H activity. Rothman et al. (1997) also observed that Chinese workers homozygous for the mutant allele and with high chlorzoxazone excretion (a measure of CYP2E1 activity) had a 7.6-fold higher risk of developing benzene toxicity. Thus, there is good experimental evidence to indicate that benzene-sensitive human subpopulations may exist.

Several experimental animal studies have observed that ethanol and benzene have an interactive effect on the production of hydroxylated benzene metabolites (Nakajima et al., 1987). When administered at the same time, ethanol and benzene enhanced the number of erythroid progenitor cells in C57BL/6J mice (Baarson and Snyder, 1991), decreased the number of CFU-E cells per femur in BDF1 mice (Seidel et al., 1990), and produced a 70% reduction of CFU-E in male Swiss-Webster mice livers (Corti and Snyder, 1996). On the other hand, a 40% elevation of CFU-E occurred in female Swiss-Webster mice exposed to benzene and ethanol compared to unexposed controls (Corti and Snyder, 1996). There are no relevant published data on the interactive effects of benzene exposure and ethanol use in the human population.

5. DOSE-RESPONSE ASSESSMENTS

The primary target organs for benzene toxicity are the hematopoietic, immune, and nervous systems. There are several reports of short-term exposure in humans and animals. Data on the effects of benzene on the human hematopoietic system following occupational inhalation exposure are scant, but they indicate effects such as leukopenia, anemia, and thrombocytopenia. Data on hematologic effects in experimental animals from short-term inhalation/oral exposures are extensive, indicating changes in peripheral erythrocytes (e.g., Cronkite et al., 1985; Rozen et al., 1984), in peripheral leukocytes (e.g., Aoyama, 1986; Li et al., 1986; Green et al., 1981a), and in bone marrow cells (e.g., Cronkite et al., 1989; Dempster and Snyder, 1991). However, these studies were generally determined to be unsuitable for derivation of the RfD/RfC because they involved short-term exposures and small numbers of experimental animals; in addition, exposure levels were often too high to establish meaningful LOAELs or NOAELs.

Data on adverse hematologic effects in humans following intermediate and chronic duration exposures to benzene in occupationally exposed individuals are available (Aksoy et al., 1971, 1972, 1974, 1987; Cody et al., 1993; Greenberg et al., 1939; Kipen et al., 1989; Li et al., 1994; Townsend et al., 1978; Yin et al., 1987b). However, in the majority of these studies the exposure level estimates and duration of exposure are poorly defined and/or characterized, and potential confounding is problematic; therefore, these studies could not be used in determinating

the RfC and RfD, with the exception of the Rothman et al. (1996a) study, which is used as the key study. The study of occupational inhalation exposure to benzene by Tsai et al. (1983) seems to suggest a NOAEL for hematologic effects; however, the study had no appropriate screening guidelines or referral criteria with which to contrast results, no follow-up, a very pronounced healthy-worker effect, and a very young employed survivor population. No relevant human data are available to evaluate hematologic effects following oral exposure.

Several subchronic and chronic studies in experimental animals following inhalation exposure (see Table 6) and oral exposure (NTP, 1986; Huff et al., 1989; Wolf et al., 1956; see also Section 4.4.2.1) identified NOAELs and LOAELs for hematologic effects and were considered in the derivation of the RfC and RfD. Ultimately, the results of the Ward et al. (1985) subchronic inhalation study and the NTP (1986) chronic gavage study were selected as the most appropriate animal data for deriving an RfC and an RfD. These RfC and RfD values are calculated for comparison with the values derived from the Rothman et al. (1996a) human study.

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 yet been developed. In addition, the hematotoxicity/immunotoxicity of benzene leads to significant health effects apart from potential induction of leukemia. Although the decreased ALC and leukemias observed from benzene exposure both result from bone marrow toxicity, they do not necessarily result from the same mechanisms, and decreased ALC may not be a necessary precursor for leukemia. For example, decreased ALC may be the result of cytotoxicity independent of the interaction of benzene metabolites with DNA and/or DNA-associated proteins and tumor formation. Thus, integration of the cancer and noncancer health assessments of benzene is not considered possible given the current state of knowledge regarding mode of action.

5.1. INHALATION REFERENCE CONCENTRATION (RfC)

5.1.1. Choice of Principal Study and Critical Effect

The human occupational inhalation study by Rothman et al. (1996a) was selected as the principal study for the derivation of the RfC because it is a well-conducted human exposure study that demonstrates a dose-response relationship for hematologic effects, responses that are considered to be among the more sensitive indices of benzene toxicity. The California EPA (2001) selected the Tsai et al. (1983) study to develop a drinking water noncancer protective concentration of 0.026 mg/L, because no increased hematological changes were observed among U.S. refinery workers chronically exposed to an average 0.53 ppm of benzene. This value was assumed to be a NOAEL by the California EPA. The U.S. EPA did not choose this study to develop an RfC or an RfD because of deficiencies in the study design. However, the California EPA's numbers were similar to those of the U.S. EPA, although different studies were used. The cross-sectional Rothman et al. (1996a) study provides exposure-response data for some of the lowest exposure concentrations at which effects have been observed, as discussed in Section 4.1.2.1. These exposure-response data are suitable for BMD modeling to derive a point of

departure for the calculation of the RfC. Reduced ALC is the most sensitive of the hematologic effects reported in this study, and it was selected as the critical effect. For comparison purposes, a chronic inhalation RfC is also derived from the subchronic experimental animal study of Ward et al. (1985), based on the critical effect of decreased HCT (see Section 5.1.5).

Rothman et al. (1996a) compared the hematologic outcomes of 44 workers occupationally exposed to benzene with those of 44 age- and gender-matched unexposed controls, all based in Shanghai, China. ALC, WBCs, RBCs, and platelets were all significantly decreased and MCV was significantly increased in the exposed group (median 8-hour TWA of 31 ppm [99 mg/m^3]) as compared to those in the control group. These effects are consistent with the hematotoxic effects of benzene shown in Aksoy (1989), Goldstein (1988), and Dosemeci et al. (1997). In the low-dose subjects (< 31 ppm [99 mg/m^3], median 8-hour TWA of 13.6 ppm [43.4 mg/m^3]), the ALC, RBCs, and platelet count were reduced compared with controls. Similarly, in a selected subgroup exposed to a median 8-hour TWA of 7.6 ppm (24 mg/m^3) benzene, a statistically significant difference in ALC versus controls was observed (p<0.03).

The study by Rothman et al. (1996a) is notable among epidemiology studies because benzene exposures were monitored; exposure to other chemicals, including toluene, was minimal; and subjects were compared with matched controls. Furthermore, a dose-response relationship was established between ALC and benzene exposure, as monitored by organic vapor passive dosimetry and the level of benzene metabolites in the urine. As discussed in Section 4.1.2.1, the median 8-hour TWA of 7.6 ppm (24 mg/m^3) was designated the LOAEL for these effects and is used herein to calculate a chronic inhalation RfC for benzene hematotoxicity in humans for comparison with the RfC derived by BMD modeling of the ALC exposure-response data.

The choice of a reduction in ALC as the primary effect in an RfD/RfC derivation is partially based on the response's potential role as a "sentinel" effect for a cascade of early hematologic and related biological changes that might be expected to result in the more profound examples of benzene poisoning observed in other cohorts of the National Cancer Institute/Chinese Academy of Preventive Medicine study, as described by Dosemeci et al. (1996). Considered together, the statistically significant inverse correlation between ALC and the level of benzene metabolites in the urine of exposed subjects in the Rothman et al. (1996a) study and the strong relationship between exposure to benzene in persons with benzene poisoning observed by Dosemeci et al. (1996) point clearly to the utility of fluctuations in ALC as a marker for the onset of potentially harmful hematologic changes. That ALC depletion is accompanied by gene-duplicating mutations in somatic cells under the same range of exposure conditions suggests that benzene can cause repeated damage to longer-lived stem cells in human bone marrow, further implicating the compound as etiologically important in the onset of benzene-associated leukemia. These findings underline the importance of basing public health concern for benzene on a toxicological effect that is representative of the earliest biological changes induced by the compound.

In summary, there is overwhelming evidence, in both experimental animals and humans, in the published literature cited in this report and in the cancer update documents (U.S. EPA, 1998a, 1999a), that chronic exposure to benzene leads to the onset of irreversible bone marrow

depression, which is characterized by anemia, leukocytopenia with emphasis on lymphocytopenia, and/or thrombocytopenia.

It is a well-known fact that benzene is a leukemogen. The mechanistic steps that lead to that outcome involve decreases in circulationg blood cells, as characterized by abnormal marrow architecture, inadequate hematopoiesis, and demonstration of chromosomal damage in many cells. Benzene toxicity is a continuum of events, leading from decreases in circulating blood cells to pancytopenia and aplastic anemia or to MDS and acute nonlymphocytic leukemia. Although the role of several metabolites of benzene and the "dose x times" relationship are only partially understood, the ultimate result as a function of marrow dysplasia or aplasia or leukemia is undisputable. In the Rothman et al. (1996a) study, benzene air levels were inversely correlated with the absolute lymphocyte counts among exposed workers. This has been the subject of more detailed discussion in several sections of this document as well as in the cancer update documents (U.S. EPA, 1998a, 1999a). The reader's attention is drawn to sections 4.1, 4.2, 4.5, as well as to some selected references: Rothman et al. (1996a, b), Dosemeci et al. (1997), Bogardi-Sari et al. (2000), Hsieh et al. (1988b), NTP (1986), and Ward et al. (1985).

5.1.2. Benchmark Dose Modeling

The exposure-response data from Rothman et al. (1996a, Table IV) that were used for the BMD modeling are reproduced below.

