Public Health Goal for BENZENE In Drinking Water

Prepared by

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PREFACE

Drinking Water Public Health Goals

Pesticide and Environmental Toxicology Section

Office of Environmental Health Hazard Assessment

California Environmental Protection Agency

This Public Health Goal (PHG) technical support document provides information on health effects from contaminants in drinking water. PHGs are developed for chemical contaminants based on the best available toxicological data in the scientific literature. These documents and the analyses contained in them provide estimates of the levels of contaminants in drinking water that would pose no significant health risk to individuals consuming the water on a daily basis over a lifetime.

The California Safe Drinking Water Act of 1996 (amended Health and Safety Code, Section 116365), amended 1999, requires the Office of Environmental Health Hazard Assessment (OEHHA) to perform risk assessments and publish PHGs for contaminants in drinking water based exclusively on public health considerations. Section 116365 specifies that the PHG is to be based exclusively on public health considerations without regard to cost impacts. The Act requires that PHGs be set in accordance with the following criteria:

1. PHGs for acutely toxic substances shall be set at levels at which no known or anticipated adverse effects on health will occur, with an adequate margin of safety.
2. PHGs for carcinogens or other substances which can cause chronic disease shall be based upon currently available data and shall be set at levels which OEHHA has determined do not pose any significant risk to health.
3. To the extent the information is available, OEHHA shall consider possible synergistic effects resulting from exposure to two or more contaminants.
4. OEHHA shall consider the existence of groups in the population that are more susceptible to adverse effects of the contaminants than a normal healthy adult.
5. OEHHA shall consider the contaminant exposure and body burden levels that alter physiological function or structure in a manner that may significantly increase the risk of illness.
6. In cases of insufficient data to determine a level of no anticipated risk, OEHHA shall set the PHG at a level that is protective of public health with an adequate margin of safety.
7. In cases where scientific evidence demonstrates that a safe dose-response threshold for a contaminant exists, then the PHG should be set at that threshold.
8. The PHG may be set at zero if necessary to satisfy the requirements listed above.
9. OEHHA shall consider exposure to contaminants in media other than drinking water, including food and air and the resulting body burden.
10. PHGs published by OEHHA shall be reviewed every five years and revised as necessary based on the availability of new scientific data.

PHGs published by OEHHA are for use by the California Department of Health Services (DHS) in establishing primary drinking water standards (State Maximum Contaminant Levels, or MCLs). Whereas PHGs are to be based solely on scientific and public health considerations without regard to economic cost considerations, drinking water standards adopted by DHS are to consider economic factors and technical feasibility. Each standard adopted shall be set at a level that is as close as feasible to the corresponding PHG, placing emphasis on the protection of public health. PHGs established by OEHHA are not regulatory in nature and represent only non-mandatory goals. By federal law, MCLs established by DHS must be at least as stringent as the federal MCL if one exists.

PHG documents are used to provide technical assistance to DHS, and they are also informative reference materials for federal, state and local public health officials and the public. While the PHGs are calculated for single chemicals only, they may, if the information is available, address hazards associated with the interactions of contaminants in mixtures. Further, PHGs are derived for drinking water only and are not to be utilized as target levels for the contamination of other environmental media.

Additional information on PHGs can be obtained at the OEHHA Web site at www.oehha.ca.gov.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PUBLIC HEALTH GOAL FOR BENZENE IN DRINKING WATER

SUMMARY

Benzene has long been known to cause cancer, as observed in both animal experimental studies and in human epidemiology investigations. In animals, benzene has been shown to be carcinogenic by different routes of administration, including inhalation, oral intubation and subcutaneous injection. In rodents, increased incidences of tumors have been observed at multiple sites, including Zymbal gland, mammary gland, ovary, uterus, nasal cavity, oral cavity, skin, Harderian gland, preputial gland, liver, and lung. Leukemias and lymphomas have been observed in some inhalation and subcutaneous injection studies of rats and mice employing high exposures, and malignant lymphomas have been observed in oral (gavage) studies. In humans, the tumors most commonly observed to be increased after benzene exposures are various types of leukemia. Benzene is classified as a known human carcinogen by the United States Environmental Protection Agency (U.S. EPA, 1999) and by the International Agency for Research on Cancer (IARC, 1982). It is also listed as "known to be a human carcinogen" by the National Toxicology Program (NTP, 1998) and as a carcinogen by the State of California under Proposition 65 (OEHHA, 1999b).

A Public Health Goal (PHG) was therefore developed for benzene in drinking water based on cancer risk from leukemias. The PHG is established as 0.00015 mg/L (0.15 ppb) of benzene in drinking water, based on a *de minimis* estimated excess individual cancer risk level of 10^{-6} from lifetime exposure. The cancer potency estimate is consistent with observations of leukemia among U.S. rubber hydrochloride workers exposed to benzene (Pliofilm Cohort) (Rinsky et al., 1987; Paxton et al., 1994) and benzene-exposed workers from various industries in China (Chinese Worker Cohort) (Hayes et al., 1997). Cancer potencies were estimated from the cohort data using Poisson regression and linear relative risk models. Cancer potency estimates for the general population from constant exposure to benzene were calculated using life table analyses. The pattern of changing leukemia risk following exposure to benzene appears consistent with that observed for radiation and chemotherapeutic agents: excess relative risk increases significantly within 10 to 15 years and then declines towards background levels in subsequent years. This concept of changing leukemia risk over time was incorporated into the analyses. Information on differences in uptake of benzene by different routes of exposure was used to scale the inhalation-based estimates from the worker studies to those expected from exposures via drinking water. Studies in human volunteers, workers, and animals suggest that humans retain 50 percent of inhaled benzene and absorb 100 percent of ingested benzene. Use of benzene-contaminated tap water in homes would result in exposures equivalent to an intake of 4.7 L, which accounts for direct ingestion (2 L), dermal exposure (1 L), and inhalation of benzene (1.7 L) transferred to indoor air (e.g., from showering).

The best upper-bound estimates of leukemia risk resulting from continuous lifetime air exposures of the general population to benzene were similar for the U.S. rubber workers (0.044 ppm^{-1}), the Chinese workers (0.056 ppm^{-1}), and for the mean of the two studies combined (0.050 ppm^{-1}). The three lifetime risk estimates all convert to a population-based cancer potency of 0.1 $(mg/kg\text{-}d)^{-1}$ for oral exposures after rounding, which can be scaled to 0.05 $(mg/kg\text{-}d)^{-1}$ for inhalation exposures. Due to the shape of the dose-response curves and other considerations, the best estimates were calculated from workers in the lowest exposure groups only. However, the range of mean and upper bound estimates within the scientific band of uncertainty would include those based on the use of the absolute risk model and the use of U.S. EPA proposed methodology (see

below). Thus, population adjusted cancer potency estimates for total leukemia ranged from 0.19 to 0.012 $(mg/kg\text{-}d)^{-1}$ (oral) which correspond to potential PHG values of 0.00008 mg/L (0.08 ppb) to 0.0012 mg/L (1.2 ppb), respectively. Cancer potency estimates for other cancer endpoints, such as acute non-lymphocytic leukemia, generally fell within this range.

The cancer potency estimates described above were consistent with estimates from (1) other epidemiological studies of benzene-exposed workers, (2) animal bioassays, and (3) epidemiological studies of leukemia from cigarette smoking (which results in exposure to benzene). This provides strong support for the PHG.

This assessment reviewed the scientific evidence for associations of benzene and various human cancers. The strongest association is for acute non-lymphocytic leukemia. Evidence suggests that benzene causes other forms of leukemia as well. Thus, total leukemia (e.g., all subtypes of leukemia as a related class of diseases) is found to be the most appropriate for the basis of risk assessment. Benzene exposure also may be associated with non-Hodgkin's lymphoma and multiple myeloma, but causal associations have not been established. If the basis for the risk assessment had included non-Hodgkin's lymphoma, the cancer potency for benzene would be increased by about 30 percent.

The evidence regarding the carcinogenic mode of action of benzene was also reviewed. Benzene and its metabolites potentially induce or affect leukemogenesis through multiple modes of action including genetic mechanisms involving direct and indirect DNA damage, and epigenetic mechanisms affecting bone marrow cell proliferation, differentiation and clonal selection. It is likely that benzene induces leukemia through multiple mechanisms. The available evidence also suggests varying susceptibility of humans to the toxic effects of benzene, for example due to genetic variability in the key enzymes involved in benzene metabolism as well as numerous dietary and environmental factors. Inter-individual variability, uncertainty in the analyses, and uncertainty in our understanding of benzene-induced hematotoxic effects are discussed.

A health protective concentration for benzene in drinking water (0.026 mg/L) was also derived for non-cancer endpoints. This value was based on no observed adverse hematological effects in a cohort of U.S. refinery workers exposed to an average concentration of 0.53 ppm benzene in air (Tsai et al., 1983). The average air concentration was used to estimate average daily intake expected from constant exposure of the general population of 0.087 mg/kg-d, by applying standard conversion factors and absorption efficiencies for oral and inhalation of benzene as described above. Additionally, this value was adjusted using an adjustment factor of ten to account for inter-individual variability, a relative source contribution (RSC) of 0.2, and a daily water consumption-equivalent factor of 4.7 L.

The PHG of 0.00015 mg/L developed in this assessment is similar to the previous health-based toxicity value of 0.00018 mg/L for exposures via multiple pathways resulting from benzene in drinking water (DHS, 1987). The current California drinking water standard, the maximum contaminant level (MCL), is 0.001 mg/L and the current federal MCL is 0.005 mg/L, which are both based in part on technical feasibility.

INTRODUCTION

The purpose of this document is to develop a PHG for benzene. The PHG describes concentrations of contaminants at which adverse health effects are not expected to occur, even over a lifetime of exposure. The California Department of Health Services (DHS) uses this health-based information, along with other criteria, such as economic and technical feasibility, to set a MCL, which serves as the State's drinking water standard.

The current California MCL of 0.001 mg/L was established by DHS in 1989. The MCL was selected based on economic and technical feasibility as well as health considerations. Before the advent of the PHG, DHS produced similar health-based evaluations and estimated health-based levels called Proposed Maximum Contaminant Levels (PMCLs). A PMCL was developed for benzene in support of the MCL determination (DHS, 1987). A range of PMCLs was estimated for leukemia risk in humans and from animal bioassays utilizing six different risk assessment models. The inhalation lifetime risk estimates ranged from 1×10^{-2}/ppm to 1×10^{-1}/ppm, which represent the upper-bound estimates of excess cancers arising from constant lifetime exposure of the general population. Corresponding PMCLs were calculated to range from 0.00016 mg/L to 0.00163 mg/L for two L daily water consumption and from 0.00005 mg/L to 0.0046 mg/L for seven L equivalent daily water consumption (which takes into account exposures from bathing, showing and other water uses). "Best judgment" estimates based on the geometric mean from two epidemiological studies were 0.00063 mg/L for two L daily consumption and 0.00018 mg/L for seven L daily consumption equivalent.

The California MCL (0.001 mg/L) is lower (more stringent) than the current federal MCL of 0.005 mg/L for benzene, last revised in 1987. U.S. EPA considered economic and technical feasibility criteria along with health concerns in selecting the federal MCL. More specifically, U.S. EPA set the MCL based on detection limits of benzene as measured by specific U.S. EPA analytical methods (US EPA, 1987). The U.S. EPA Maximum Contaminant Level Goal (MCLG) which is the federal equivalent of the PHG is, as a matter of policy, set to zero mg/L for carcinogens.

Under the Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65), benzene was listed as a chemical known to the state to cause cancer in 1987 and as a reproductive and developmental toxicant in 1997. In 1988, the Office of Environmental Health Hazard Assessment (OEHHA) evaluated the carcinogenic risks of exposure to benzene and recommended a potency value of 0.1 $(mg/kg\text{-}d)^{-1}$ and a risk specific intake level of 7 µg/day associated with a 10^{-5} risk (DHS, 1988). The International Agency for Research on Cancer (IARC) has classified benzene as Group 1, known to be carcinogenic to humans (IARC, 1987). Benzene is also listed in the National Toxicology Program's (NTP) Eighth Report on Carcinogens as a compound known to be a human carcinogen (NTP, 1998). U.S. EPA classifies benzene as a Group A, human carcinogen (U.S. EPA, 1999).