Median exposure (ppm; 8-hr TWA)	Number of subjects	ALC (mean ± SD × 10^3/μL blood)	Transformed exposures
0.02 (control)	44	1.9 ± 0.4	0.0198
13.6	22	1.6 ± 0.3	2.68
91.9	22	1.3 ± 0.3	4.53

The modeling was done using EPA's Benchmark Dose Modeling Software (version 1.20). The data are fairly supralinear, that is, the change in ALC per unit change in exposure decreases with increasing exposure; therefore, in order to fit the data with one of the available continuous models, the exposure levels were first transformed according to the equation $d' = \ln(d + 1)$. Then the exposure-response data were fit using the continuous linear model, which has the form

$$Y[dose] = \beta_0 + \beta_1 * dose,$$

where, in this case, Y[dose] is the mean ALC and dose is the transformed exposure, d'. The parameters were estimated using the method of maximum likelihood. A constant variance model was used. The resulting parameter estimates are:

Variable	Estimate	SE

β_0	1.91029	26.8966
β_1	-0.129822	70.7846

The model fit was good, as is apparent from the *p*-value of 0.5443 and the graphical display in Figure 2.

In the absence of a clear definition for an adverse effect for this continuous endpoint, a default benchmark response (BMR) of one standard deviation change from the control mean was selected, as suggested in EPA's draft *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b). This default definition of a benchmark response for continuous endpoints corresponds to an excess risk of approximately 10% for the proportion of individuals below the 2nd percentile (or above the 98th percentile) of the control distribution for normally distributed effects (see U.S. EPA, 2000b). The software uses the estimated standard deviation. For the resulting BMC, a 95% lower confidence limit (BMCL) was calculated using the likelihood profile method. A BMC of 2.69 and a BMCL of 2.10 were obtained with the transformed exposures. Transforming these values back to the original exposure scale yields the following values: BMC = 13.7 ppm, 8-hour TWA; BMCL = 7.2 ppm, 8-hour TWA.

A two-degree restricted polynomial model also adequately fits the data, yielding a BMC of 3.00 and a BMCL of 2.14 with the transformed doses (i.e., 19.1 and 7.5 ppm 8-hour TWA, respectively); however, the linear model was selected because it is the most parsimonious (i.e., it

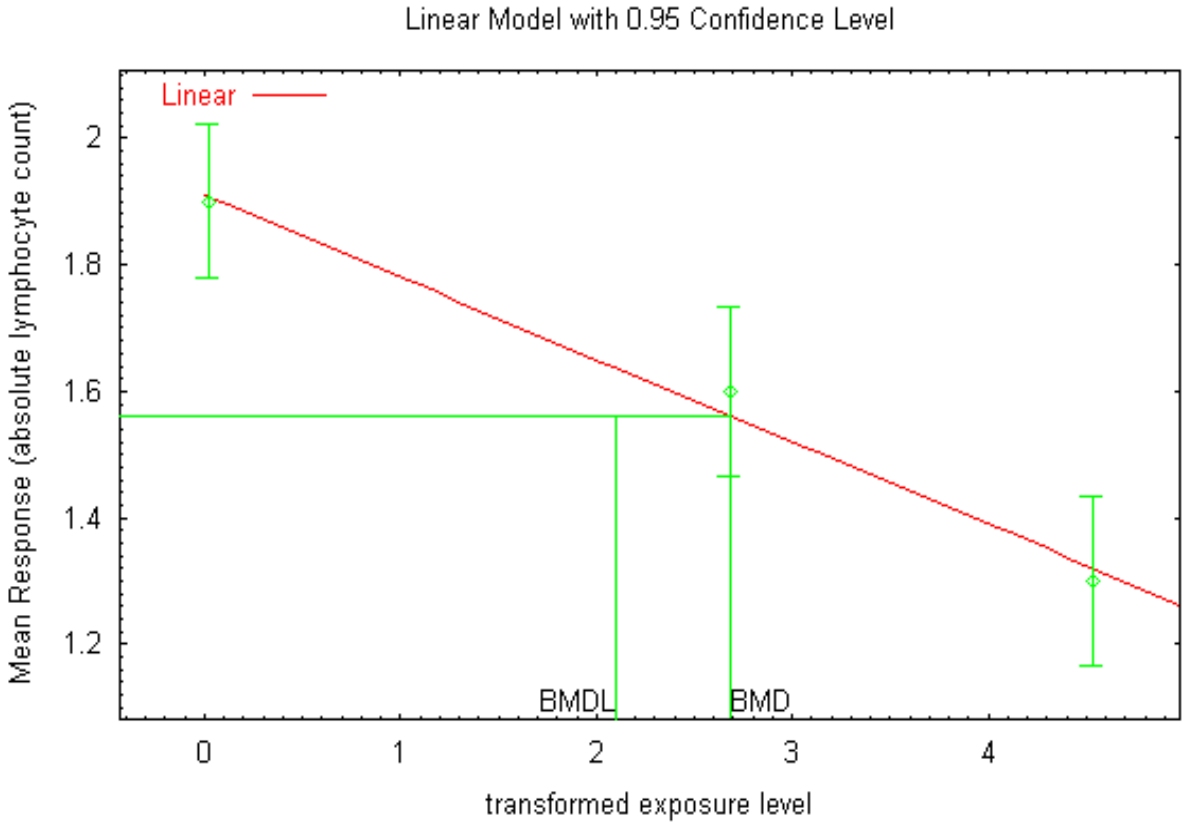


Figure 2. Linear model of ALC data from Rothman et al. (1996a).

is the model with the fewest parameters that fits the data) and 2 × (the absolute difference in the log-likelihood values between the two models) < χ_1^2, that is, the parameter β_2 for the coefficient of dose^2 in the two-degree polynomial model is not statistically different from 0. Results using a restricted power model were identical to those using the two-degree polynomial.

Note that the 7.6 ppm exposure subgroup (11 subjects) was not included explicitly in the exposure-response modeling because it was a subset of the 13.6 ppm group (22 subjects) and the data were not available for the remaining 11 subjects in the 13.6 ppm group (nor were the individual data available). Thus, using the 7.6 ppm group rather than the 13.6 ppm group would have meant that some of the data, for one-fourth of the exposed subjects, would not have been used at all. Comparison analyses were conducted to examine the effects of using the 7.6 ppm group and either omitting the subjects in the 2nd exposure quartile or estimating the exposure level of the 2nd quartile as the midpoint between 13.6 and 31 ppm. The BMCLs were similar to those obtained when the 7.6 ppm group was not included separately (see Table 10).

5.1.3. RfC Derivation

As suggested in the draft BMD technical guidance document (U.S. EPA, 2000b), the BMCL is chosen as the point of departure for the RfC derivation. An adjusted BMCL is calculated by converting ppm to mg/m^3 and adjusting the 8-hour TWA occupational exposure to

Table 10. Results of BMC modeling of Rothman et al. (1996a) data on benzene and ALC[a]

Dataset	BMC (ppm)	BMCL (ppm)
without 7.6 ppm subgroup	13.7	7.2
with 7.6 ppm subgroup, omitting 2nd quartile	14.7	7.4
with 7.6 ppm subgroup, estimating 2nd quartile	17.1	8.4

[a]Using log-transformed exposures, a linear model, and a BMR level of one standard deviation change from the control mean.

an equivalent continuous environmental exposure. The BMCL is first converted to mg/m^3 using the molecular weight of 78.11 for benzene and assuming 25°C and 760 mm Hg:

$$BMCL\ (mg/m^3) = 7.2\ ppm \times 78.11/24.45 = 23.0\ mg/m^3$$

The converted value is then adjusted from the 8-hour TWA to an exposure concentration adjusted for continuous exposure using the default occupational minute volume (U.S. EPA, 1994).

$$BMCL_{ADJ} = BMCL\ (mg/m^3) \times (VEho/VEh) \times 5\ days/7\ days$$

$$BMCL_{ADJ} = 23.0\ mg/m^3 \times (10\ m^3/20\ m^3) \times 5\ days/7\ days = 8.2\ mg/m^3$$

where:

$BMCL_{ADJ}$ = the BMCL dosimetrically adjusted to account for continuous exposure

BMCL = occupational exposure level (8-hour TWA)

VEho = human occupational default minute volume (10 m^3/8 hours)

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

To calculate an RfC using the $BMCL_{ADJ}$ value of 8.2 mg/m^3, several UFs were applied. First, because the BMC is considered to be an adverse-effect level, an effect-level extrapolationfactor analogous to the LOAEL-to-NOAEL UF is used. EPA is planning to develop guidance for applying an effect-level extrapolation factor to a BMD. In the interim, a factor of 3 is used in this analysis, based on the professional judgement that, while the BMC corresponds to an adverse-effect level at the low end of the observable range, the endpoint is not very serious in and of itself. Decreased ALC is a very sensitive sentinel effect that can be measured in the blood, but it is not a frank effect, and there is no evidence that it is related to any functional impairment at levels of decrement near the BMR. For a more serious effect, a larger factor, such as 10, might be selected.

Second, a factor of 10 was used for intraspecies differences in response (human variability) as a means of protecting potentially sensitive human subpopulations. Third, a subchronic-to-chronic extrapolation factor was applied because the mean exposure duration for the subjects in the principal study was 6.3 years, which is less than the exposure duration of 7 years (one tenth of the assumed human life span of 70 years) which has been used by the Superfund program as a cut-off for deriving a subchronic human reference dose (U.S. EPA, 1989). However, because the mean exposure duration (range 0.7 to 16 years) was near the borderline of what would be considered chronic (i.e., 6.3 years vs. 7 years), a partial value of 3 (vs. 10) was felt to be appropriate for the UF.

Finally, a UF of 3 was chosen to account for database deficiencies because, despite the extensive nature of the overall toxicological database for benzene, no two-generation reproductive/developmental toxicity study is available. Therefore, an overall UF of 3 × 10 × 3 × 3 = 300 is used to calculate the chronic inhalation RfC, as follows:

$$RfC = BMCL_{ADJ} \div UF = 8.2 \text{ mg/m}^3 \div 300 = \mathbf{3 \times 10^{-2} \text{ mg/m}^3}$$

5.1.4. Comparison Analysis Based on the LOAEL

A median 8-hour TWA concentration of 7.6 ppm was designated the LOAEL for the Rothman et al. (1996a) study for a comparison calculation of a chronic inhalation RfC for the critical effect of reduced ALC. A $LOAEL_{ADJ}$ can be calculated, as described for the $BMCL_{ADJ}$ in Section 5.1.3, by converting to mg/m^3 and adjusting from the occupational ventilation rate and intermittent work-week schedule to a continuous 24-hour exposure, 7 days/week:

$$LOAEL\ (mg/m^3) = 7.6 \text{ ppm} \times 78.11/24.45 = 24.3 \text{ mg/m}^3$$

$$LOAEL_{ADJ} = 24.3 \text{ mg/m}^3 \times (10 \text{ m}^3/20 \text{ m}^3) \times 5 \text{ days}/7 \text{ days} = 8.7 \text{ mg/m}^3$$

To calculate an RfC using the $LOAEL_{ADJ}$ value of 8.7 mg/m^3, the following UFs were selected. A factor of 10 was applied to account for using a LOAEL because of the lack of an appropriate NOAEL. A factor of 10 was used for intraspecies differences in response (human variability). A partial UF of 3 for subchronic-to-chronic extrapolation was applied and a UF of 3 was chosen to account for database deficiencies, as discussed in Section 5.1.3 above. Therefore, an overall UF of 10 × 10 × 3 × 3 = 1000 is used in the calculation of the chronic inhalation RfC from the LOAEL, as follows:

$$RfC = LOAEL_{ADJ} \div UF = 8.7 \text{ mg/m}^3 \div 1000 = 9 \times 10^{-3} \text{ mg/m}^3$$

This value is in good agreement with the RfC of 3×10^{-2} mg/m^3 calculated from the BMC.

5.1.5. Comparison Analysis Based on the Ward et al. (1985) Experimental Animal Study

A chronic inhalation RfC was also derived from the subchronic experimental animal study of Ward et al. (1985) for comparison with the RfC of 3×10^{-2} mg/m^3 based on the Rothman et al. (1996a) human study. The Ward et al. (1985) study was selected because it used a relatively long inhalation exposure duration and an adequate number of animals, and it

provided dose-response data. The study identified both a LOAEL of 300 ppm and a NOAEL of 30 ppm. The investigators exposed male and female CD-1 mice and Sprague-Dawley rats to 0, 1, 10, 30, or 300 ppm (0, 3.2, 32, 96, or 960 mg/m^3) benzene 6 hours/day, 5 days/week for 91 days and measured various hematologic endpoints. The male mouse appears to be the most sensitive sex/species in this study. The exposure-response relationships for the different hematologic endpoints were modeled using a BMD modeling approach, and decreased HCT was chosen as the critical effect. The exposure-response data from Ward et al. (1985, Table II, 91 days) for the male mouse are reproduced below.

Exposure (ppm)	Number of subjects	% HCT (mean ± SD)
0	20	41.1 ± 1.61
1	20	38.4 ± 3.93
10	20	40.8 ± 3.22
30	20	38.4 ± 5.65
300	20	27.9 ± 4.75

EPA's Benchmark Dose Modeling Software (version 1.20) was used for the modeling. An assumption of constant variance was used, although the test for homogeneity of the variances failed. The continuous linear, polynomial, and power models all resulted in the same BMC and BMCL estimates; however, the linear model had better results for the fit statistics. The linear model had a *p*-value of 0.09, which is of borderline adequacy (EPA's draft *Benchmark Dose Technical Guidance Document* [U.S. EPA, 2000b] recommends a *p*-value of $\geq$ 0.1), whereas the other models had *p*-values of 0.04. Thus the continuous linear model was selected. This model has the form

$$Y[dose] = \beta_0 + \beta_1 * dose$$

where, in this case, Y[dose] is the mean HCT level and dose is the experiment exposure concentration. The parameters were estimated using the method of maximum likelihood. The resulting parameter estimates are

Variable	Estimate	SE
β_0	40.0962	2.44042
β_1	-0.0407075	329.232

See Figure 3 for a graphical display of the data and model.

In the absence of a clear definition for an adverse effect for this continuous endpoint, a default BMR of one standard deviation from the control mean was selected, as suggested in the EPA's draft BMD technical guidance document (U.S. EPA, 2000b). The software uses the estimated standard deviation. A 95% lower confidence limit (BMCL) on the resulting BMC was calculated using the likelihood profile method. A BMC of 100.7 ppm and a BMCL of 85.0 ppm

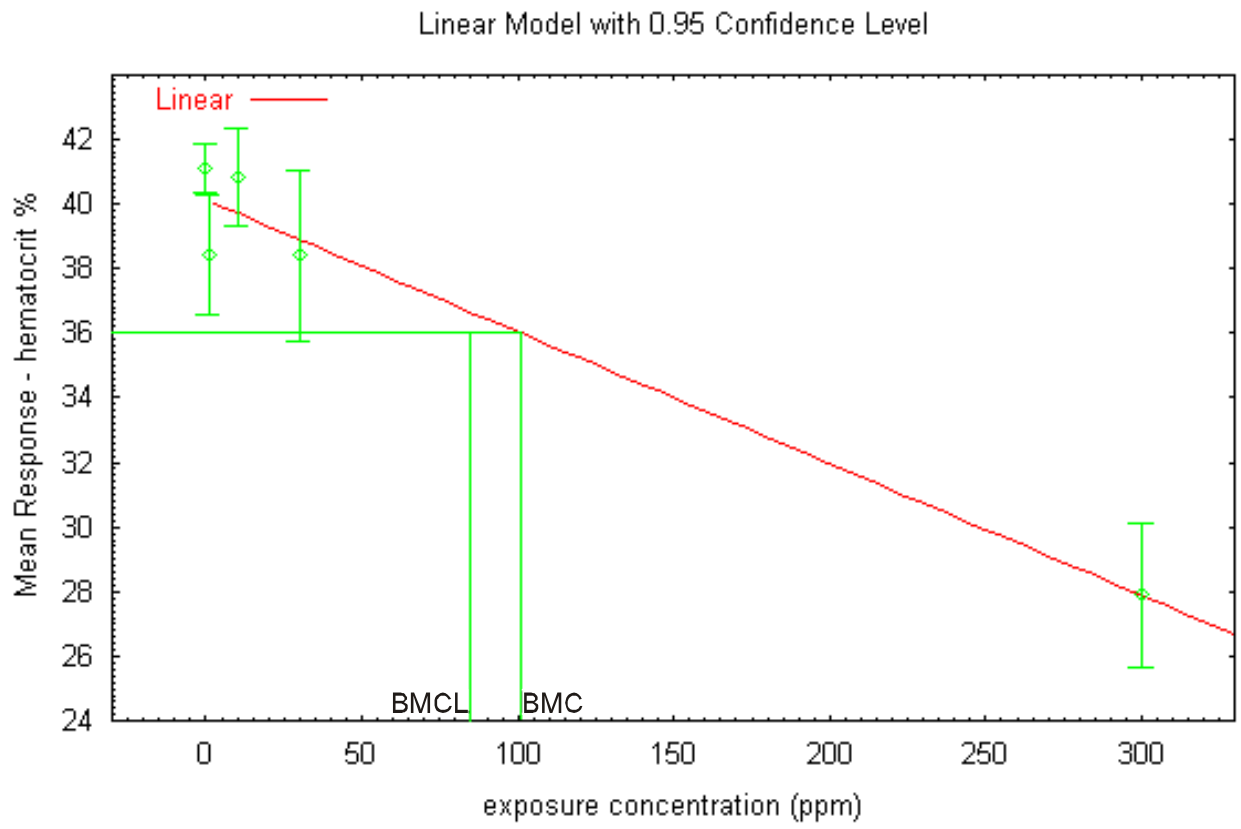


Figure 3. Linear model of HCT data from Ward et al. (1985)

were obtained.

It should be noted that the dose spacing in this study was less than ideal. Responses in the three lower-exposure groups for all the hematologic endpoints tended to clump near control group levels, and significant deviations in response were generally seen only in the 300 ppm group, with a large exposure range in between, including where the BMC is located, for which there are no response data (see Figure 3). Therefore, there is some uncertainty about the actual shape of the dose-response curve in the region of the BMR and, thus, some corresponding uncertainty about the values of the BMC and BMCL estimates.

ALCs were not reported in Ward et al. (1985), so this endpoint could not be compared with the human ALC results. Total WBC counts were reported, and they exhibited the largest percent change in response between the control and the 300 ppm group. However, the data for this endpoint also had substantial variance, and because the BMR used for this analysis is a function of the standard deviation, WBC counts did not yield the lowest BMC estimate. The actual lowest BMC estimates were obtained for increased mean cell Hgb (78 ppm; BMCL = 67 ppm) and increased mean cell volume (79 ppm; BMCL = 68 ppm); however, these endpoints are probably not adverse per se. On the other hand, they are likely to be compensatory effects—and thus markers of toxicity—and one could probably justify using them as the critical effects. In any event, the BMC estimates are not much different from the BMC of 100 ppm obtained for decreased HCT. The results are also similar for total blood Hgb (BMC = 104 ppm; BMCL = 88

ppm). RBC results were in between those for MCV and mean corpuscular Hgb (MCH) and those for HCT and total Hgb; however, the model fits were not adequate for the RBC data; thus, the RBC results have more uncertainty.

To derive the RfC, the BMCL is used as the point of departure, as suggested in the draft BMD technical guidance document (U.S. EPA, 2000b). An adjusted BMCL is calculated by converting ppm to mg/m^3 and adjusting from animal experiment exposure to equivalent continuous exposure. (For conversion of the inhalation exposures across species, ppm equivalence was assumed; this is identical to using EPA's inhalation dosimetry methodology with the regional gas dose ratio for the respiratory tract region ($RGDR_r$) = 1 [U.S. EPA, 1994].) The BMCL is first converted to mg/m^3 using the molecular weight of 78.11 for benzene and assuming 25°C and 760 mm Hg:

$$\text{BMCL } (mg/m^3) = 85.0 \text{ ppm} \times 78.11/24.45 = 272 \text{ mg/m}^3$$

The converted value is then adjusted to an equivalent continuous exposure as follows:

$$BMCL_{ADJ} = 272 \text{ mg/m}^3 \times 6 \text{ hours}/24 \text{ hours} \times 5 \text{ days}/7 \text{ days} = 48.5 \text{ mg/m}^3$$

To derive the RfC, several UFs are applied to the $BMCL_{ADJ}$. As discussed in Section 5.1.3, a UF of 3 is used as an effect-level extrapolation factor, analogous to a LOAEL-to-NOAEL UF, because the BMC is considered an adverse-effect level. In addition, the standard UFs of 3 for interspecies extrapolation for inhalation studies and of 10 for intraspecies variability are used. Also as discussed in Section 5.1.3, a UF of 3 for database deficiencies is used due to the absence of a two-generation reproductive/developmental toxicity study for benzene. Finally, a partial UF of 3 was used to extrapolate from subchronic to chronic exposure. This partial value was selected on the basis of the observation that hematologic fluctuations such as reductions in RBCs and WBCs in the high-dose mice were noted at interim sacrifice (14 days) as well as at termination (91 days), suggesting that the responses occurred early in the exposure cycle and then remained comparatively unchanged. Therefore, an overall UF of $3 \times 3 \times 10 \times 3 \times 3 = 1000$ is used in the calculation of the chronic inhalation RfC from the $BMCL_{ADJ}$, as follows:

$$RfC = BMCL_{ADJ} \div UF = 48.5 \text{ mg/m}^3 \div 1000 = 5 \times 10^{-2} \text{ mg/m}^3$$

This value is in good agreement with the RfC of 3×10^{-2} mg/m^3 calculated from the BMC from the Rothman et al. (1996a) human study.