In this document, the available data on the toxicity of benzene was evaluated and included information available since the previous assessments by ARB (1984) and DHS (1987). No long-term studies of humans exposed to benzene via ingestion were available. Of the available information, data from occupational cancer studies in which benzene was inhaled by workers were utilized preferentially over ingestion studies in animals for several reasons. First, data from the human inhalation studies are of good quality and using human data reduces the level of uncertainty associated with the estimation of the cancer potency since there is no need for interspecies extrapolation. Second, for typical uses of tap (drinking) water in the home, inhalation exposures to benzene resulting from volatilization from showering and washing were estimated to be equal in magnitude to those expected from ingestion. Third, benzene absorbed either through ingestion or inhalation appears to be metabolized and distributed within the body in an equivalent manner. Fourth, cancer potency estimates derived from animal studies of orally administered benzene were essentially the same as potency estimates derived from the occupational cohort studies (Table 29). To determine a public health-protective level of benzene in drinking water, sensitive groups were identified and considered, and relevant studies were identified, reviewed and evaluated.

CHEMICAL PROFILE

Chemical Identity

Benzene is an unsubstituted, six-carbon aromatic ring. The chemical formula, structure, synonyms and identification numbers are listed in Table 1.

Physical and Chemical Properties

Important physical and chemical properties of benzene are given in Table 2.

Production and Uses

Benzene is one of the top 20 production chemicals in the U.S. (ATSDR, 1997). A reported 12.32 billion pounds was produced in the U.S. in 1993 (HSDB, 1997). Greater than 98 percent of the benzene in the U.S. is produced from petroleum, primarily by companies in the petroleum and petrochemical industries (ATSDR, 1997). Benzene is manufactured by catalytic cracking of petroleum, aromatic ring formation by hydrogenating straight-chain paraffins, or transalkylation of toluene (HSDB, 1997).

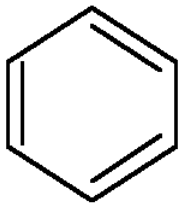
Benzene has many industrial uses. Roughly 55 percent of the manufactured benzene is used to produce ethylbenzene, an intermediate in the synthesis of styrene which is used to make plastics and elastomers (ATSDR, 1997). About 24 percent of the benzene is converted to cumene to produce phenol for the manufacture of phenolic resins, and to acetone, a common solvent. Roughly 12 percent of the benzene is converted to cyclohexane primarily for the production of nylon resins (ATSDR, 1997). The balance of the manufactured benzene is utilized to synthesize other organic chemicals, detergents, pesticides, and solvents (OHM/TADS, 1997).

Gasoline is also a major source of benzene; past formulations of gasoline contained about one to two percent benzene (ARB, 1997; HSDB, 1997; OHM/TADS, 1997). In California, current formulations of gasoline are required to contain no more than one percent benzene by volume.

Sources

The major sources of benzene come from petrochemical production and industrial use of benzene and through the widespread use of gasoline. However, there are additional sources of benzene in the environment. Benzene is formed as a minor combustion product of cigarette and wood smoke, and is generated by volcanoes and forest fires (IPCS, 1993; HSDB, 1997). Improper handling or storage of benzene, such as leaking underground storage tanks or landfills, can result in contamination of drinking water or outgassing to nearby airsheds (IPCS, 1993). Benzene also occurs naturally as a component of petroleum (IPCS, 1993).

Table 1. Chemical Identity of Benzene (adapted from IPCS, 1993; ATSDR, 1997)

Chemical name	Benzene
Synonyms	Annulene, benzeen (Dutch), benzen (Polish) benzine; benzol, benzole, benzolo (Italian), bicarburet of hydrogen, coal naphtha, cyclohexatriene, fenzen (Czech), mineral naphtha, motor benzol, NCI-C55276, phene, phenyl hydride, pyrobenzol, pyrobenzole
Registered trade name	Polystream
Chemical formula	C_6H_6
Chemical structure	

Identification numbers

Chemical Abstracts Service (CAS) Registry No.:	71-43-2
NIOSH Registry of Toxic Effects of Chemical Substances (RTECS)® No.:	CY1400000
U.S. EPA Hazardous Waste No.:	U019 (in commercial product) F005 (non-specified source, spent solvent)
Oil and Hazardous Materials/Technical Assistance Data System (OHM/TADS) No.:	7216601
Hazardous Substances Data Bank (HSDB) No.:	2554
National Cancer Institute (NCI) No.:	C55276

Table 2. Physical and Chemical Properties of Benzene

Property	Value	References
Molecular weight	78.11 g/mol	HSDB (1997)
Color	Clear, colorless	HSDB (1997)
Physical state	Liquid at ambient temperatures	HSDB (1997)
Odor	Aromatic	HSDB (1997)
Odor threshold		
Lower	0.84 ppm	OHM/TADS (1997)
Upper53 ppm	OHM/TADS (1997)	
Taste threshold	0.5 to 4.5 mg/L	HSDB (1997)
Melting point	5.5^0C	HSDB (1997)
Boiling point	80.1^0C (at 760 mm Hg)	HSDB (1997)
Flash point	-11^0C (closed cup)	HSDB (1997)
Flammability limits (air)	1.3 % (lower limit)	OHM/TADS (1997)
	7.1 % (upper limit)	OHM/TADS (1997)
Autoignition temperature	580^0C	OHM/TADS (1997)
Solubility		
Water	1800 mg/L (25^0C)	HSDB (1997)
Organic solvents	Soluble in alcohol, chloroform, ether, carbon disulfide, acetone, oils, carbon tetrachloride, glacial acetic acid	HSDB (1997)
Density/specific gravity	0.8787 g/mL (15^0C)	HSDB (1997)
Partition coefficients		
Log octanol-water (Log K_{ow})	2.13, 2.15	HSDB (1997)
Log soil-organic carbon-water (Log K_{oc})	1.8 to 1.9	HSDB (1997); ATSDR (1997)
Vapor pressure	95.2 mm Hg (25^0C)	OHM/TADS (1997)
	40 mm Hg (7^0C)	OHM/TADS (1997)
Henry's law constant (25^0C)	5.5 X 10^{-3} atm-m^3/mol	ATSDR (1997)
Conversion factors	1 ppm = 3.19 mg/m^3 (25^0C)	Calculated[1]

[1] 1.0 μg/g or ppm = (1 parts/10^6 parts) (78.11 mg/mmol) (24.45 L/mol, molar gas constant at 25^0C)$^{-1}$ (1000 mmol/mol) (1000 L/m^3) = 3.19 mg/m^3

ENVIRONMENTAL OCCURRENCE AND HUMAN EXPOSURE

Air

Due to the extensive production and use of benzene and benzene-containing products such as gasoline, benzene is a ubiquitous air contaminant. U.S. EPA Total Exposure Assessment Methodology studies of benzene found that more than 99 percent of personal exposures of the general population were through air sources (Wallace, 1996). The Cal/EPA Air Resources Board routinely monitors ambient air concentrations of benzene throughout California through its air toxics network. In 1982, when the monitoring program began, the estimated population-weighted annual concentration of benzene was roughly five ppb (1.6 $\mu g/m^3$) (ARB, 1984). Concentrations have declined steadily over time such that in 1994 average estimates across the state were approximately 1.2 ppb (0.376 $\mu g/m^3$) (ARB, 1995). Moreover, since the recent statewide use of oxygenated gasoline, the ambient air concentrations have dropped even lower. Based on a typical respiration rate of 24 m^3/day, an air concentration of 1.0 ppb (0.313 $\mu g/m^3$) would correspond to an intake of 7.5 μg benzene per day.

Other than urban air, exposures to the general population from benzene occur primarily from automobile-related activities and cigarette use. The average smoker (32 cigarettes/day with an average tar content) inhales approximately 1.8 mg of benzene per day. This is approximately ten times the daily intake of a non-smoker (Wallace, 1989, 1996). Exposure to environmental tobacco smoke can also result in measurable increases in benzene intake (Wallace, 1989; Thomas et al., 1993). Automobile-related activities can result in benzene exposures from evaporation of gasoline and from tailpipe emissions. A task force team from U.S. EPA related increases in benzene exposures to driving times, filling gas tanks, and indoor air of homes with attached garages (Wallace, 1989). Concentrations of benzene in indoor air (stemming from sources such as cigarette and wood smoke, heating or cooking systems, and vaporization of gasoline in attached garages) were found to be about 4-fold to 11-fold higher than surrounding outdoor air (Sheldon et al., 1991; Thomas et al., 1993). In homes with attached garages and environmental tobacco smoke, benzene concentrations ranged from 8 to 31 $\mu g/m^3$. Concentrations in garages ranged from 3 to 196 $\mu g/m^3$ (Thomas et al., 1993).

Occupational exposures to benzene are widespread since benzene is used or produced in large quantities by a wide variety of industries, including petroleum refining, and chemical and rubber manufacturing. The reader is directed to the section below on Toxicological Effects in Humans for descriptions of exposure estimates in different industries.

Soil

Benzene contamination of soil is generally a result of spillage or leakage of gasoline or other benzene-containing petroleum products or wastes, such as leaking underground storage tanks. Human exposure from direct contact or ingestion of soil is, in general, not a primary concern since benzene volatilizes rapidly from soil (IPCS, 1993).

Benzene levels in the soil surrounding industrial facilities that produced or used benzene were measured to be <2 ppb to 191 ppb (U.S. EPA, 1979a; IARC, 1982).

Water

Benzene has been detected in surface water at levels as high as 7 μg/L in polluted areas, tap water ranging from <0.1 to 0.3 μg/L, groundwater ranging from 0.005 to 300 μg/L, and waste water ranging from <1 to 179 μg/L (IPCS, 1993; ATSDR, 1997). Of the 41,742 sites sampled from 1992 to 1994 from 11 states including California, 0.63 percent had detectable levels of benzene and only 0.11 percent were above the federal MCL (U.S. EPA, 1997). Page et al. (1993) surveyed bottled drinking water sold in Canada for benzene and other volatile organic compounds. Benzene was detected in only one of 182 samples at a concentration of 2 ppm.

U.S. EPA estimated that between 100,000 to 400,000 of the three to five million underground storage tanks in the U.S. have been leaking at some point in their lifetime. These statistics, coupled with the fact that roughly 50 percent of the U.S. population depends on groundwater for its drinking water, result in significant potential for human exposure (Lindstrom et al., 1994).

Exposure estimates from benzene-contaminated tap water

In addition to direct ingestion of drinking water, estimates of human exposure to contaminants in tap water must take into account potential exposures from dermal contact during showering or bathing and from inhalation of volatile compounds which are transferred from water to household air (e.g., showering). Total exposure estimates from two studies (Lindstrom et al., 1994; Beavers et al., 1996) in which benzene was present in the tap water are presented in Table 3. In addition to these empirical studies, CalTOX, a multimedia total exposure program, was used to estimate exposure from different pathways resulting from benzene-contaminated tap water (DTSC, 1999).

Lindstrom et al. (1994) conducted a study in North Carolina of exposures to benzene in a home whose water source (groundwater) was contaminated with gasoline (292 μg benzene/L). A primary focus of the study was to determine the exposures expected from inhalation (e.g., benzene stripped into the air during showering). A combination of peak grab samples and personal monitors were used to measure exposures. On three consecutive days, benzene concentrations in air were measured in and near the shower stall and throughout the house, during and following 20-minute showers. Bathroom air concentrations of 0.11 to 0.16 ppm benzene were measured, with shower stall concentrations ranging 0.24 to 0.52 ppm. Concentrations in the rest of the house were highest approximately 0.5 to 1 hour following showering and ranged from 0.012 to 0.045 ppm.

Table 3 provides dose estimates that stem from different pathways of exposure. Estimates of the oral daily dose assumed ingestion of 2.0 L/day of tap water. Daily intake from inhalation was estimated from the measured concentrations and assumed a single 20-minute shower per day and 24-hour occupancy of the house. Lindstrom et al. noted that the experimental showering time was higher than the typical showering time (~ 10 min), which would overestimate the inhalation dose for a typical individual. However, they also noted that a typical household would be expected to have multiple users and multiple daily showers. Seventy percent retention of inhaled benzene was assumed, whereas 50 percent appears to be a more accurate value (see Appendix C); thus, values for inhalation were adjusted accordingly (Table 3). The dermal dose was calculated assuming exposure to 75 percent of a skin surface area of 20,900 cm^2 for 0.33 hr with a dermal permeability constant of 0.11 cm/hr. The Department of Toxic Substances Control uses a dermal permeability constant for benzene of 0.19 cm/hr in its CalTOX program (DTSC, 1999). This value represents the mean of two studies, one that was used by Lindstrom et al. (1994). Dermal exposure estimates (Table 3) were recalculated using the CalTOX exposure factor.