Similarly, for comparison purposes, a chronic inhalation RfC can be derived from the NOAEL of 30 ppm observed for hematologic effects in the Ward et al. (1985) experimental animal study. First, the NOAEL is converted to mg/m^3 and adjusted to equivalent continuous exposure, as above:

$$30 \text{ ppm} \times 78.11/24.45 = 95.8 \text{ mg/m}^3$$
$$95.8 \text{ mg/m}^3 \times (6 \text{ hours}/24 \text{ hours}) \times 5 \text{ days}/7 \text{ days} = 17.1 \text{ mg/m}^3$$

(ppm equivalence across species was assumed; this is identical to using EPA's inhalation dosimetry methodology with $RGDR_r$=1 [U.S. EPA, 1994]). UFs are identical to those employed above, except that no NOAEL-to-LOAEL UF is used, thus the overall UF is 300, resulting in an RfC of

$$NOAEL_{ADJ} \div UF = 17.1 \div 300 = 6 \times 10^{-2} \text{ mg/m}^3$$

This value is also in good agreement with the RfC of 3×10^{-2} mg/m^3 derived from the Rothman et al. (1996a) study.

It should be noted, however, that other experimental animal studies have reported significant hematologic effects at benzene exposures of 10–25 ppm, which are lower than the NOAEL of 30 ppm from the Ward et al. (1985) study. These studies have insufficient data for dose-response modeling, and they used shorter exposure durations and/or fewer experimental animals than did the Ward et al. study; nonetheless, they observed statistically significant hematologic effects at 10–25 ppm. Baarson et al. (1984), for example, exposed male C57BL/6J mice (five/group) to 10 ppm benzene 6 hours/day, 5 days/week for 178 days and observed statistically significant reductions in blood lymphocytes at each of the three monitoring time points (32, 66, and 178 days) as compared with controls. The magnitude of the reduction in lymphocytes ranged from about 53% at 32 days to about 68% at 178 days. Cronkite et al. (1985) exposed male and female C57BL/6 BNL mice to various concentrations of benzene 6 hours/day, 5 days/week for 2 weeks and observed no decrease in blood lymphocytes at 10 ppm; however, the investigators did observe a statistically significant reduction of about 21% at 25 ppm as compared with controls (5–10 mice/group). Thus, lower RfCs than those calculated above for the Ward et al. (1985) study are possible, based on other experimental animal results. In the most extreme case, using a LOAEL of 10 ppm and an overall UF of 3000 yields a $LOAEL_{ADJ}$ of 5.7 mg/m^3 and an RfC of 2×10^{-3} mg/m^3.

5.2. ORAL REFERENCE DOSE (RfD)

5.2.1. Choice of Principal Study and Critical Effect

As with the inhalation RfC (Section 5.1), the human occupational inhalation study of Rothman et al. (1996a) was selected as the principal study, and reduced ALC was selected as the critical effect, for the derivation of the chronic oral RfD. This study was selected because it was a well-conducted human exposure study that demonstrated a dose-response relationship for hematologic effects, responses that are considered to be among the more sensitive indices of benzene toxicity (see Section 5.1.1 for more details). Furthermore, no relevant human data are available to evaluate hematologic effects following oral exposure. For comparison purposes, a chronic oral RfD is also derived from the chronic experimental animal gavage study of the NTP (1986) on the basis of the same critical effect of reductions in lymphocyte count (see Section 5.2.5).

5.2.2. Conversion of Inhalation Exposure to Equivalent Oral Dose Rate

As discussed in Section 5.1.2, BMD modeling of the ALC data of Rothman et al. (1996a) yielded a BMC of 13.7 ppm (8-hour TWA) and a BMCL of 7.2 ppm (8-hour TWA) for the

default BMR of one standard deviation change from the control mean response. This default definition of a BMR for continuous endpoints corresponds to an excess risk of approximately 10% for the proportion of individuals below the 2nd percentile (or above the 98th percentile) of the control distribution for normally distributed effects (see U.S. EPA, 2000b). As suggested in the draft *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b), the BMCL is chosen as the point of departure for the RfD derivation. Calculation of an equivalent oral dose rate from the inhalation BMCL of 7.2 ppm (8-hour TWA) is shown below.

The BMCL is first converted to mg/m^3 using the molecular weight of 78.11 for benzene and assuming 25°C and 760 mm Hg:

$$BMCL\ (mg/m^3) = 7.2\ ppm \times 78.11/24.45 = 23.0\ mg/m^3$$

The converted value is then adjusted from the 8-hour TWA to an exposure concentration adjusted for continuous exposure using the default occupational minute volume (U.S. EPA, 1994).

$$BMCL_{ADJ} = BMCL\ (mg/m^3) \times (VEho/VEh) \times 5\ days/7\ days$$

$$BMCL_{ADJ} = 23.0\ mg/m^3 \times (10\ m^3/20\ m^3) \times 5\ days/7\ days = 8.2\ mg/m^3$$

where:

$BMCL_{ADJ}$ = the BMCL dosimetrically adjusted to account for continuous exposure

BMCL = occupational exposure level (8-hour TWA)

VEho = human occupational default minute volume (10 m^3/8 hours)

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

In the support document for the benzene cancer assessment on IRIS (U.S. EPA, 1999a), EPA provided a simple method for extrapolating benzene-induced cancer risk from the inhalation to oral route. The same method is applied here for noncancer (hematopoietic) effects. The method is based on the relative efficiency of benzene absorption across routes of exposure, especially pulmonary and gastrointestinal barriers. An inhalation absorption rate of 50% and an oral absorption rate of 100% were used to calculate the absorbed benzene dose. These values are based on human inhalation absorption studies (Nomiyama and Nomiyama, 1974; 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 ^{14}C-benzene by rats and mice during a 6-hour exposure decreased as exposure concentration increased. Retention decreased from 33 ± 6% to 15 ± 9% for rats and from 50 ± 1% to 10 ± 2% for mice as exposure concentration increased from 32 to 3,200 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 weight. At oral doses below 15 mg/kg, > 90% of the ^{14}C excreted was in the urine as

nonethyl acetate-extractable material. At higher doses, an increasing percentage of the orally administered benzene was exhaled unmetabolized. Thus, in the dose range represented by the BMCL from the study by Rothman et al. (a, b), absorption of a comparable oral dose was assumed to be 100%. See also U.S. EPA (1999a) for more details about the route-to-route extrapolation of benzene inhalation results to oral exposures.

To calculate an equivalent oral dose rate, the $BMCL_{ADJ}$ 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):

$$8.2\ mg/m^3 \times 20\ m^3/day \times 0.5 \div 70\ kg = 1.2\ mg/kg/day$$

5.2.3. RfD Derivation

To calculate an RfD using the $BMCL_{ADJ}$-equivalent oral dose rate value of 1.2 mg/kg/day, several UFs were applied. First, because the BMC is considered to be an adverse effect level, an effect-level extrapolation factor analogous to the LOAEL-to-NOAEL UF is used. EPA is planning to develop guidance for applying an effect level extrapolation factor to a BMD. A factor of 3 will be used in this analysis, as in Section 5.1.3. For a more serious effect, a larger factor, such as 10, might be selected. Second, a factor of 10 was used for intraspecies differences in response (human variability) as a means of protecting potentially sensitive human subpopulations. Third, a partial UF of 3 was used for subchronic-to-chronic extrapolation, as discussed in Section 5.1.3. Finally, a UF of 3 was chosen to account for database deficiencies due to the lack of a two-generation reproductive/developmental toxicity study for benzene. Therefore, an overall UF of 3 x 10 x 3 x 3 = 300 is used to calculate the chronic oral RfD, as follows:

$$RfD = \text{equivalent oral dose rate} \div UF = 1.2\ mg/kg/day \div 300 = \mathbf{4 \times 10^{-3}\ mg/kg/day}.$$

Use of a modifying factor of 3 to account for uncertainty in the route-to-route extrapolation was considered; however, it was deemed unnecessary. The RfD is based on human data for a sensitive endpoint; thus, it was felt that the composite UF of 300 provides sufficient protection.

5.2.4. Comparison Analysis Based on the LOAEL

A median 8-hour TWA concentration of 7.6 ppm was identified from the Rothman et al. (1996a) study as a LOAEL for the critical effect of reduced lymphocyte counts. An equivalent oral dose rate can be calculated from the LOAEL, as shown in Section 5.2.2 above and in abbreviated form below. First, a $LOAEL_{ADJ}$ is calculated by converting to mg/m^3 and adjusting from the occupational ventilation rate and intermittent work-week schedule to a continuous 24-hour exposure, 7 days/week:

$$LOAEL\ (mg/m^3) = 7.6\ ppm \times 78.11/24.45 = 24.3\ mg/m^3$$

$$LOAEL_{ADJ} = 24.3\ mg/m^3 \times (10\ m^3/20\ m^3) \times 5\ days/7\ days = 8.7\ mg/m^3$$

Next, an inhalation absorption rate of 50% was used to calculate the absorbed benzene dose, and oral absorption was assumed to be 100%, as discussed in Section 5.2.2. To calculate an equivalent oral dose rate, the $LOAEL_{ADJ}$ 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):

$$8.7\ mg/m^3 \times 20\ m^3/day \times 0.5 \div 70\ kg = 1.2\ mg/kg/day$$

To calculate an RfD using the $LOAEL_{ADJ}$-equivalent oral dose rate value of 1.2 mg/kg/day, the following UFs were selected: a factor of 10 to account for using a LOAEL due to the lack of an appropriate NOAEL, a factor of 10 for intraspecies differences in response (human variability), a factor of 3 for subchronic-to-chronic extrapolation, and a factor of 3 for database deficiencies, as above. Therefore, an overall UF of $10 \times 10 \times 3 \times 3 = 1000$ has been used to calculate the chronic oral RfD, as follows:

$$RfD = \text{equivalent oral dose rate} \div UF = 1.2\ mg/kg/day \div 1000 = 1 \times 10^{-3}\ mg/kg/day.$$

This value is in good agreement with the RfD of 4×10^{-3} mg/kg/day calculated from the BMC.

5.2.5. Comparison Analysis Based on the NTP (1986) Experimental Animal Study

The proposed RfD is based on the Rothman et al. (1996a) study because it was decided that it was preferable to use the human data rather than the experimental animal data. The NTP (1986) study rather than the Hsieh et al. (1988b) study was used for the comparison analysis because it was much larger (50 vs. 5 animals/dose group), it was conducted for 2 years (vs. 28 days) with blood measurements made at multiple time points, and it examined both male and female rats and mice (vs. just male mice). The Hsieh et al. (1988b) study provided a lower LOAEL (8 mg/kg/day vs. 18 mg/kg/day adjusted to 7 days/week); however, the lower LOAEL is not crucial, because the main analysis currently uses a BMD approach. Both studies yielded similar BMDLs for decreased ALC (0.7 mg/kg/day for the male rat NTP data vs. 1.4 mg/kg/day for the Hsieh et al. data; both based on a linear model with transformed doses and a BMR of one standard deviation change from the control mean).

A chronic oral RfD was also derived from the NTP (1986) experimental animal study for comparison with the RfD of 1×10^{-3} mg/kg/day based on the Rothman et al. (1996a) human study. In the NTP (1986) study, F344 rats and B6C3F1 mice of both sexes were administered benzene by gavage 5 days/week for 103 weeks (see also Section 4.2.1.2). Male rats (50/group) were administered doses of 0, 50, 100, or 200 mg/kg and females (50/group) were administered doses of 0, 25, 50, or 100 mg/kg. B6C3F1 mice (50/sex/group) were administered doses of 0, 25, 50, or 100 mg/kg. Blood was drawn from 10 randomly preselected animals per species/sex/dose group at 12, 15, 18, and 21 months and from all animals at the terminal kill at 24 months. Additional groups of 10 animals of each sex and species were administered benzene for 51 weeks at the same doses of the 2-year study, and blood was drawn at 0, 3, 6, 9, and 12 months. This study identified a LOAEL of 25 mg/kg for leukopenia and lymphocytopenia in female F344 rats and male and female B6C3F1 mice and of 50 mg/kg in male F344 rats. These were the lowest doses tested and, thus, no NOAEL was identified.

Reduction in lymphocyte count was selected as the critical effect, and attempts were made to model the dose-response relationships using a BMD modeling approach. However, the dose-response analysis of the NTP data was problematic for a number of reasons. First, only the grouped data, not the individual animal data, were provided. Second, as discussed in Appendix N of the NTP (1986) report, not all of the groups were comparable for a variety of reasons, including experiment design, differences in bleeding methods and time of bleeding, and differences in measurement instrumentation. Third, the grouped data exhibited substantial variability, and baseline measurements (vehicle control groups) were not constant over time. Furthermore, for the rats, the strong dose effect in lymphocyte count was accompanied by significant temporal variability, which appears to be largely due to temporal variability in the vehicle control group means. For these reasons, it was not possible to do a pooled analysis across time points. Instead, modeling was performed for each dataset in two data groupings within which the datasets are comparable (months 6 and 9; and months 12, 15, 18, and 21), and ranges of results are presented. Each of these datasets had at most 10 animals/dose, so the dose-response results are not very robust. The males of both species exhibited more dramatic and consistent reductions in lymphocyte count than did the females, but it was not clear a priori which species was more sensitive; therefore, dose-response analyses were performed for both the male mouse (NTP Table N8) and the male rat (NTP Table N4).

The continuous linear, polynomial, and power models in EPA's Benchmark Dose Modeling Software (version 1.20) were used for the modeling. The software estimates the parameters using the method of maximum likelihood. Most of the data were supralinear (i.e., the magnitude of the reductions in lymphocyte count decreased with increasing unit dose) and not amenable to modeling with restricted models (in this case, with parameters constrained to be nonpositive), as suggested in EPA's draft *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b). Therefore, two different strategies were attempted. The first was to use unrestricted polynomial models and exclude the high-dose group when necessary to fit the models, and the second was to transform the dose data before modeling. Dropping the high-dose groups alone did not resolve the problem because there was still too much supralinear curvature in the two lower dose groups to fit restricted models. Results are summarized below in Tables 11 and 12, for untransformed and transformed data, respectively.

For each dataset, the selected model was chosen on the basis of the lowest Akaike's Information Criterion value with consideration of the graphical display, as suggested in the draft BMD technical guidance document. For selecting between models within a family of models (e.g., between a linear and a two-degree polynomial model), consideration was given to the log-likelihood values to evaluate the statistical significance of adding an extra parameter. In some cases there was no model that adequately fit the data. The draft technical guidance document suggests a *p*-value of 0.1 for model fit. In addition, there was substantial variability in these data, but the variability appeared to be random and not amenable to modeling. Therefore, constant variance was assumed for all the models, although in some cases the variances failed the test for homogeneity.

In the absence of a clear definition for an adverse effect for this endpoint, a default BMR of one standard deviation change from the control mean response was selected as suggested in the draft BMD technical guidance document. This definition of the BMR is highly sensitive to the substantial variability in data such as these; thus, the BMR itself is not very robust. The usefulness of this default definition would be strengthened by the use of a larger dataset of historical control data, but such data were not located. The software uses the estimated "constant" standard deviation as the standard deviation for all the group means. The 95% lower confidence limit (BMDL) on the resulting BMD was then calculated using the likelihood profile method.

Because many of the datasets could not be modeled using the available models without dropping the parameter constraints and sometimes even excluding the highest dose group (Table 11), a second modeling approach was tried in which the dose levels were transformed before the dose-response modeling was conducted, as was done for the human data modeled in Section 5.1.2. The dose levels were transformed according to the formula: transformed dose = ln(dose + 1). Results of the dose-response modeling using the transformed doses are presented in Table 12.

The results in Table 12 suggest that the male rat is more sensitive than the male mouse to lymphocyte count reductions from exposure to benzene in this NTP (1986) gavage bioassay, because the ranges of BMDs/BMDLs are substantially lower for the male rat, especially for year 2. The ranges for the male rat are also fairly tight, and the models selected provide good fits to all the male rat datasets. However, all but one of the calculated BMDs for the male rat are over an order of magnitude below the lowest exposure dose of 50 mg/kg. Ideally, BMDs should be closer to the low end of the range of observation (i.e., the range of the actual exposure doses) to reduce the impacts of model selection and the uncertainties inherent in extrapolating to lower doses.

Nevertheless, data from two drinking water studies (Hsieh et al., 1988b; White et al., 1984) provide support for selecting a BMD in this range. These two studies were of shorter duration and used fewer experimental animals than did the NTP (1986) study; however, they do provide dose-response data for BMD modeling and they also have the advantage of being drinking water studies; thus the benzene exposure scenario is more relevant to human oral benzene exposures. In one study, Hsieh et al. (1988b) exposed male CD-1 mice (five/group) to 0, 8, 40, or 180 mg/kg/day benzene in drinking water for 28 days. Hematologic effects were

Table 11. BMD modeling results of the NTP (1986) male mouse and male rat lymphocyte counts, with untransformed data

Dataset	Model	Variance homogeneity	Fit	BMD[a] (mg/kg)	BMDL[a] (mg/kg)
Male mouse					
6-month	unrestricted 2-degree polynomial	OK	yes p=0.13	20.52	12.96
9-month	unrestricted 2-degree polynomial	no	yes p=0.72	23.06	13.70
year 1 range				20.52–23.06	12.96–13.70
12-month	unrestricted 2-degree polynomial	OK	no[b] p=0.005	16.68[b]	11.41[b]
15-month	linear	no	yes, p=0.98	51.94	36.80
18-month	linear	no	yes, p=0.16	35.50	26.92
21-month	linear	no	yes, p=0.25	42.91	30.44
year 2 range				35.50–51.94	26.92–36.80
Male rat					
6-month	unrestricted 2-degree polynomial	OK	yes p=0.56	15.70	11.79
9-month	unrestricted 2-degree polynomial	no	yes p=0.37	16.88	12.72
year 1 range				15.70–16.88	11.79–1272
12-month; w/o high-dose group	unrestricted 2-degree polynomial	OK	yes[c]	10.51	7.3
15-month; w/o high-dose group	unrestricted 2-degree polynomial	OK	yes[c]	7.56	5.56
18-month; w/o high-dose group	unrestricted 2-degree polynomial	no	yes[c]	13.84	9.07
21-month; w/o high-dose group	unrestricted 2-degree polynomial	OK	yes[c]	7.21	5.03
year 2 range				7.21–13.84	5.03–9.07

[a]Unadjusted animal doses in mg/kg.
[b]These data could not be fit, and the BMD and BMDL are excluded from the reported ranges.
[c]Models were judged to fit on the basis of the graphical displays and χ^2-residuals; however, there were insufficient degrees of freedom for calculation of a p-value.