Beavers et al. (1996) assessed household exposure to benzene and other hydrocarbons from gasoline-contaminated drinking water in a New England home. On two days, five weeks apart, personal air samples were taken on a test subject to estimate breathing zone concentrations while showering, bathroom concentrations after showering, and personal air concentrations over a four- to five-hour period following showering. Because the subject was a smoker, additional samples were taken to account for benzene stemming from environmental tobacco smoke. Concentrations of benzene in the tap water were 16-fold higher on the first sampling day (414 μg/L) compared with the second (25.4 μg/L). Median personal air concentrations during showering were 1.04 ppm and 0.033 ppm, for day one and day two, respectively. Median air concentrations of personal air over a five-hour period following showering were 0.021 ppm and 0.001 ppm, respectively for day one and day two.

Table 3 shows the estimated inhalation, dermal and ingestion doses received on day one of exposure (Beavers et al., 1996). These estimates assumed 20 m^3 daily inhaled volume, 50 percent absorption of inhaled benzene, 0.5 hr shower per day, 15.5 hr of daily non-shower activity in the home, ingestion of two L of tap water per day and 100 percent absorption of ingested benzene. Dermal intake was assumed to be equivalent to ingestion intake, which the authors suggested was possible under "maximum" conditions. Dermal absorption estimates were recalculated using methods employed by Lindstrom et al. (1994) and the CalTOX dermal permeability factor (DTSC, 1999), but retaining the Beaver et al. selection of 30 min as the showering time (Table 3). The 30-minute showering time likely represents an upper-end estimate (DTSC, 1999); thus, the 6.2 L-equivalents estimated from the Beavers et al. (1996) study is also likely to represent an upper-end estimate.

Table 3. Estimates of dose of benzene received by an individual from household use of gasoline-contaminated tap water

	Dermal	Ingested	Inhaled	Total
Lindstrom et al. (1994)				
Daily intake (as reported)	160 μg (11 %)	584 μg (41 %)	697 μg (48 %)	1441 μg
Daily intake (adjusted)[a]	276 μg (20 %)	584 μg (43 %)	498 μg (37 %)	1358 μg
L (equivalents)	1.0	2.0	1.7	4.7
Beavers et al. (1996)				
Daily intake (as reported)	828 μg (30 %)	828 μg (30 %)	1126 μg (40 %)	2782 μg
Daily intake (adjusted)[b]	632 μg (24 %)	828 μg (32 %)	1126 μg (44 %)	2586 μg
L (equivalents)	1.5	2.0	2.7	6.2
CalTOX (DTSC, 1999)[c]				
Daily intake	0.49 μg (16 %)	1.36 μg (43 %)	1.28 μg (41 %)	3.13 μg
L (equivalents)	0.7	2.0	1.9	4.6

[a] Adjusted from 70 percent retention to 50 percent retention of inhaled benzene, and for dermal permeability.
[b] "Maximum" dermal intake estimates reported by Beavers et al. (1996) were recalculated using methods employed by Lindstrom et al. (1994) using the CalTOX exposure factor.
[c] Assumes tap water concentration of 1.0 μg/L.

The CalTOX program (DTSC, 1999) can be used to generate dose estimates for a variety of exposure scenarios. Distributions of values pertaining to average residential housing features such as room volume, ventilation and water use; behavior factors such as water intake and bathing time; physiological parameters such as body weight, surface area, breathing rate; and chemical specific factors such as molecular weight and partition coefficients are built into the model, but may be modified by the user. CalTOX assumes complete absorption by the ingestion and inhalation routes, whereas dermal dose is computed according to a formula that accounts for a benzene-specific skin-water partition coefficient and skin penetration rate. Using default assumptions for the residential and physiological parameters for an average Californian, a tap water concentration of 1.0 μg/L benzene, and correcting the respired dose to reflect 50 percent absorption by this route (See Appendix C), CalTOX predicts that 16 percent, 43 percent, and 41 percent of total dose is derived from the dermal, ingestion, and inhalation routes, respectively. These percentages result in a daily intake of 4.6 L-equivalents of tap water, if 2.0 L/day are ingested. The relative dose fractions obtained for water at 1.0 μg/L were unchanged when the higher tap water concentrations measured by Beavers et al. (1996) and Lindstrom et al. (1994) were substituted (Table 3). The CalTOX estimates are in good agreement with the findings based on empirical measurements. Possible sources for the differences include the longer showering times used in the Beavers et al. (1996) and Lindstrom et al. (1994) studies.
If bathing time is increased in CalTOX to 20 minutes, the estimated daily intake increases to 5.0 L-equivalents. In addition, CalTOX uses a mass transfer efficiency of benzene from shower water to air of 0.77; Lindstrom et al. (1994) measured an average value of 0.88 (range 0.73-0.99).

Food

Although data are limited, early studies reported benzene in a variety of foods and drinks. Some of the highest reported levels have been in Jamaican rum (120 μg/L), irradiated beef (19 μg/kg), heat-treated canned beef (2 μg/kg), eggs (500 to 1900 μg/kg), oysters (220 to 260 μg/kg), and fish (3 to 88 μg/kg) (IPCS, 1993). Recent studies of benzene levels in food found negligible quantities in nearly all food measured; levels were all below 40 ppb, most less than 2 ppb (Wallace, 1996).

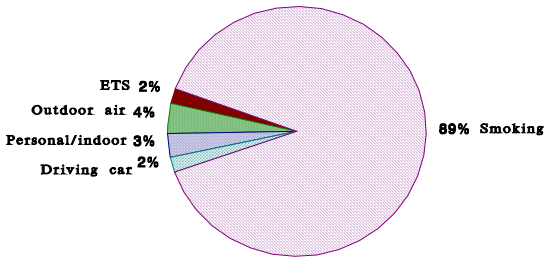
Other Sources of Exposure

Benzene is a byproduct of cigarette smoke and wood smoke. Residues of benzene were measured in numerous chemical products, including paints, primers, paint strippers, lubricants, and model and hobby glues (Rastogi et al., 1993).

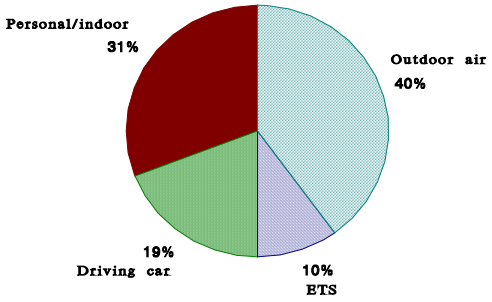
Wallace (1996) provided an updated review of environmental exposure to benzene. New studies conducted in Europe confirmed the original findings from the U.S. EPA TEAM studies regarding the primary sources of benzene exposure experienced by the general population (Figure 1). On average smokers take in roughly 2.0 mg of benzene per day compared with approximately 0.2 mg per day for a typical non-smoker. Smoking is by far the greatest contributor of benzene exposure by smokers, accounting for approximately 89 percent of the daily benzene exposure. Among non-smokers, personal/indoor outdoor air concentrations are major sources accounting for 31 percent and 40 percent, respectively. Driving in cars and environmental tobacco smoke (ETS) were other significant sources for non-smokers, 19 percent and 10 percent, respectively. Food,

beverages, and drinking water were generally not significant contributors to a person's overall exposure to benzene in the general population.

Figure 1. Sources of Benzene Exposure for Smokers and Non-smokers (from Wallace, 1996)



Smokers



Non-smokers

Biomarkers of Exposure

Biomarkers of exposure, which provide a measure of internal or "biologically-effective" dose, have been developed or employed for studies of benzene toxicity. These include benzene in exhaled breath or excreted unchanged in the urine, and urinary phenol, catechol, hydroquinone, 1,2,4-trihydroxybenzene, muconic acid and phenylmercapturic acid. Additionally, macromolecular adducts with DNA or proteins have been investigated including phenylguanine, and benzoquinone and benzene oxide adducts with blood proteins. Due to a high background of phenolic compounds stemming from dietary and endogenous sources, biomarkers of phenol, hydroquinone or adducts of benzoquinone are not sufficiently specific for benzene, especially at lower doses (McDonald et al., 1993; ATSDR, 1997; also see section on Inter-individual Variability to Benzene Hematotoxicity, page 71). Biomarkers of benzene or benzene oxide (e.g., phenylmercapturic acid or albumin adducts of benzene oxide) may prove to have greater utility as biomarkers of exposure in future molecular epidemiological studies (Yeowell-O'Connell et al., 1998).

Recently, Zhang et al. (1998) observed significant increases in the rates of monosomy of chromosomes five and seven ($p<0.001$, $p<0.0001$, respectively) as well as trisomy and tetrasomy frequencies of these chromosomes in the blood of benzene-exposed workers (Zhang et al., 1998). Loss of long (q) arm deletions and aneusomy of chromosomes five and seven are the most common cytogenetic changes in therapy- and chemical-induced leukemia; thus, aberrations in chromosomes five and seven may be useful biomarkers of exposure or early effect.

METABOLISM AND PHARMACOKINETICS

Absorption

Absorption of benzene via oral and inhalation exposures is extensively reviewed in Appendix C. Absorption efficiencies depend on dose. A greater proportion of benzene is retained at lower exposures via inhalation or oral routes. Humans exposed experimentally to low to moderate air concentrations of benzene (approximately 1.7 to 32 ppm, Appendix C, Table 3) absorbed 50 percent of the benzene inhaled. These observations are consistent with animal inhalation studies using low concentrations of benzene (11 to 29 ppm, Appendix C, Table 2). Based on animal studies, benzene is completely absorbed by the oral route at lower concentrations, and complete absorption via the oral route for humans is expected as well. Benzene is absorbed via dermal exposure, dependent on skin permeability and other factors.

Metabolism

There is strong evidence that metabolism plays a critical role in benzene toxicity (Snyder and Hedli, 1996). For example, competitive inhibition of metabolism by toluene decreases benzene toxicity. Rodents given a partial hepatectomy (Sammett et al., 1979) or mice lacking the CYP2E1 gene (Valentine et al., 1996) had decreased metabolism of benzene and, correspondingly, decreased toxicity. There is no indication that the route of administration has a marked effect on the metabolites formed (IPCS, 1993).

The metabolism of benzene is complex (Figure 2) and has been extensively reviewed elsewhere (ATSDR, 1997; Snyder and Hedli, 1996; Snyder et al., 1993; IPCS, 1993; Subramanyam et al., 1991). To briefly summarize, benzene is metabolized primarily in the liver by cytochrome P450

2E1 and to a lesser degree by other P450 isozymes to form benzene oxide (or its oxepin) which spontaneously rearranges to phenol. Valentine et al. (1996) confirmed the central role of P450 2E1 by demonstrating that transgenic mice lacking CYP2E1 expression had decreased benzene metabolism, cytotoxicity, or genotoxicity compared to wild type mice. Phenol may also be formed by hydroxylation of benzene by hydroxyl radicals generated from hydrogen peroxide. Phenol is subsequently oxidized by the mixed function oxidases to hydroquinone. Hydroquinone, in turn, can go through an additional oxidation step to form 1,2,4-trihydroxybenzene. Benzene oxide may also be acted upon by epoxide hydrolase in the liver to form benzene-1,2-dihydrodiol which is converted to catechol by dehydrogenases. Catechol is formed by oxidation of phenol, but this pathway may only be a significant pathway at high doses. Hydroquinone, catechol and 1,2,4-trihydroxybenzene can be further oxidized either spontaneously or enzymatically, such as by myeloperoxidase, to form 1,4-, 1,2- or 1,2,4-benzoquinone (or corresponding semiquinones), respectively. Alternately, a fraction of the benzene oxide is oxidized to a ring-open product, *trans,trans*-muconaldehyde, and subsequently, to muconic acid.

Conjugation of benzene metabolites occurs primarily through sulfation, glucuronidation and linkage to glutathione (Snyder and Hedli, 1996; IPCS, 1993). Benzene oxide can bind covalently to glutathione leading to the formation of phenylmercapturic acid. Phenol, catechol, hydroquinone and 1,2,4-trihydroxybenzene are readily conjugated through sulfation and glucuronidation. The relative contribution of these pathways are highly dependent on dose and species (Henderson, 1996).