Table 12. BMD modeling results of the NTP (1986) male mouse and male rat lymphocyte counts, with transformed dose data

Dataset	Model	Variance homogeneity	Fit	BMD[a] (mg/kg)	BMDL[a] (mg/kg)
Male Mouse					
6-month	2-degree polynomial	OK	borderline p=0.047	19.68	6.57
9-month	linear	no	yes, p=0.35	9.07	4.05
year 1 range				9.07–19.68	4.05–6.57
12-month	linear	OK	yes, p=0.30	3.74	2.32
15-month	power	no	yes, p=0.31	47.46	18.55
18-month	power	no	borderline p=0.09	28.93	13.99
21-month	power	no	yes, p=0.15	23.34	5.80
year 2 range				3.74–47.46	2.32–18.55
Male rat					
6-month	power	OK	yes, p=0.30	9.92	4.52
9-month	linear	no	yes, p=0.11	3.71	2.30
year 1 range				3.71–9.92	2.30–4.52
12-month	linear	no	yes, p=0.22	1.34	0.95
15-month	linear	OK	yes, p=0.93	1.34	0.95
18-month	linear	no	yes, p=0.22	2.73	1.74
21-month	linear	OK	yes, p=0.54	1.69	1.10
year 2 range				1.34–2.73	0.95–1.74

[a]Unadjusted animal dose in mg/kg, after transforming the results back according to the formula: dose = exp(transformed dose) − 1.

observed at all exposure levels. BMD modeling of the ALC yielded a BMD of 2.2 mg/kg/day and a BMDL of 1.4 mg/kg/day, based on a linear model with transformed doses and a BMR of one standard deviation change from the control mean, as above. In the second study, White et al. (1984) exposed female B6C3F1 mice to 0, 12, 195, or 350 mg/kg/day benzene in drinking water for 30 days. BMD modeling of the ALC (five to six mice/group) resulted in a BMD of 11.6 mg/kg/day and a BMDL of 5.3 mg/kg/day (also based on a linear model with transformed doses and a BMR of one standard deviation change from the control mean, as above).

The results in Table 12 from BMD modeling of the male rat ALC data from the NTP (1986) study show the lowest BMDL of about 1 mg/kg at three time points in the second year; therefore, this was selected as the point of departure for an RfD calculation. Adjusting for

exposure 7 days/week yields a $BMDL_{ADJ}$ of 0.7 mg/kg/day. As discussed in previous subsections, a UF of 3 is used as an effect-level extrapolation factor, analogous to a LOAEL-to-NOAEL UF, because the BMD is considered to be an adverse-effect level. In addition, the standard UFs of 10 for interspecies extrapolation for oral studies and for intraspecies variability are used. Finally, a UF of 3 for database deficiencies is used, as in the previous derivations. These values yield an overall UF of 1000 and an RfD as follows:

$$RfD = BMDL_{ADJ} \div UF = 0.7 \text{ mg/kg/day} \div 1000 = 7 \times 10^{-4} \text{ mg/kg/day}$$

This RfD value is in reasonably good agreement (within an order of magnitude) with the RfD value of 4×10^{-3} mg/kg/day derived from the Rothman et al. (1996a) human inhalation study.

For comparison purposes, a chronic oral RfD can be derived from the LOAEL of 25 mg/kg identified for hematologic effects in the NTP (1986) study (there was no NOAEL). Adjusting from 5-day to 7-day exposure yields a $LOAEL_{ADJ}$ of 18 mg/kg/day, which can be used to calculate an RfD for benzene as follows:

$$RfD = LOAEL_{ADJ} \div UF = 18 \text{ mg/kg/day} \div 3000 = 6 \times 10^{-3} \text{ mg/kg/day},$$

where the combined UF of 3000 is made up of component factors of 10 for LOAEL-to-NOAEL extrapolation, 10 for interspecies extrapolation, 10 for intraspecies variability, and 3 for database deficiencies. This value is in good agreement with the RfD of 4×10^{-3} mg/kg/day calculated from the BMD analysis of the Rothman et al. (1996a) human data.

5.3. DOSE-RESPONSE SUMMARY

For the derivation of a chronic inhalation RfC, the human occupational inhalation study of Rothman et al. (1996a) was selected as the principal study, and the inhalation study of Ward et al. (1985) was selected as a supporting experimental animal study. These two studies provided the best dose-response data for quantitatively evaluating the potential human health risks due to inhalation of benzene. The cross-sectional study of Rothman et al. (1996a) was designated as the principal study because it is a human exposure study with well-quantified benzene exposure estimates and data on sensitive hematological endpoints. The study compared the hematologic evaluations of 44 workers occupationally exposed to benzene with those of 44 age- and gender-matched unexposed controls, all based in Shanghai, China. ALC, WBCs, RBCs, and platelets were all significantly decreased, whereas MCV was significantly increased in the group of exposed workers (median 8-hour TWA of 31 ppm [99 mg/m^3]), compared with the age- and sex-matched control group. In the low-dose subjects (< 31 ppm, median 8-hour TWA of 13.6 ppm [43.4 mg/m^3]), the ALC, RBCs, and platelet count were reduced as compared with controls. Similarly, in a selected subgroup exposed to a median 8-hour TWA of 7.6 ppm (23 mg/m^3) benzene, a statistically significant difference in ALC versus controls was observed ($p<0.03$). Furthermore, a dose-response relationship was established between the ALC and benzene exposure, as monitored by organic vapor passive dosimetry and the level of benzene metabolites in the urine. The median 8-hour TWA of 7.6 ppm was designated the LOAEL for these effects and was used to calculate a chronic inhalation RfC for benzene hematotoxicity in humans for comparison with the RfC derived in the primary analysis using BMD modeling of the ALC exposure-response data.

BMD modeling of the ALC exposure-response data was conducted using the continuous models from EPA's Benchmark Dose Modeling Software (version 1.20). In order to fit the data with one of the available models, the data were first transformed according to the equation $d' = \ln(d + 1)$. The exposure-response data were then fit using the continuous linear model, which provided a good fit. The two-degree polynomial model and the power model also provided adequate fits to the data; however, the linear model was selected because it was more parsimonious and the additional parameters were not statistically significant. In the absence of a clear definition for an adverse effect for this continuous endpoint, a default BMR of one standard deviation change from the control mean response was selected, as suggested in EPA's draft *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b). This default definition of a BMR for continuous endpoints corresponds to an excess risk of approximately 10% for the proportion of individuals below the 2nd percentile (or above the 98th percentile) of the control distribution for normally distributed effects. Transforming the resulting BMC and BMCL back to the original exposure scale yielded a BMC of 13.7 ppm (8-hour TWA) and a BMCL of 7.2 ppm (8-hour TWA).

As suggested in the draft BMD technical guidance document, the BMCL was chosen as the point of departure for the RfC derivation. After converting to mg/m^3 and adjusting for continuous exposure, a $BMCL_{ADJ}$ of 8.2 mg/m^3 was obtained. Dividing this value by an overall UF of 300 yields a **chronic inhalation RfC of 3×10^{-2} mg/m^3**, based on BMD modeling of the ALC data from the Rothman et al. (1996a) human study. Because the BMC is considered to be an adverse-effect level, an effect-level extrapolation factor analogous to the LOAEL-to-NOAEL UF was used. EPA is planning to develop guidance for applying an effect-level extrapolation factor to a BMD. In the interim, a factor of 3 was used in this analysis. For a more serious effect, a larger factor, such as 10, might be selected. Additional factors of 10 for intraspecies variability, 3 for subchronic-to-chronic extrapolation (exposure range 0.7 to 16 years), and 3 for database deficiencies, due to the absence of a two-generation reproductive/developmental toxicity study for benzene, comprise the remainder of the 300 composite UF. These UFs, as well as the UFs for the comparison analyses and the RfD calculations, are summarized in Table 13.

For comparison, an RfC was also calculated from the LOAEL of 7.6 ppm (8-hour TWA) from Rothman et al. (1996a). After converting to mg/m^3 and adjusting for continuous exposure, a $LOAEL_{ADJ}$ of 8.7 mg/m^3 was obtained. Dividing this value by an overall UF of 1000 yields an RfC of 9×10^{-3} mg/m^3. The UF of 1000 was based on a factor of 10 to account for the use of a LOAEL because of the lack of an appropriate NOAEL, a factor of 10 for intraspecies differences in response (human variability), a partial UF of 3 for subchronic-to-chronic extrapolation, and a factor of 3 for database deficiencies in the absence of a two-generation reproductive/ developmental toxicity study for benzene. This result of 9×10^{-3} mg/m^3 based on the LOAEL is in good agreement with the result of 3×10^{-2} mg/m^3 based on the BMCL. The BMD modeling approach is chosen as the primary analysis in this document because BMD modeling is a generally superior methodology that addresses some of the limitations of the LOAEL/NOAEL approach. For example, BMD modeling makes use of all the dose-response data, and the BMD is not restricted to being one of the doses used in the study. The BMD also provides a more

Table 13. Summary of uncertainty factors used for deriving the RfC and RfD[a]

Basis	BMCL or BMDL (NOAEL or LOAEL)[b]	Effect level extrapolation factor (LOAEL-to-NOAEL UF)	Interspecies UF	Intraspecies UF	Subchronic-to-chronic UF	Database deficiencies UF	Composite UF	RfD or RfC
RfC based on Rothman et al. (1996a) (human)	8.2 mg/m^3 (8.7 LOAEL)	3 (10)	NA	10	3	3	300 (1000)	0.03 mg/m^3 (0.009)
Comparison RfC based on Ward et al. (1985) (rodent)	48.5 mg/m^3 (17 NOAEL)	3 (NA)	3	10	3	3	1000 (300)	0.05 mg/m^3 (0.06)
RfD based on Rothman et al. (1996a) (human)	1.2 mg/kg/day[c] (1.2 LOAEL)	3 (10)	NA	10	3	3	300 (1000)	4×10^{-3} mg/kg/day (1×10^{-3})
Comparison RfD based on NTP (1986) (rodent)	0.7 mg/kg/day (18 LOAEL)	3 (10)	10	10	NA	3	1000 (3000)	7×10^{-4} mg/kg/day (6×10^{-3})

[a]Primary results are based on BMD analysis; comparison results using NOAEL/LOAEL approach are presented in parentheses where different.
[b]The BMC was based on a BMR of one standard deviation change from the control mean. All values are adjusted for continuous exposure.
[c]Oral BMDL (and LOAEL) for Rothman et al. (1996a) inhalation study was derived by route-to-route extrapolation with the assumptions that inhalation absorption was 50% and oral absorption was 100% in the dose range near the BMC.