Henderson (1996) recently 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 rats and primates. This finding may explain why mice are more sensitive to benzene than are rats. In all species, a greater proportion of benzene is converted to hydroquinone and ring-open metabolites at low doses than at high doses.

Recently 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, 10 or 100 mg benzene/kg body weight, in male B6C3F$_1$ mice at oral doses of 0.1 and 100 mg/kg, and in male LVG 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 percent of the dose was recovered in the urine within 48 hours and a small percentage (about three percent) was recovered in the breath. At higher doses, 10 and 100 mg/kg, the percentage eliminated in the breath increased to about nine percent and 50 percent, 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 percent), mice (~5 percent), and hamsters (~7 percent). However, the percentage of hydroquinone and related conjugates ranged from about three percent at the highest dose to as much as seven percent at the lowest doses. A higher percentage of hydroquinone metabolites was seen in mice (~ 30 percent) and in hamsters (~30 percent), but the percentage did not appear to be dose-dependent.

A concern central to benzene carcinogenesis is the conversion of benzene to reactive metabolites capable of damaging DNA or altering other critical targets either directly or indirectly. The number of potential reactive metabolites of benzene is large (Table 4). They include various epoxides, quinones, aldehydes, and free radical species (Snyder and Hedli, 1996; Witz et al.,

1996; Subramanyam et al., 1991). The relative importance and contribution that these metabolites play in the carcinogenic process is not well understood.

Figure 2. Benzene Metabolism

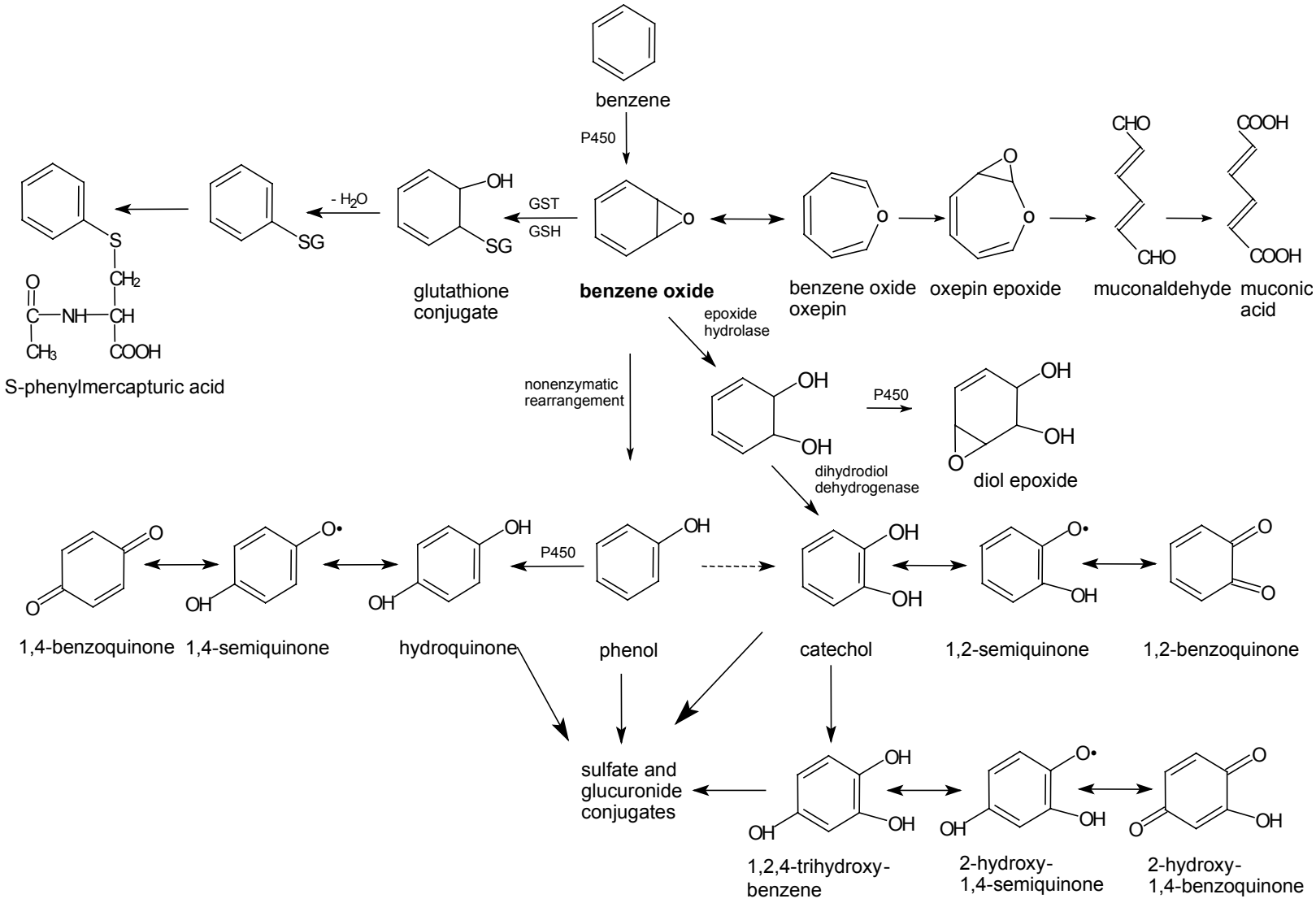


Table 4. Reactive Compounds Associated with Benzene Metabolism

Benzene oxide (or oxepin)
1,4-Benzoquinone (or semiquinone)
1,2-Benzoquinone (or semiquinone)
Hydroxy-1,4-benzoquinone (or semiquinone)
Glutathione conjugated quinones
Oxygen free radicals
trans-*trans*-Muconaldehyde
6-Hydroxy-*trans*-*trans*-2,4-hexadienal
Lipid peroxidation products
Hydroquinone epoxide
Catechol epoxide
Benzene dihydrodiol epoxide
Glutathione free radicals
4,4'-Dibenzophenone (or semiquinone)
2,2'-Biphenol semiquinone
Polymeric coupling products

Lindstrom et al. (1997) measured benzene oxide in the blood of rats administered at a dose of 400 mg benzene/kg. The half-life of benzene oxide in blood was estimated to be 7.9 minute, a sufficient time to be transported to all tissues. Lovern et al. (1997) demonstrated that benzene oxide is produced by incubation of one mM benzene with liver microsomes from humans, rats and mice. McDonald et al. (1994) measured dose-related increases in benzene oxide adducts with bone marrow proteins following oral administration of benzene to rats and mice.

A leading theory of benzene-induced hematotoxicity and carcinogenesis involves the metabolism of phenolic metabolites of benzene, particularly the metabolism of hydroquinone to benzoquinone, semiquinones, and free radical species (Smith and Fanning, 1997; Smith,1996a; Snyder and Kalf, 1994). The phenolic metabolites of benzene namely, phenol, hydroquinone, catechol and 1,2,4-trihydroxybenzene, are transported to the bone marrow via the blood where peroxidases and other enzymes can convert them to reactive species. For example, hydroquinone may be oxidized to 1,4-benzoquinone (or semiquinone) by myeloperoxidase in the marrow. The redox cycling that accompanies these reactions generates oxygen free radicals, lipid peroxidation products, and other free radicals (Subramanyam et al., 1991). The bone marrow contains approximately three percent dry weight of myeloperoxidase as well as other peroxidases such as eosinophil peroxidase and prostaglandin synthetase (Smith, 1996a). An enzyme that efficiently reduces (detoxifies) quinones is NQO1 (NADPH-dependent quinone oxidoreductase). Bone marrow cells contain low concentrations of NQO1 relative to other tissues, and may explain in part why the bone marrow is a target tissue for benzene toxicity (Snyder and Kalf, 1994). Glutathione conjugates of hydroquinone and 1,2,4-benzenetriol readily autooxidize to quinone species which may react with cellular macromolecules directly or generate free radical species (Snyder and Hedli, 1996).

Some researchers have hypothesized that metabolites of benzene in which the aromatic ring has been broken may significantly contribute to benzene hematotoxicity (reviewed in Witz et al., 1996). *Trans,trans*-muconaldehyde has been shown to be toxic to bone marrow in a manner similar to that of benzene, and co-administration with other metabolites, especially hydroquinone, is very potent in damaging bone marrow cells. Another reactive, open-ring metabolite, namely 6-

hydroxy-*trans*-*trans*-2,4-hexadienal, has also been implicated in causing mutations and hematotoxicity.

Distribution

Benzene is rapidly distributed via the blood throughout the body. Owing to its lipophillicity, benzene reaches higher concentrations in lipid rich tissues, such as bone marrow and fat, than other tissues (ATSDR, 1997). Autopsies of individuals who died from inhalation exposure to benzene showed benzene in the blood, brain, liver, kidney, stomach, bile, abdominal fat, and urine (ATSDR, 1997). Benzene has also been found in maternal and umbilical cord blood (OEHHA, 1997a). Rickert et al. (1979) exposed F344 rats to benzene at 500 ppm for six hours and observed wide distribution of benzene throughout the body. Steady state concentrations of benzene in the bone marrow and fat were considerably higher than other tissues tested. Estimates of tissue half-lives of elimination were rapid, less than one hour for the blood, bone marrow, liver, lung, kidney, spleen, and brain.

The metabolites of benzene also appear to be widely distributed (IPCS, 1993; Henderson et al., 1992). Sabourin et al. (1988) examined the distribution of benzene metabolites in F344 rats and $B6C3F_1$ mice exposed by inhalation to 50 ppm tritiated benzene for six hours. Labeled phenol and hydroquinone were found in liver, lung, and blood of mice. Labeled catechol was found in the liver of mice, but was below detection limits in the lung or blood. Labeled phenol, hydroquinone, and catechol were below detection limits in the liver, lung, and blood of rats. It should be noted that high background concentrations of the phenolic metabolites of benzene, stemming from endogenous and environmental sources, were also shown to be distributed in both the blood and bone marrow of mice (Bechtold et al., 1996).

The distribution of potentially reactive metabolites of benzene has also been studied. For example, benzene oxide was measured in the blood of F344 rats given an oral dose of benzene (400 mg/kg) (Lindstrom et al., 1997). The half-life of benzene oxide in blood was estimated to be about eight minutes, which is stable enough to be distributed to all tissues. The distribution of benzene metabolites also can be inferred from the presence of benzene-derived adducts in various tissues. Rats and mice administered labeled benzene were found to have labeled benzoquinone and benzene oxide protein adducts in both the blood and bone marrow 24 hours later (Bechtold et al., 1992a,b; McDonald et al., 1993, 1994). Benzene oxide adducts of hemoglobin and albumin have been observed in humans occupationally exposed to benzene (Bechtold et al., 1992b; Yoewell-O'Connell et al., 1998).

Excretion

Benzene and its metabolites are eliminated rapidly, with most eliminated in hours. The main route of elimination for unmodified benzene is by exhaled air. The fraction eliminated by exhaled air increases with higher exposures (from any route of exposure), due to saturation of metabolic pathways. Benzene metabolites are excreted primarily via the urine. The metabolites found in the highest concentrations in the urine were phenol, catechol, hydroquinone, benzenetriol, and muconic acid (either as free metabolite or sulfate and glucuronide conjugates). Phenyl sulfate and phenylmercapturic acid were also excreted to a significant degree. A small proportion of the dose is excreted in the feces (Matthews et al., 1998; ATSDR, 1997; IPCS, 1993).

In humans, benzene excretion is multiphasic (ATSDR, 1997). In one study, a human was exposed to benzene at 31 ppm for eight hours, and elimination in breath followed for 125 hours.

A four component exponential model was fit to the data, with half-lives of 19 minutes, 1.8 hours, 4.2 hours, and 27 hours for each compartment. Further experiments on the same individual using different exposure conditions found similar half-lives (Sherwood, 1988). In three humans who had inhaled 25 ppm benzene and 100 ppm toluene for two hours, approximately 90-95 percent of the benzene was eliminated from blood in 300 minutes. Elimination was fit to a three component exponential model with rate constants corresponding to half-lives of 1.7, 25, and 219 minutes.

In rats and mice exposed to 100 or 300 ppm benzene for six hours per day, five days per week, for 20 exposures, elimination of benzene from the blood was monitored after the 1st, 6th, and 20th exposures. Elimination could be fitted to a one component exponential model for all exposures except for mice after 20 exposures, which required a two-component model. The rate constants corresponded to half-lives of 15.4-16.3 minutes for mice at 100 ppm, 21.1-37.5 minutes for mice at 300 ppm, 51-100 minutes for rats at 100 ppm, and 128-154 minutes for rats at 300 ppm. Thus, mice eliminated benzene from the blood more rapidly than rats. Elimination from both species was slower at the higher exposure level (Snyder et al., 1981).

In rats, following exposure to 500 ppm for six hours, the elimination of benzene from various tissues was rapid. The half-lives ranged from 0.4 hours for lung and kidney to 1.6 hours for fat, with blood intermediate at 0.7 hours (Rickert et al., 1979). In rats and mice exposed to 50 ppm benzene for six hours, the elimination of several metabolites (muconic acid, hydroquinone glucuronide and phenyl sulfate) from blood, liver, and lung occurred with half-lives of less than two hours (Sabourin et al., 1988).

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

Physiologically-based Pharmacokinetic (PBPK) Models

Several investigators have applied mathematical modeling to benzene metabolism and distribution (reviewed in ATSDR, 1997). Medinsky et al. (1989) developed a model using data from benzene-exposed rodents. The model predicts levels of hydroquinone produced from a range of exposures that correlate well with observations of bone marrow toxicity in mice. Bois et al. (1996) developed a model of benzene distribution and excretion of phenol in humans. Employing a population-based approach and Bayesian numerical techniques, the model parameters were statistically derived to fit data from a human exposure chamber study. However, this model has not been tested for its ability to predict data from other studies (Smith and Fanning, 1997).

The Bois et al. (1996) model predicts a linear relationship between benzene exposure (up to ten ppm) and the fraction of benzene metabolized in the bone marrow. The studies by Medinsky et al. (1989, 1996) suggest that the fractions of total metabolites represented by phenol and hydroquinone will vary with exposure. The Medinsky et al. (1989) model predicts that the internal dose of hydroquinone will increase in a supralinear fashion for benzene concentrations of 100 ppm (i.e., the trend in hydroquinone levels falls below a linear trend with dose). The authors suggest that the non-linearity may be due to competition between liver cytochrome P450 2E1 and conjugation enzymes.

Extensive variability in metabolism of benzene has been shown in a number of studies (see section on Inter-individual Variability and Benzene Hematotoxicity). Also, there are also

significant species differences in metabolism (Henderson et al., 1996). More work is needed to validate these PBPK models before the production and distribution of individual benzene metabolites can be accurately predicted for exposures in a heterogeneous human population (Smith and Fanning, 1997).

TOXICOLOGY

Toxicological Effects in Animals

Acute Toxicity

Exposure of animals to single, high doses of benzene resulted in narcotic effects and death. Oral LD_{50} values for rats ranged from 300 to 8100 mg/. Short-term exposure to air concentrations of approximately 10,000 ppm benzene in air or higher were lethal to 50 percent or more (LC_{50}) to rats, mice, rabbits and guinea pigs (Paustenbach et al., 1993; IPCS, 1993).

Subchronic and Chronic Toxicity

Moderate and long-term exposure of animals to benzene resulted in numerous adverse effects including bone marrow damage, changes in circulating blood cells, developmental and reproductive effects, alterations of the immune response, and cancer. The responses among animals were variable and depended on numerous factors, including species, strain, sex, dose, and exposure duration and pattern. Carcinogenicity, immunotoxicity, and developmental and reproductive effects are described in separate sections devoted to those topics. This section will address endpoints not covered elsewhere, primarily non-cancer hematotoxicity (e.g., alterations in specific blood cells and bone marrow cell depression).

Studies reporting non-cancer hematological changes following subchronic and chronic exposures to benzene in animals are summarized in Table 5, and descriptions of selected studies are provided in the text below. These studies taken together clearly demonstrate benzene's potential to damage the lymphoid and hematopoietic systems.

Wolf et al. (1956) studied the effects of repeated exposure to benzene in rabbits (80 ppm, 175 total exposures), rats (88 ppm, 136 total exposures) and guinea pigs (88 ppm, 193 total exposures). The observed effects included leukopenia, increased spleen weight, and histological changes to bone marrow. Wolf et al. (1956) also reported dose-related hematological changes in female rats administered repeated oral doses, 0 (olive oil), 1, 10, 50 and 100 mg benzene/kg-d, for about 26 weeks. No effects were seen at 1 mg/kg-d, slight leukopenia was observed at 10 mg/kg-d, and clear leukopenia and erythrocytopenia were observed at 50 and 100 mg/kg-d.

Gill et al. (1980) demonstrated that intermittent exposure of male C57 Bl/6 mice to 4000 ppm benzene produced leukopenia without altering bone marrow cellularity; however, the number of colony forming units (CFU-S) were decreased by 30 percent compared to controls. Snyder et al. (1978) exposed Sprague-Dawley rats and AKR/J mice to 300 ppm benzene, six hours per day, five days per week for life. Lymphocytopenia, anemia and decreased survival were observed in both species. Cronkite et al. (1982) exposed male mice to 400 ppm benzene, six hours per day, five days per week for 9 ½ weeks. Depressed bone marrow cellularity, decreased stem cell count, and altered morphology in spleen colony-forming cells were observed in the mice.

Toft et al. (1982) observed reduced numbers of nucleated marrow cells per tibia and numbers of colony-forming units of granulopoietic cells (CFU-C) in mice exposed continuously to 21 ppm benzene for four to ten days. Intermittent exposure (21 ppm benzene, eight hours per day, five days per week, for two weeks) reduced the number of CFU-C only. Similarly, Cronkite et al. (1985) exposed C57B/6 BNL mice via inhalation to air concentrations of 10, 25, 100, 300 or 400 ppm benzene for two to 16 weeks. No adverse effects were observed for the 10 and 25 ppm dose groups. However, in the higher dose groups, significant reductions in bone marrow cellularity and number of pluripotent stem cells were reported.

Baarson et al. (1984) observed a progressive depression in the colony-forming ability of erythroid progenitor cells (CFU-E) in mice exposed to ten ppm benzene, six hours per day, five days per week. Burst-forming cell growth was depressed by 55 percent in exposed animals relative to controls after 66 days of exposure, but returned to control growth levels by 178 days.

Ward et al. (1985) exposed 50 Sprague-Dawley rats and 150 CD-1 mice of both sexes to air concentrations of 0, 1, 10, 30, or 300 ppm benzene, six hours per day, five days per week for 13 weeks. Thirty mice and ten rats of each sex were sacrificed after 7, 14, 28, 56 and 91 days of treatment. No hematological changes were found for rats or mice at doses of 1, 10 or 30 ppm. However, at 300 ppm male and female mice exhibited significant increases ($p<0.05$) in mean cell volume and mean cell hemoglobin values and decreases in hematocrit, hemoglobin, lymphocyte percentages, and decreases in red cell, leukocyte and platelet counts. These changes were first observed after 14 or 28 days of exposure, and in males only persisted to the end of the study. Histological changes in mice included myeloid hypoplasia of the bone marrow, splenic periarteriolar lymphoid sheath depletion, lymphoid depletion in the mesenteric lymph node, increased extramedullary hematopoiesis in the spleen, and plasma cell infiltration of the mandibular lymph node. Histological changes were present at early time points and persisted throughout the study. Effects were less severe in rats. At 300 ppm rats exhibited significant decreases ($p<0.05$) in leukocyte counts in males (day 14 only) and females (day 91 only) and decreases in the percentage of lymphocytes in male and females (day 14 through day 91). The only benzene-related histological change in rats was a slight decrease in femoral marrow cellularity at the 300 ppm dose.

Li et al. (1986) exposed female Wistar rats to benzene at air concentrations of 0, 20, 50, 100, 300, 1000 or 3000 ppm for seven days to study the effect on blood alkaline phosphatase levels. Serum alkaline phosphatase levels were not affected at any dose. Leukocyte levels of the enzyme were unaffected at the 20 and 50 ppm levels but showed a dose-related increase between 100 and 300 ppm. No further increase was seen at the 1000 or 3000 ppm levels. As enzyme levels increased, leukocyte levels correspondingly decreased, suggesting a linkage between enzyme level and leukopenia.

NTP (1986) administered benzene to F344 rats and B6C3F_1 mice by gavage in corn oil at 0, 25, 50, 100, 200, 400 and 600 mg/kg, five days per week for 17 weeks. Rats exhibited dose-related leukopenia at all doses, while mice exhibited leukopenia in the 400 and 600 mg/kg dose groups. In long-term studies in which rats and mice were administered 0, 25, 50, 100 mg/kg (females) or 0, 50, 100, 200 mg/kg (males) five days per week for 103 weeks, dose-related lymphocytopenia and leukocytopenia were observed in both species. In addition, mice exhibited lymphoid depletion of the thymus and spleen, and hyperplasia of the bone marrow.

Table 5. Non-cancer Hematological Changes in Animals Following Inhalation and Oral Subchronic and Chronic Exposures to Benzene

Study	Species	Dose[1]	Adverse Effects
Inhalation			
Wolf et al. (1956)	Rats, guinea pigs, rabbits	80 to 88 ppm repeated exposures	Leukopenia, erythrocytopenia, increased spleen weight, and histological changes to bone marrow
Snyder et al. (1978)	Rats, mice	300 ppm, 6 h/d, 5 d/wk, life	Lymphocytopenia, anemia, decreased survival
Gill et al. (1980)	Mice	4000 ppm, intermittent	Leukopenia, decreased CFU-S
Green et al. (1981)	Mice	103, 306, 603, 1276, 2416, 4862 ppm 6h/d, 5d/wk, 50d	Reduced femoral and splenic cellularity
Toft et al. (1982)	Mice	21 ppm, 4 to 10 days or 21 ppm, 8 h/d, 5 d/wk, 2 wk	Reduced CFU-C and nucleated marrow cells per tibia
Cronkite (1982)	Mice	400 ppm, 6 h/d, 5 d/wk for up to 9.5 wk	Depressed bone marrow cellularity and stem cell count, altered morphology in spleen colony-forming cells
Baarson et al. (1984)	Mice	10 ppm, 6 h/d, 5 d/wk, 178d	Depressed CFU-E
Snyder et al. (1984)	Rats	100 ppm, 6 h/d, 5 d/wk, life	Depressed peripheral erythrocyte and lymphocyte counts, morphological changes
Cronkite (1985)	Mice	100, 300, 1000 ppm, 2 to 16 wk	Depressed bone marrow cellularity and stem cell count
Ward et al. (1985)	Rats, mice	300 ppm, 6h/d, 5d/wk, 13 wk	Multiple changes to circulating blood cells and marrow morphology
Li et al. (1986)	Rats	100, 300, 1000, 3000 ppm, 7 d	Leukopenia, increased leukocyte alkaline phosphatase
Aoyama (1986)	Mice	200 ppm 6 h/d, 7 d or 50 ppm for 14 d	Leukopenia

[1] Doses that exhibited at least one of the listed adverse effects are shown in the table.