NA = not applicable.

standardized point of comparison across endpoints and studies because it corresponds to a specific response level rather than being merely one of the doses used in a particular study.

Support for this chronic inhalation RfC was provided by the experimental animal study of Ward et al. (1985). The subchronic inhalation study of Ward et al. (1985) was selected as a supporting study because it used a relatively long inhalation exposure duration and an adequate number of animals; it also provided dose-response data. The study identified both a LOAEL of 300 ppm and a NOAEL of 30 ppm. Ward et al. exposed male and female CD-1 mice and Sprague-Dawley rats to 0, 1, 10, 30 or 300 ppm (0, 3.2, 32, 96 or 960 mg/m^3) benzene 6 hours/day, 5 days/week for 91 days and measured various hematologic endpoints. The male mouse appears to be the most sensitive sex/species in this study. The exposure-response relationships for the different hematologic endpoints in male mice were modeled using a BMD modeling approach, and decreased HCT was chosen as the critical effect.

BMD modeling was conducted using the continuous models from EPA's Benchmark Dose Modeling Software (version 1.20). The linear, polynomial, and power models all resulted in the same BMC and BMCL estimates; however, the linear model had better results for the fit statistics. Thus, the continuous linear model was selected. In the absence of a clear definition for an adverse effect for this continuous endpoint, a default BMR of one standard deviation for an adverse effect for this continuous endpoint, a default BMR of one standard deviation change from the control mean response was selected, as suggested in EPA's draft *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b). A BMC of 100.7 ppm and a BMCL of 85.0 ppm were obtained. It should be noted that because of the large dose spacing between the 30 ppm and the 300 ppm groups, there is some uncertainty about the actual shape of the dose-response curve in the region of the BMC and, thus, some corresponding uncertainty about the values of the BMC and BMCL estimates.

ALCs were not reported in Ward et al. (1985), so this endpoint could not be compared with the human ALC results. Total WBC counts were reported and exhibited the largest percent change in response between the control and the 300 ppm group; however, the data for this endpoint also had substantial variance. In addition, because the BMR used for this analysis is a function of the standard deviation, WBC counts did not yield the lowest BMC estimate. The actual lowest BMC estimates were obtained for increased mean cell Hgb (78 ppm; BMCL = 67 ppm) and increased mean cell volume (79 ppm; BMCL = 68 ppm); however, these endpoints are probably not adverse per se. On the other hand, they are likely to be compensatory effects—and thus markers of toxicity—and one could probably justify using them as the critical effects. In any event, the BMC estimates are not much different from the BMC of 100 ppm obtained for decreased HCT. The results are also similar for total blood Hgb (BMC = 104 ppm; BMCL = 88 ppm). RBC results were in between those for MCV and MCH and those for HCT and total Hgb; however, the model fits were not adequate for the RBC data. Thus, the RBC results have more uncertainty.

As suggested in the draft BMD technical guidance document, the BMCL was chosen as the point of departure for the RfC derivation. After converting to mg/m^3 and adjusting for continuous exposure, a $BMCL_{ADJ}$ of 48.5 mg/m^3 was obtained for the critical effect of decreased HCT. To derive the RfC, several UFs are applied to the $BMCL_{ADJ}$. As discussed above, a UF of 3 is used as an effect-level extrapolation factor, analogous to a LOAEL-to-NOAEL UF, because

the BMC is considered an adverse-effect level. In addition, the standard UFs of 3 for interspecies extrapolation for inhalation studies and of 10 for intraspecies variability are applied. A UF of 3 for database deficiencies is used due to the absence of a two-generation reproductive/developmental toxicity study for benzene. Finally, a partial UF of 3 was used to extrapolate from subchronic to chronic exposure. This partial value was selected on the basis of the observation that hematologic fluctuations such as reductions in RBCs and WBCs in the high-dose mice were noted at interim sacrifice (14 days) as well as at termination (91 days), suggesting that the responses occurred early in the exposure cycle and then remained comparatively unchanged. Dividing the $BMCL_{ADJ}$ by the overall UF of 1000 yields a chronic inhalation RfC of 5×10^{-2} mg/m^3. This value is in good agreement with the RfC of 3×10^{-2} mg/m^3, based on BMD modeling of the ALC data from the Rothman et al. (1996a) human study.

Similarly, for comparison purposes, a chronic inhalation RfC can be derived from the NOAEL of 30 ppm observed for hematologic effects in the Ward et al. (1985) experimental animal study. First, the NOAEL is converted to mg/m^3 and adjusted to equivalent continuous exposure, yielding 17.1 mg/m^3. UFs are identical to those employed above, except that no NOAEL-to-LOAEL UF is used; thus, the overall UF is 300. Dividing 17.1 by 300 results in an RfC of 6×10^{-2} mg/m^3. This value is also in good agreement with the RfC derived from the Rothman et al. (1996a) study.

For derivation of an RfD, the human occupational inhalation study of Rothman et al. (1996a) was again selected as the principal study, and the 103-week gavage study conducted by the NTP (1986) in F344 rats and B6C3F1 mice was selected as a supporting experimental animal study. These studies provided the best dose-response data for quantitatively evaluating the potential human health risks due to oral benzene exposure. The cross-sectional study of Rothman et al. (1996a) is designated as the principal study because it is a human exposure study, with well-quantified exposure estimates and data on sensitive hematological endpoints.

As with the RfC, the BMCL of 7.2 ppm (8-hour TWA) for the default BMR of one standard deviation change from the control mean response for the critical effect of reduced ALC in Rothman et al. (1996a) was used as the point of departure for the derivation of the RfD. After converting the units, correcting for continuous exposure, and adjusting for the route-to-route extrapolation from inhalation to oral exposure, a $BMCL_{ADJ}$-equivalent oral dose rate of 1.2 mg/kg/day was obtained. The route-to-route extrapolation assumes 50% absorption of inhaled doses and 100% absorption of oral doses. These assumptions were based on experimental data (Nomiyama and Nomiyama, 1974; Pekari et al., 1992; Srbova et al., 1950; Sabourin et al., 1987) and are the same as those used by EPA for route-to-route extrapolation of the inhalation cancer risk estimates to obtain cancer risk estimates for oral exposures (U.S. EPA, 1999a). Dividing the $BMCL_{ADJ}$-oral of 1.2 mg/kg/day by an overall UF of 300 yields a **chronic oral RfD of 4×10^{-3} mg/kg/day**, based on BMD modeling of the ALC data from the Rothman et al. (1996a) human study. Because the BMC is considered to be an adverse-effect level, an effect-level extrapolation factor analogous to the LOAEL-to-NOAEL UF was used. EPA is planning to develop guidance for applying an effect-level extrapolation factor to a BMD. In the interim, a factor of 3 was used in this analysis. Additional factors of 10 for intraspecies variability, 3 for subchronic-to-chronic extrapolation (exposure range 0.7 to 16 years), and 3 for database deficiencies due to the absence of a two-generation reproductive/developmental toxicity study for benzene comprise the remainder of the 300 composite UF.

For comparison, an RfD was also calculated from the LOAEL of 7.6 ppm (8-hour TWA) from Rothman et al. (1996a). After unit conversion, correction for continuous exposure, route-to-route extrapolation, and division by a combined UF of 1000, an RfD of 1×10^{-3} mg/kg/day was derived from the LOAEL. The route-to-route extrapolation was based on an assumption of 50% absorption of inhaled doses and 100% absorption of oral doses, as above. The combined UF of 1000 was based on a factor of 10 to account for using a LOAEL because of the lack of an appropriate NOAEL, a factor of 10 for intraspecies variability, a factor of 3 for subchronic-to-chronic extrapolation, and a factor of 3 for database deficiencies. This RfD value of 1×10^{-3} mg/kg/day is in good agreement with the value of 4×10^{-3} mg/kg/day calculated from the BMC.

Support for this chronic oral RfD was provided by the experimental animal study of the NTP (1986). In this study, F344 rats and B6C3F1 mice of both sexes were administered benzene by gavage 5 days/week for 103 weeks (see also Section 4.2.1.2). For rats, males (50/group) were administered doses of 0, 50, 100, or 200 mg/kg, and females (50/group) were administered doses of 0, 25, 50, or 100 mg/kg. B6C3F1 mice (50/sex/group) were administered doses of 0, 25, 50, or 100 mg/kg. Blood was drawn from 10 randomly preselected animals per species/sex/dose group at 12, 15, 18, and 21 months and from all animals at the terminal kill at 24 months. Additional groups of 10 animals of each sex and species were administered benzene for 51 weeks at the same doses of the 2-year study, and blood was drawn at 0, 3, 6, 9, and 12 months. This study identified a LOAEL of 25 mg/kg for leukopenia and lymphocytopenia in female F344 rats and male and female B6C3F1 mice and 50 mg/kg in male F344 rats. These were the lowest doses tested and, thus, no NOAEL was identified.

Reduction in lymphocyte count was selected as the critical effect, and attempts were made to model the dose-response relationships using a BMD modeling approach. The males of both species exhibited more dramatic and consistent reductions in lymphocyte count than did the females, but it was not clear a priori which species was more sensitive; therefore, dose-response analyses were performed on datasets for various time points for both the male mouse (NTP Table N8) and the male rat (NTP Table N4). Various continuous models were used to fit the different datasets, as appropriate, and one standard deviation change from the control mean was used as the BMR, as discussed above. The modeling results suggested that the male rat is more sensitive than the male mouse to lymphocyte count reductions from exposure to benzene in this NTP gavage bioassay. However, all but one of the calculated BMDs for the male rat were over an order of magnitude below the lowest exposure dose of 50 mg/kg. Ideally, BMDs should be closer to the low end of the range of observation (i.e., the range of the actual exposure doses) to reduce the impacts of model selection and the uncertainties inherent in extrapolating to lower doses. Nonetheless, BMD modeling of two subchronic drinking water studies (Hsieh et al., 1988b; White et al., 1984) supported the selection of a BMD in the range of BMDs estimated from the NTP (1986) rat data, and a BMDL of 1 mg/kg was selected as the point of departure.