Table 5 (continued). Non-cancer Hematological Changes in Animals Following Inhalation and Oral Subchronic and Chronic Exposures to Benzene

Study	Species	Dose[1]	Adverse Effects
Keller and Snyder (1988)	Mice	*in utero*, 5, 10, 20 ppm (dose to dams), day 6 to day 15 of gestation.	Reduced circulating erythroid precursor cells, increased blast and precursor cells
Cronkite et al. (1989)	Mice	25, 100, 300, 400 ppm 6 h/d, 5 d/wk for up to 16 wk	Decreased blood lymphocytes, marrow cellularity, CFU-S
Luke et al. (1988a,b) Tice et al. (1989)	Mice	300 ppm 6 h/d for 13 wk at (1) 5 d/wk or (2) 3 d/wk	Depression of erythropoiesis
Dempster and Snyder (1990, 1991)	Mice	10 ppm, 6 h/d, 5 d	Reduced CFU-E per tibia, CFU-E, CFU-E, GM-CFU-C
Chertkov et al. (1992)	Mice	300 ppm 6 h/d, 5 d/wk, 2 wk	Decreased marrow cellularity, hematocrit, leukocytes
Neun et al. (1992)	Mice	300 ppm	Reduced marrow cellularity CFU-E
Necas et al. (1992)	Mice	300 ppm 6 h/d, 5 d/wk up to 6 wk	Reduced CFU-E, 59Fe incorporation in red cell
Farris et al. (1993)	Mice	300 ppm, 6 h/d,5 d/wk,16wk	Granulocytic hyperplasia
Plappert et al. (1994), Seidel et al. (1989a,b)	Mice	100, 300, 900 ppm, 6 h/d, 5 d/wk up to 8 wk	Anemia, altered CFU-S CFU-C, BFU-E, CFU-E
Abraham (1996)	Mice	300 ppm, 6 h/d, 5 d/wk, 2 wk	Reduced hematopoiesis in long-term marrow cultures
Farris et al. (1997a)	Mice	100, 200 ppm 6 h/d, 5 d/wk, 1, 2, 4, or 8 wk	Multiple changes in number and replication rate of blood and marrow cells in different stages of differentiation
Oral (gavage)			
Wolf et al. (1956)	Rats	50, 100 mg/kg-d, ~ 26 wk	Leukopenia, erythrocytopenia
Maltoni et al. (1985)	Rats	500 mg/kg, 84 wk	Leukopenia
NTP (1986)	Rats, mice	25, 50, 100, 200, 400, 600 mg/kg, 17 or 103 wk	Leukopenia, lymphocytopenia, lymphoid depletion, marrow hyperplasia
MacEachern and Laskin (1992)	Mice	660 mg/kg-d, 3 d	Stimulation of marrow leukocytes to produce growth regulatory cytokines

[1] Doses that exhibited at least one of the listed adverse effects are shown in the table.

Keller and Snyder (1988) exposed Swiss Webster mice to benzene *in utero* from day six through day 15 of gestation. Pregnant mice were administered benzene via inhalation at doses of 5, 10 or 20 ppm. On day 16 of gestation, two days after birth and six weeks after birth, offspring were tested for levels of blood hemoglobin and levels of hematopoietic cells in the blood, bone marrow and liver. No adverse effects were observed for fetuses at day 16 of gestation. However, two days after birth, offspring exhibited reduced numbers of circulating erythroid precursor cells (all doses), increased numbers of hematopoietic blast cells in the liver (20 ppm only), increased granulopoietic precursor cells (20 ppm only) and decreased numbers of erythropoietic precursor cells (20 ppm only). Mice examined six weeks after birth also demonstrated increased granulopoiesis in the 20 ppm dose group.

The effect of exposure regimen and duration on benzene-induced bone marrow damage was investigated in three strains of male and female mice, $B6C3F_1$, C57Bl/6, and DBA/2 (Luke et al., 1988a,b; Tice et al., 1989). Mice were exposed via inhalation to 300 ppm benzene for different durations (one to 13 weeks, six hours per day), or different regimens (five day per week or three days per week). The ratio of polychromatic erythrocytes (PCE) to normochromatic erythrocytes was used to evaluate benzene-induced alterations in erythropoiesis. Both inhalation regimens reduced the erythropoiesis; however, the depression persisted longer with the three days per week regimen than the five day per week regimen. All benzene-exposed mice also exhibited reduced packed cell volume and bone marrow cellularity. The magnitude of these effects was dependent on sex, strain and regimen. Bone marrow damage was directly dependent on regimen and duration, but not on sex or strain.

Cronkite et al. (1989) exposed CBA/Ca mice (a sensitive strain) to air concentrations of benzene at 10, 25, 100, 300, 400, and 3000 ppm, six hours per day, five days per week for up to 16 weeks. No effects were observed for the ten ppm dose group. Lymphopenia was observed in the 25 ppm dose group. Higher doses of benzene produced dose-dependent decreases in blood lymphocytes, bone marrow cellularity, spleen colony-forming-units (CFU-S), and an increased percentage of CFU-S in S-phase synthesis.

Farris et al. (1997a) exposed groups of $B6C3F_1$ mice to benzene at air concentrations of 1, 10, 100 or 200 ppm six hours per day, five days per week for one, two, four or eight weeks. A separate study administered 1, 5, or 10 ppm benzene for four weeks. Numerous hematotoxic measures and markers were evaluated. One assay examined the colony forming units of high proliferative potential (CFU-HPP) (i.e., primitive precursor hematopoietic cells). The number of CFU-HPP forming cells per femur was decreased in the 100 and 200 ppm dose groups only. However, the percentage of CFU-HPP cells in the S-phase in mice exposed to 0, 1, 10, 100 and 200 ppm for four weeks was 8, 15, 33, 49, and 55 percent, respectively, demonstrating a trend over all doses. This trend was not seen in the four-week study using exposures of 1, 5, or 10 ppm. Later stage (or committed) progenitor cells were measured as erythrocytic- and granulocytic-colony forming units (CFU-E and CFU-GM, respectively). Exposure to 100 or 200 ppm benzene induced a statistically significant increase in CFU-E per femur after one week of exposure and a significant decrease after two and eight weeks. Results for CFU-E and CFU-GM counts for the 1, 5, or 100 ppm dose groups were not reported. Blood leukocytes and platelets were decreased for weeks two, four and eight in the 100 and 200 ppm-dosed mice.

Genetic Toxicity

Benzene is clearly genotoxic and has been extensively studied in animals and *in vitro* test systems. Extensive reviews of the literature can be found elsewhere (Dean, 1978, 1985; ATSDR,

1997; U.S. EPA, 1998). Benzene is both a mutagen and clastogen in whole animal systems, although its clastogenic activity is likely to be of greatest concern (see Mode of Action section).

In *in vitro* test systems, benzene has exhibited mixed results, although positive findings have been reported for gene mutations in bacteria, and DNA or RNA synthesis inhibition in mammalian cells (ATSDR, 1997). However, metabolites of benzene including various phenolic, quinone, epoxide and aldehyde species exhibited myriad effects such as mutations in bacteria; sister chromatid exchanges, micronuclei formation, DNA strand breaks, DNA adducts, and oxidative DNA damage in mammalian cells (ATSDR, 1997).

In animals *in vivo*, benzene induced chromosomal aberrations in lymphocytes of mice and in bone marrow cells of rats and hamsters. Benzene caused increases in micronuclei in bone marrow of mice and hamsters, in peripheral erythrocytes of mice, and in lymphocytes of rats. Administration of benzene increased incidences of gene mutation and polyploidy in lymphocytes of mice. Benzene caused sister chromatid exchanges in bone marrow, fetus, and liver of mice, and lymphocytes of rats and mice. Administration of benzene also caused increases in DNA or RNA synthesis inhibition in bone marrow of mice and rabbits and in liver mitochondria of rats. Sperm head abnormalities have also been observed in benzene-exposed mice (reviewed in ATSDR, 1997). Benzene administered to transgenic mice by inhalation (Mullin et al., 1995; 1998) and orally (Provost et al., 1996) induced significant increases in mutations of the *lacI* transgene in multiple tissues.

Developmental and Reproductive Toxicity

In 1997, the Developmental and Reproductive Toxicant Identification Committee which is part of the OEHHA Science Advisory Board recommended that benzene be listed on the Proposition 65 list of chemicals "known to the State" of California to cause developmental or reproductive harm. The Committee noted that sufficient evidence exists to designate benzene as a developmental toxicant and a male reproductive toxicant. In the hazard identification document that formed the basis of this decision, OEHHA (1997) extensively reviewed the available literature on benzene's reproductive and developmental toxicity. Portions of the summary sections of the hazard identification document are reproduced below (OEHHA, 1997a).

Developmental Toxicity: "Delayed intrauterine development has been a consistent finding of studies in rats, rabbits and mice which used benzene exposure by inhalation to pregnant dams during organogenesis. The relevant endpoints are fetal weight reduction (in the range of 7-10 percent) and delayed ossification. In some of these studies, but not all, maternal toxicity was reported to occur concurrent with exposures that produced adverse fetal effects. There is little indication that benzene causes structural malformations. There are no studies with postnatal endpoints. Dose dependence is seen and some benzene concentrations produce these effects in the absence of reported maternal toxicity" (OEHHA, 1997a).

"Two benzene effects seen in adults, chromosome damage and changes in populations of hematopoietic precursors, are also seen in mouse fetuses whose dams are treated with benzene. Some effects on hematopoietic cell populations persist in the postnatal period. Hematopoietic effects occur at distinctly lower benzene exposure concentrations than delayed development. Functional consequences at the organismic level have not been explored" (OEHHA, 1997a).

Female Reproductive Toxicity: "There are relatively few studies of female reproductive toxicity in animals and they vary in quality and completeness of presentation. One large, well-conducted single generation study in rats by the inhalation route failed to find effects on female reproductive toxicity measures. There is no multigeneration study or continuous breeding study" (OEHHA, 1997a).

Male Reproductive Toxicity: "Studies in animals have demonstrated testicular damage and effects on sperm count, morphology and chromosome damage after benzene exposure. Associated general toxicity was not reported in many of these studies. Separate studies of the potential consequences of sperm effects in terms of fertility or dominant lethal measures failed to find such effects in rats or mice. Of potential interest in this regard are human studies reporting associations between paternal exposure to benzene and increased risk of stillbirth, small-for-gestational age infants, and childhood leukemia. These studies, while suggestive, are not definitive and further research is needed" (OEHHA, 1997a).

Immunotoxicity

Benzene suppresses the immune system. Rozen et al. (1984) exposed mice to benzene via inhalation at concentrations of 10, 30, 100 and 300 ppm, six hours per day, for six days. Liposaccharide-induced B-cell proliferation was decreased at all dose levels. Phytohemagglutinin-induced T-cell response was depressed in mice exposed to 30 ppm and higher. Peripheral lymphocyte counts were decreased at all levels and erythrocyte counts were decreased only in the 100 and 300 ppm dose groups. Rozen and Snyder (1985) exposed mice to 300 ppm benzene, six hours per day, five days per week, for 115 days. The number of B-cells in the spleen and bone marrow and the number of T-cells in the thymus and spleen were reduced in the benzene-exposed mice. Benzene exposure also reduced the immune response to other mitogens.

CD-1 mice were orally administered 8, 40 or 180 mg/kg benzene daily for four weeks. A dose-related reduction in peripheral blood lymphocytes was observed. However, levels of neutrophils and other white blood cells were unaffected. When challenged by B- and T-cell mitogens, the splenic lymphocyte proliferative response was enhanced in the 8 mg/kg dose group and depressed in the 40 and 180 mg/kg dose groups (Hsieh et al., 1988).

Antibody response to tetanus toxin was reduced in mice exposed to high levels of benzene in air, and mice exposed to benzene were more susceptible to infection by *Listeria monocytogene* (reviewed in IPCS, 1993). Treatment of Balb/c mice with 660 mg/kg benzene for three days resulted in marked changes to the morphology and functional activity of the bone marrow phagocytes (MacEachern et al., 1992). Exposure of mice to inhaled benzene (10 or 100 ppm, five days per week for four weeks) reduced some processes involved with tumor surveillance (Rosenthal and Snyder, 1987).

Farris et al. (1997b) evaluated the effects of low levels of benzene exposure on lymphocytes of mice. Male $B6C3F_1$/CrlBr mice were exposed to air concentrations of benzene at 1, 5, 10, 100 and 200 ppm, six hours per day, five days per week for up to eight weeks. Exposure to 100 and 200 ppm benzene resulted in rapid and persistent reductions in the levels of femoral B-, splenic T- and B-, and thymic T-lymphocytes. The percentage of femoral B- and thymic T-lymphocytes in apoptosis was increased 6- to 15-fold in the 200 ppm group compared to controls. Compensatory replication of femoral B-lymphocytes was increased in the bone marrow in the 100 and 200 ppm dose groups. Mice exposed to 10 ppm or less did not show statistically significant effects relative to controls for the endpoints evaluated. Slight reductions in total nucleated cells of the thymus and spleen were observed for the 10 ppm group which was part of a dose-related trend over the administered doses at the early time points.

Robinson et al. (1997) administered benzene to Sprague-Dawley rats via inhalation at 0, 30, 200 or 400 ppm, six hours per day, five days per week for two or four weeks. In the 400 ppm dose group, a reduction in the number of B-lymphocytes was observed after two weeks and reductions in thymus weight, and in the numbers of spleen B-, CD4+/CD5+, and CD5+ T-lymphocytes were

observed after four weeks. Rats challenged with sheep red blood cells did not develop an immune response different from controls, except for a transient reduction in spleen B-lymphocytes after two weeks in the high dose group.

Carcinogenicity

Benzene has been shown to be carcinogenic in numerous animal studies either by inhalation or oral routes of administration. Carcinogenicity studies in which animals were exposed to benzene via inhalation are summarized in Table 6. Carcinogenicity studies in which animals were exposed to benzene via oral ingestion (gavage) are summarized in Table 7. Statistically significant increased incidences of cancer were observed at multiple sites, including Zymbal gland, mammary gland, ovary, uterus, nasal cavity, oral cavity, skin, Harderian gland, preputial gland, liver, and lung. Leukemias and lymphomas have been observed in some inhalation studies of rats and mice employing high exposures, and malignant lymphomas have been observed in oral (gavage) studies. Benzene administered via subcutaneous injection to multiple strains of mice also produced leukemias and sarcomas in several studies conducted before 1965 (reviewed in Maltoni et al., 1989).

Inhalation studies

Male AKR/J mice were exposed to 100 ppm (Snyder et al., 1980) or 300 ppm (Snyder et al., 1978) of benzene for six hours per day, five days per week for life. No treatment related tumors were observed, although early death, aplastic anemia and bone marrow hyperplasia were clearly evident. Snyder et al. (1980) exposed male C57BL/6J mice to 300 ppm benzene, six hours per day, five days per week for approximately 70 weeks and observed the development of malignant lymphoma in six of 40 mice compared to two of 40 unexposed controls. Goldstein et al. (1982) reported findings in CD-1 mice exposed to 300 ppm benzene six hours per day, five days per week for life. Of 40 CD-1 mice exposed, one case of acute myelogenous leukemia, one case of chronic myelogenous leukemia, and one case of granulocytic hyperplasia were reported compared to 0/40 in the controls. Snyder et al. (1978) exposed 40 Sprague-Dawley rats to 300 ppm benzene six hours per day, five days per week for 99 weeks. Twenty-five rats served as controls. No treatment-related tumors were reported. Snyder et al. (1984) exposed groups of 40 male Sprague-Dawley rats to 0 or 100 ppm benzene, six hours per day, five days per week for 123 weeks. Rats exhibited 4/40 liver tumors, 2/40 Zymbal gland tumors and 1/40 chronic myelogenous leukemia (a rare cancer in this strain). No tumors were observed at these sites in the control rats.

Maltoni et al. (1983, 1985, 1989) chronically exposed pregnant Sprague-Dawley rats (breeders) and their offspring to high concentrations of benzene. Dosing regimens were complex (see Table 6) but exposure concentrations ranged from 200 to 300 ppm. Among the breeders, a slight increase in the incidences of Zymbal gland carcinoma and mammary tumors were reported. Among offspring, significant increased incidences in Zymbal gland tumors and slight (non-significant) increases in cancers of the oral and nasal cavity, mammary gland and liver were reported. In a separate experiment, Sprague-Dawley rats were exposed to benzene *in utero* from day 12 of gestation and during lactation. Dams were exposed to a regimen (see Table 6) of 200 to 300 ppm benzene. Slight increases in the incidences of Zymbal gland carcinoma, oral cavity carcinoma, hepatoma, and leukemia were observed (Maltoni et al., 1989).

In a series of experiments reported by Cronkite et al. (1984, 1985, 1989) and Cronkite (1986), C57BL/6 and CBA/Ca mice were exposed to air concentrations of benzene, 300 ppm six hours per day, five days per week for 16 weeks at variable intervals that mimicked patterns of human exposure to benzene. A significant increase in leukemia and lymphoma was reported in both

strains of mice as well as solid tumors (mammary and hepatoma) for CBA/Ca mice. Cronkite et al. (1989) observed an increased incidence of leukemia in male and female CBA/CA mice exposed to 300 ppm and 3000 ppm for 16 weeks; however, exposure to 3000 ppm did not shorten the latency or increase the incidence compared to 300 ppm.

Snyder et al. (1988) exposed CD-1 and C57BL male mice to benzene employing two different exposure patterns to compare the effect of long-term discontinuous exposures to that of continuous exposure. The first exposure pattern was representative of an occupational setting (intermittent for life with short-term high doses). Mice were exposed to an alternating pattern of 300 ppm benzene six hours per day, five days per week, for one week followed by two weeks without exposure. The alternating pattern of exposure was continued until death. Statistically significant increased incidences of Zymbal gland tumors in C57BL mice and lung adenomas in CD-1 mice were observed. The second exposure pattern was 1200 ppm benzene six hours per day, five days per week for ten weeks. Significant increases in lung adenoma and Zymbal gland carcinoma in CD-1 mice were reported. Both exposure protocols were markedly hematotoxic to both mouse strains as measured by peripheral blood counts. Both strains of mice responded to the intermittent 300 ppm benzene exposures with elevated incidences of malignant tumors. However, only the CD-1 mice exhibited increases in tumor incidences following the second exposure pattern to 1200 ppm benzene delivered over 10 weeks.

Farris et al. (1993) exposed 125 male CBA/Ca mice to 300 ppm benzene, six hours per day, five days per week for 16 weeks and sacrificed the animals after 18 months. Sham-exposed controls (n=125) were treated with filtered air. Significant increases in incidences of malignant lymphoma (14/118 in exposed mice, 2/119 in controls), preputial gland squamous cell carcinoma (71/118 exposed, 0/118 controls), and lung adenoma (42/118 exposed, 1/125 controls) were observed. Carcinomas of the Zymbal gland and forestomach squamous cells were also significantly increased over controls; however, for these endpoints, histopathology was conducted only when gross lesions were observed. Increased granulocytic hyperplasia of the bone marrow and spleen were also reported.

Table 6. Summary of Inhalation Studies of the Carcinogenicity of Benzene in Animals

Species	Exposure Concentration	Dose Regimen	Tumor Type	Incidence: Dosed Group (Controls)	Reference
AKR/J mice (male, 8 wk old)	100 ppm 300 ppm	6 hr/d, 5 d/wk, life (~70wk)	No tumors	0/60 (0/60) 0/50 (0/50)	Snyder et al., 1980, 1978
Sprague-Dawley rats (male)	300 ppm	6 hr/d, 5 d/wk, 99 wk	No tumors	0/45 (0/25)	Snyder et al., 1978
CD-1 mice (male)	100 ppm 300 ppm	6 hr/d, 5 d/wk, life	Leukemia	2/40 (0/40)	Goldstein et al., 1982
C57BL/6J mice (male, 8 wk old)	300 ppm	6 hr/d, 5 d/wk, life (~70wk)	Lymphocytic lymphoma with thymic involvement	6/40 (2/40)	Snyder et al., 1980
Sprague-Dawley rats (male, 6 wk old)	100 ppm	6 hr/d, 5 d/wk, life (~123 wk)	Liver Zymbal gland Chronic myelogenous leukemia	4/40 (0/40) 2/40 (0/40) 1/40 (0/40)	Snyder et al., 1984
CD-1 mice (male)	300 ppm (see regimen)	Intermittent: 6 hr/d, 5 d/wk for 1 wk, then 2 wk with no exposure, repeat for life	Lung adenoma	14/54 (3/46)	Snyder et al., 1988
C57BL/6 mice (male)	300 ppm (see regimen)	Intermittent: 6 hr/d, 5 d/wk for 1 wk, then 2 wk with no exposure, repeat for life	Zymbal gland carcinoma	19/54 (0/46)	Snyder et al., 1988

Table 6 (Continued). Summary of Inhalation Studies of the Carcinogenicity of Benzene in Animals

Species	Exposure Concentration	Dose Regimen	Tumor Type	Incidence: Dosed Group (Controls)	Reference
CD-1 mice	1200 ppm	6 h/d, 5 d/wk for 10 wk	Lung adenoma Zymbal gland carcinoma	33/70 (17/71) 4/71 (0/71)	Snyder et al. 1988
C57BL/6 mice (7 to 9 wk old)	300 ppm	6 h/d, 5 d/wk for 16 wk, observed for life	Leukemia (all types) Lymphoma (thymic) Lymphoma (non-thymic)	20/89 (8/88) 10/89 (1/88) 6/89 (2/88)	Cronkite et al., 1985
CBA/Ca BNL mice (male)	100 ppm 300 ppm	6 h/d, 5 d/wk for 16 wk, observed for life	"Myelogenous neoplasms"	2/85 (0/70) 11/57 (0/60)	Cronkite et al., 1989
CBA/Ca BNL mice (female)	300 ppm	6 h/d, 5 d/wk for 16 wk, observed for life	"Myelogenous neoplasms"	6/54 (1/60)	Cronkite et al., 1985
Sprague-Dawley rats (Breeders, 13 wk old)	200 to 300 ppm (see regimen)	200 ppm 4 h/d, 5 d/wk for 7 wk, then 7 h/d, 5 d/wk for 12 wk, then 300 ppm 7 h/d, 5 d/wk for 85 wk	Zymbal gland carcinoma Mammary (malignant and benign combined)	3/54 (1/60) 30/54 (24/60)	Maltoni et al., 1983, 1985, 1989
(Offspring)	200 to 300 ppm	See regimen for Breeders. Offspring exposed in utero, during lactation, and for 85 wk (104 wk total). Sacrifice at 150 wk.	Zymbal gland carcinoma Mammary (malignant and benign combined) Nasal carcinoma Hepatoma	14/75 (2/158) m[a] 8/65 (0/149) f[a] 6/75 (11/158) m 35/65 (84/149) f 1/75 (0/158) m 2/65 (0/149) f 2/75 (1/158) m 7/65 (0/149) f	

[a] m, males; f, females

Table 6 (Continued). Summary of Inhalation Studies of the Carcinogenicity of Benzene in Animals

Species	Exposure Concentration	Dose Regimen	Tumor Type	Incidence: Dosed Group (Controls)	Reference
Sprague-Dawley rats	200 ppm (dose to the dams)	In utero from day 12 of gestation and during lactation. 4 hr/d, 5 d/wk for 7 wk, then 7 hr/d, 5 d/wk for 12 wk. Sacrifice 150 wk	Zymbal gland carcinoma	4/70 (2/158) m[a] 1/59 (0/149) f[a]	Maltoni et al., 1983, 1985, 1989
			Oral cavity carcinoma	2/70 (0/158) m 6/59 (0/149) f	
			Leukemia	4/70 (12/158) m 4/58 (1/148) f	
			Hepatoma	2/70 (1/158) m 5/59 (0/149) f	
CBA/Ca mice	300 ppm	6 hr/d, 5 d/wk, 16 wk Sacrificed at 18 months	Malignant lymphoma	14/118 (2/119)	Farris et al., 1993
			Preputial gland squamous cell carcinoma	71/118 (0/118)	
			Lung adenoma	42/118 (17/119)	
			Zymbal gland carcinoma (only gross lesions examined)	14/125 (1/125)	
			Forestomach squamous cell carcinoma (only gross lesions examined)	9/125 (0/125)	

[a] m, males; f, females

Oral (gavage) studies

In a series of carcinogenicity studies reported by Maltoni et al. (1982, 1983, 1985, 1989), different strains of rats and mice were administered benzene orally via gavage. Incidence data for these studies are reported in Table 7 and briefly summarized here. Male and female Sprague-Dawley rats 13 weeks of age were administered 0, 50 or 250 mg benzene/kg dissolved in olive oil for four to five times per week for 52 weeks and sacrificed at 144 weeks. Dose-related increases were observed for Zymbal gland carcinoma in the female rats only. Male and female Sprague-Dawley rats seven weeks of age were administered 0 or 500 mg/kg benzene in olive oil, four to five times per week for 104 weeks, then observed until natural death. Significant increases relative to controls were reported for Zymbal gland carcinoma (males and females), oral cavity carcinoma (males and females), nasal cavity carcinoma (males), skin carcinoma (males), and forestomach carcinoma (females). Wistar rats, Swiss mice and RF/J mice (approximately 40 animals/sex/group) were exposed to 0 or 500 mg/kg benzene, four to five times per week for 52 weeks in RF/J mice, 78 weeks in Swiss mice, and 104 weeks in Wistar rats. Animals were observed until natural death. In Wistar rats, increased incidences compared to controls were reported for Zymbal gland carcinoma (males) and oral cavity carcinoma (females). In Swiss mice, increased incidences relative to controls were reported for Zymbal gland carcinoma (males), mammary gland carcinomas (females), and pulmonary tumors (males and females). In RF/J mice, increased incidences relative to controls were reported for pulmonary tumors (males and females) and mammary gland carcinomas (females).