Adjusting the BMDL of 1 mg/kg for exposure 7 days/week yielded a $BMDL_{ADJ}$ of 0.7 mg/kg/day. A UF of 3 was used as an effect-level extrapolation factor, analogous to a LOAEL-to-NOAEL UF, because the BMD is considered to be an adverse-effect level. Also, UFs of 10 for interspecies extrapolation for oral studies, 10 for intraspecies variability, and 3 for database deficiencies due to the absence of a two-generation reproductive/developmental study were applied, resulting in a composite UF of 1000. Dividing the $BMDL_{ADJ}$ by 1000 yields an RfD of 7×10^{-4} mg/kg/day. This RfD value is in reasonably good agreement (within an order of

magnitude) with the RfD value of 4×10^{-3} mg/kg/day derived from the Rothman et al. (1996a) human inhalation study.

For comparison purposes, a chronic oral RfD was also calculated using the LOAEL from the NTP (1986) study. The LOAEL of 25 mg/kg was adjusted to a continuous exposure level of 17.9 mg/kg/day and then divided by a UF of 3000 to derive an RfD of 6×10^{-3} mg/kg/day. The combined UF of 3000 is based on a factor of 10 for the absence of a NOAEL, a factor of 10 for interspecies extrapolation, a factor of 10 for intraspecies differences in response (human variability), and a factor of 3 for database deficiencies, as above. This value of 6×10^{-3} mg/kg/day is in good agreement with the value 4×10^{-3} mg/kg/day derived from the Rothman et al. (1996a) human study.

In summary, the chronic inhalation RfC values calculated on the basis of the human and the experimental animal data and using BMD modeling and NOAEL/LOAEL approaches are in good agreement, yielding values that range from 9×10^{-3} to 6×10^{-2} mg/m^3 (Table 14). This consistency in results provides increased confidence in the selected chronic inhalation RfC of 3×10^{-2} mg/m^3, which is based on BMD modeling of the Rothman et al. (1996a) human data.

Similarly, the chronic oral RfD values calculated on the basis of the human and the experimental animal data and using BMD modeling and NOAEL/LOAEL approaches are in generally good agreement, yielding values that range from 7×10^{-4} to 6×10^{-3} mg/kg/day. This consistency in results provides increased confidence in the selected chronic oral RfD of 4×10^{-3} mg/kg/day, which is based on BMD modeling of the Rothman et al. (1996a) human data.

The RfC and RfD values based on the Rothman et al. (1996a) human study were selected over the values derived from experimental animal studies because they are based on good-quality human data and therefore are not subject to the uncertainties inherent in interspecies extrapolation. Reference values based on BMD modeling were selected over those calculated using the NOAEL/LOAEL approach because BMD modeling is a superior methodology that makes better use of the exposure-response data. In any event, especially in the case of the RfC, all the estimates are in sufficiently good agreement as to be effectively indistinguishable. The overall confidence in this RfC and RfD assessment is medium.

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

Table 14. Summary of RfC and RfD estimates using human and experimental animal data, as well as BMD modeling and LOAEL/NOAEL approaches[a]

Approach	RfC (mg/m^3)		RfD (mg/kg/day)	
	Human	Rodent	Human	Rodent
BMD modeling	**3×10^{-2}**	5×10^{-2}	**4×10^{-3}**	7×10^{-4}
LOAEL/NOAEL	9×10^{-3}	6×10^{-2}	1×10^{-3}	6×10^{-3}

[a]Selected values are in bold.

biological impacts of benzene exposure have been extensively studied in humans and in experimental animal models. Metabolism of benzene is necessary for the compound's toxic effects to develop. 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 genotoxicity and 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 with the greatest sensitivity. 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). Other target tissues are also characterized by high peroxidase activity (Low et al., 1995). 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, is bone marrow depression, which leads to lymphocytopenia, leukocytopenia, thrombocytopenia, anemia, and aplastic anemia (Aksoy, 1991; Goldstein, 1988; Dosemeci et al., 1996). The most sensitive effect observed in humans is the depression of ALC in peripheral blood (Rothman et al., 1996a). In test animal studies, the most sensitive effects observed are 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. The regulation of hematopoiesis is a dynamic process in which stem and progenitor cells, in conjuction with bone marrow stroma, give rise to mature blood cells. The survival and proliferation of these cells are controlled by multiple growth factors or cytokines that regulate hematopoiesis. Several studies in mice and human bone marrow cultures have shown that benzene alters cytokine production or response to cytokines (Dempster and Snyder, 1990; Farris et al., 1993; Cronkite et al., 1989; Irons et al., 1992; Irons and Stillman, 1993, 1996; Rothman et al., 1996b)

Although a large number of human and experimental animal studies have been conducted, there are few human studies with reliable estimates of exposure to benzene and few long-term, repeated-dose experiments in test animals. Human studies frequently are also complicated by exposure to other solvents. The long-term test animal studies have used 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. The use of BMD modeling obviates the need for NOAELs; however, good-quality exposure-response data from the low end of the observable response range are still required to reliably estimate BMDs.

Another area of scientific uncertainty in this assessment concerns the neurotoxic effects of benzene. As is the case 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 of the compound. 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 workday), tired easily, had difficulties sleeping, and complained of memory loss. Overall, 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 for calculating the risk to humans, 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 by 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 caused changes in colony-forming hematopoietic cells. These studies were considered supporting, because the LOAEL of 5 ppm is below the LOAEL of 7.6 ppm established for hematotoxic and immunotoxic effects observed in humans (Rothman et al., 1996a). 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). Furthermore, the responses did not establish consistent patterns in different ages of the progeny. Also, a limited number of animals were examined. These limitations make this study less useful because of the high degree of uncertainty that these are truly adverse effects. (See section 4.2.2.2.)

6.2. DOSE RESPONSE

6.2.1. Inhalation RfC

Quantitative estimates of human health risk as a result of low-level chronic exposure to benzene via the inhalation route are based on data from human occupational inhalation exposure and from a subchronic inhalation study in experimental animals. Hematotoxicity and immunotoxicity are the critical effects observed in both humans and test animals.

The air concentration of benzene considered to be without any appreciable risk with lifetime chronic human exposure (the RfC) is 3×10^{-2} mg/m^3. This value was obtained by applying a UF of 300 to the $BMCL_{ADJ}$ obtained from BMR modeling of the exposure-response data for reductions in ALC from the Rothman et al. (1996a) occupational study, using a BMR level of one standard deviation change from the control mean response. This RfC is in good agreement with the value of 9×10^{-3} mg/m^3 based on the LOAEL in the Rothman et al. (1996a) study, with a UF of 1000.

The RfC of 3×10^{-2} mg/m^3 is similarly in good agreement with the value of 5×10^{-2} mg/m^3 obtained by applying a UF of 1000 to the $BMCL_{ADJ}$ estimated from BMD modeling of the exposure-response data for the hematologic effect of decreased HCT in male mice from the Ward et al. (1985) subchronic inhalation study, using a BMR level of one standard deviation change from the control mean response. Finally, both of these values are in good agreement with the value of 6×10^{-2} mg/m^3 based on the NOAEL in the Ward et al. (1985) study, with a UF of 300.

The overall confidence in this RfC assessment is medium. The Rothman et al. (1996a) and Ward et al. (1985) studies were both well conducted, and various methodologies for deriving the RfC yielded similar results for the two studies. Furthermore, the availability of good-quality human data for a sensitive endpoint eliminates the uncertainty associated with basing the RfC on experimental animal data. However, with continuous endpoints such as the hematologic parameters measured in these studies, there is uncertainty about when a change in a parameter that has inherent variability becomes an adverse effect. Other uncertainties explicitly recognized in the quantitative derivation include intraspecies variability, that is, the need to accommodate sensitive human subgroups; the extrapolation of subchronic results to a lifetime exposure scenario; and database deficiencies.

6.2.2. Oral RfD

Quantitative estimates of human health risk as a result of low-level chronic exposure to benzene via the oral route are based on data from human occupational inhalation exposure and from a chronic gavage study in experimental animals. Once again, hematotoxic responses are the critical effects observed.

The human chronic dose of ingested benzene considered to be without any appreciable risk (the RfD) is 4×10^{-3} mg/kg/day. This value was obtained by applying a UF of 300 to the oral equivalent dose extrapolated from the $BMCL_{ADJ}$ obtained from BMD modeling of the ALC data from the human occupational study of Rothman et al. (1996a) using a BMR level of one

standard deviation change from the control mean response, as for the RfC discussed above. This RfD is in good agreement with the value of 1×10^{-3} mg/kg/day based on the oral equivalent LOAEL from the Rothman et al. (1996a) study, with a UF of 1000.

This RfD of 4×10^{-3} mg/kg/day is also in good agreement with the values of 7×10^{-4} mg/kg/day and 6×10^{-3} mg/kg/day, which are 1/1000 of the $BMDL_{ADJ}$ derived from the male rat ALC data in the NTP (1986) chronic gavage study and 1/3000 of the LOAEL from the NTP (1986) study, respectively.

The overall confidence in this RfD assessment is medium. The Rothman et al. (1996a) study was well conducted, and the availability of good-quality human data for a sensitive endpoint eliminates the uncertainty associated with basing the RfD on experimental animal data. However, with continuous endpoints such as hematologic parameters, there is uncertainty about when a change in a parameter that has inherent variability becomes an adverse effect. Other uncertainties explicitly recognized in the quantitative derivation include intraspecies variability, to accommodate sensitive human subgroups; the extrapolation of subchronic results to a lifetime exposure scenario; and database deficiencies.

A further uncertainty in the RfD ensues from the use of route-to-route extrapolation to estimate oral equivalent doses from inhalation exposures resulting from analysis of the Rothman et al. (1996a) occupational data. In experiments conducted to compare the metabolite doses to the target organ following oral or inhalation exposure, Sabourin et al. (1987, 1989) 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, route-to-route extrapolation is justified and introduces a lower degree of uncertainty than extrapolating from test animals to humans (U.S. EPA, 1999a). Use of a modifying factor of 3 to account for uncertainty in the route-to-route extrapolation was considered; however, it was deemed unnecessary. The RfD is based on human data for a sensitive endpoint; thus, it was felt that the composite UF of 300 provides sufficient protection.

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