The U.S. National Toxicology Program (NTP, 1986) conducted two-year bioassays in F344 rats and $B6C3F_1$ mice on the carcinogenic effects of oral (gavage) exposure to benzene. Female rats and mice were administered benzene in corn oil at doses of 0, 25, 50, and 100 mg/kg, five days per week, for 103 weeks. Male rats and mice were administered benzene at doses of 0, 50, 100, and 200 mg/kg, five days per week for 103 weeks. Incidence data for these studies are shown in Table 7. In female F344 rats, statistically significant dose-related increases in the incidences of neoplasms were reported for the oral cavity, Zymbal gland and uterus. In male F344 rats, statistically significant dose-related increases in the incidences of neoplasms were reported for the oral cavity, Zymbal gland and skin. In female $B6C3F_1$ mice, statistically significant dose-related increases in the incidences of tumors were reported for the Zymbal gland, ovary, mammary gland, Harderian gland, lung, and lymphoma/leukemia combined. In male $B6C3F_1$ mice, statistically significant dose-related increases in the incidences of tumors were reported for the Zymbal gland, preputial gland, Harderian gland, lung and lymphoma/leukemia combined.

Low et al. (1995) investigated possible reasons for the observed specificity of tumor formation in the various tissues of the rat following exposure to benzene. The authors reported that the Zymbal gland (i.e., the tissue most sensitive to benzene-induced carcinogenicity) exhibited phenyl- and aryl- sulfatase activity (i.e., hydrolysis or release of sulfate conjugates) and no phenol sulfoconjugating activity. High sulfatase and low sulfotranferase activities were also observed for the oral cavity, mammary gland, and bone marrow. Zymbal gland, bone marrow, nasal and oral cavities, and mammary gland homogenates also possessed much higher levels of peroxidase activity than other tissues that did not develop tumors. The authors suggested that 'sulfate shunting', in which sulfate conjugates of benzene metabolites such as hydroquinone are transported to various tissues where they are released and subsequently oxidized by peroxidases to reactive species, may represent an important mechanistic pathway in benzene-induced tumorigenesis. Information on sulfatase, sulfotransferase, and peroxidase activity in human tissues is not readily available.

Carcinogenicity of benzene metabolites

Some of the primary metabolites of benzene, namely phenol, catechol, hydroquinone and 1,4-benzoquinone, have been tested for carcinogenic activity in animal bioassays. Results of these studies are summarized briefly below. Carcinogenicity studies of phenol were conducted by the National Cancer Institute as part of the National Toxicology Program (NCI, 1980). Phenol was administered to F344 rats and $B6C3F_1$ mice for 103 weeks in drinking water at concentrations of 0, 2500 or 5000 ppm. An increased incidence of leukemia was reported for the low-dose group in male rats compared to controls; however, the incidence of leukemia was not increased in the high-dose group. No treatment-related cancers were seen in the female rats, female mice, or male mice. In other studies, phenol has been shown to act as a mouse-skin-tumor promoter following pretreatment with either 7,12-dimethylbenz[*a*]anthracene or benzo[*a*]pyrene (reviewed in NTP, 1986).

Catechol, a major phenolic metabolite of benzene, has produced mixed results in animal carcinogenicity studies. No dose-related increases in neoplasms were detected in rats fed a diet comprised of 0.0625 percent to 1.0 percent catechol for two years (Lehman et al., 1951). No tumor promoting activity was observed when catechol was applied to the skin of mice following administration of dimethylbenz[*a*]anthracene (Boutwell and Bosch, 1959) or benzo[*a*]pyrene (Van Duuren and Goldschmidt, 1976). However, catechol has been found to be a potent co-carcinogen with benzo[*a*]pyrene in a mouse skin tumor model (Hecht et al., 1981, Melikian et al., 1986, 1989, 1990).

Hydroquinone, one of the primary phenolic metabolites of benzene and a precursor to 1,4-benzoquinone, was found to be carcinogenic in rodents. In the NTP bioassays of hydroquinone, both female and male F344/N rats and $B6C3F_1$ mice were given hydroquinone in water by gavage at doses of 0, 25 or 50 mg/kg for rats and 0, 50 or 100 mg/kg for mice, five days per week for two years. Clear evidence of dose-related renal tumors was seen in male F344/N rats, while some evidence of hydroquinone-related mononuclear cell leukemia was observed in female F344/N rats. Female mice showed increased incidence of liver tumors, primarily adenomas, in both dose groups compared to control mice. No dose-related neoplasms were seen in the male mice (NTP, 1988). Studies of hydroquinone following treatment of mouse skin with DMBA or benzo[*a*]pyrene indicated that hydroquinone did not act as a tumor promoter (Boutwell and Bosch, 1959, Van Duuren and Goldschmidt, 1976). Coadministration of benzo[*a*]pyrene and hydroquinone produced fewer skin tumors than benzo[a]pyrene alone (Van Duuren and Goldschmidt, 1976). It should be noted that the sites of tumor formation in animals were different for hydroquinone and benzene.

The carcinogenicity of 1,4-benzoquinone has been investigated in three studies. In the first, 1,4-benzoquinone (diluted in benzene) painted on the skin of mice for 200 days at concentrations of 0, 0.1 or 1.25 percent produced increased incidences of lung adenocarcinomas, skin papillomas, and skin carcinomas (Takizawa et al., 1940 as reviewed in IARC, 1977). In the second, incidence of lung tumors was not increased in mice chronically exposed to five mg per day of 1,4-benzoquinone by inhalation six days per week compared to controls (Kishizawa et al., 1956 as reviewed in IARC, 1977). In the third, Umeda (1957) administered subcutaneous injections of 1,4-benzoquinone in propylene glycol to 24 rats at various doses and regimens; two injection-site fibrosarcomas were reported (as reviewed in IARC, 1977).

Table 7. Summary of Oral (Gavage) Studies of the Carcinogenicity of Benzene in Animals

Species	Dose (in oil)	Dose Regimen	Tumor Type	Incidence: Dosed Group (Controls)	Reference
Sprague-Dawley rats (13 wk old)	50 mg/kg 250 mg/kg	4 to 5 doses/wk, 52 wk. Sacrifice at 144 wk	Zymbal gland carcinoma Oral cavity carcinoma Malignant mammary	2/30 (0/30) f[a] 8/35 f 0/30 (0/30) f 2/35 f 4/30 (4/30) f 7/35 f	Maltoni et al., 1982, 1983, 1985, 1989
Sprague-Dawley rats (7 wk old)	500 mg/kg	4 to 5 doses/wk, 104 wk. Observed until natural death	Zymbal gland carcinoma Oral cavity carcinoma Nasal cavity carcinoma Skin carcinoma Forestomach acanthomas and dyplasias Forestomach carcinoma	18/40 (1/50) m[a] 16/40(0/50) f 21/40 (0/50) m 20/40 (0/50) f 3/40 (0/50) m 9/40 (1/50) m 10/40 (0/50) m 7/40 (0/50) f 6/40 (0/50) f	Maltoni et al., 1982, 1983, 1985 1989
Wistar rats (7 wk old)	500 mg/kg	4 to 5 doses/wk, 104 wk Observed until natural death	Zymbal gland carcinoma Oral cavity carcinoma	7/40 0/40 m 6/40 0/40 f 4/40 0/40 f	Maltoni et al., 1989
Swiss mice (7 wk old)	500 mg/kg	4 to 5 doses/wk, 78 wk Observed until natural death	Zymbal gland carcinoma Mammary carcinomas Pulmonary tumors	4/40 (0/40) m 19/40 (1/40) f 17/40 (3/40) m 15/40 (4/40) f	Maltoni et al., 1989
RF/J mice (6 wk old)	500 mg/kg	4 to 5 doses/wk, 52 wk Observed until natural death	Pulmonary tumors Mammary carcinomas	23/45 5/45 m 18/40 3/40 f 9/40 1/40 f	Maltoni et al., 1989

[a] m, males; f, females

Table 7 (Continued). Summary of Oral (Gavage) Studies of the Carcinogenicity of Benzene in Animals

Species and Sex	Exposure Concentration	Dose Regimen	Tumor Type	Incidence: Dosed Group (Controls)	Reference
$B6C3F_1$ mice (6 to 8 wk old) Males	50 mg/kg 100 mg/kg 200 mg/kg	5 d/wk, 103 wk	Zymbal gland carcinoma	1/34 (0/43) 4/40 21/39	NTP, 1986
			Preputial gland carcinoma	5/28 (0/21) 19/29 31/35	
			Harderian gland adenoma or carcinoma	10/46 (1/49) 13/49 14/48	
			Lung carcinoma	11/48 (5/49) 12/50 14/49	
			Malignant lymphoma	9/48 (4/49) 9/50 15/49	
$B6C3F_1$ mice (6 to 8 wk old) Females	25 mg/kg 50 mg/kg 100 mg/kg	5 d/wk, 103 wk	Zymbal gland carcinoma	0/32 (0/43) 1/37 3/31	NTP, 1986
			Ovary tumors	4/44 (1/47) 24/49 19/48	
			Mammary gland carcinoma and carcinosarcoma	2/45 (0/49) 6/50 14/49	
			Harderian gland adenoma or carcinoma	6/44 (5/48) 10/50 10/47	
			Lung adenoma or carcinoma	5/42 (4/49) 10/50 13/49	

Table 7 (Continued). Summary of Oral (Gavage) Studies of the Carcinogenicity of Benzene in Animals

Species and Sex	Exposure Concentration	Dose Regimen	Tumor Type	Incidence: Dosed Group (Controls)	Reference
			Malignant lymphoma	24/45 (15/49) 24/50 20/49	
F344 rats (7 to 8 wk old) Males	50 mg/kg 100 mg/kg 200 mg/kg	5 d/wk, 103 wk	Zymbal gland carcinomas	6/46 (2/32) 10/42 17/42	NTP, 1986
			Oral cavity squamous cell papilloma or carcinoma	9/50 (1/50) 16/50 19/50	
			Skin squamous cell papilloma or carcinoma	7/50 (0/50) 4/50 11/50	
F344 rats (7 to 8 wk old) Females	25 mg/kg 50 mg/kg 100 mg/kg	5 d/wk, 103 wk	Zymbal gland carcinomas	5/40 (0/40) 5/44 14/46	NTP, 1986
			Oral cavity squamous cell papilloma or carcinoma	5/50 (1/50) 12/50 9/50	
			Uterus	7/50 (7/50) 7/50 14/50	

Toxicological Effects in Humans

Acute Toxicity

Acute toxic effects of exposure to benzene by the general population have resulted primarily from the misuse of benzene or benzene-containing products. Individuals have died from sniffing glue containing benzene as a solvent. Blood concentrations were roughly one to 65 mg/L, and death was a result of either pulmonary hemorrhage and inflammation, renal congestion or cerebral edema, or some combination of these (IPCS, 1993).

A lethal oral dose of benzene in humans has been estimated to be about nine grams (125 mg/kg) (ATSDR, 1997). Exposure to air concentrations of benzene of roughly 20,000 ppm for five to ten minutes, 7500 ppm for 30 minutes, or 1500 ppm for 60 minutes is estimated to cause death or severe toxicity in humans (IPCS, 1993). Symptoms of headaches, lethargy, and weakness were reported from exposure to 50 to 150 ppm benzene for five hours, whereas exposure to 25 ppm for eight hours showed no clinical effect (IPCS, 1993, Paustenbach et al., 1993).

Subchronic and Chronic Toxicity

Moderate and long-term exposure of humans to benzene has resulted in numerous adverse effects including bone marrow damage, changes in circulating blood cells, developmental and reproductive effects, immunological effects, and cancer. Carcinogenicity, immunotoxicity, and developmental and reproductive effects are described in separate sections of this document devoted to these topics. This section will address other endpoints, focussing primarily on non-cancer hematotoxicity.

Many blood disorders, including aplastic anemia, pancytopenia, thrombocytopenia, granulocytopenia, lymphocytopenia, and leukemia have been associated with chronic exposure to inhaled benzene. The involvement of different hematopoietic lineages suggests that the targets of the toxic metabolites of benzene are the bone marrow stem or early progenitor cells. These effects have been extensively reviewed elsewhere (Aksoy, 1988; Cooper and Snyder, 1988; IPCS, 1993; Snyder and Kalf, 1994; ATSDR, 1997; Smith, 1996c).

Cases of benzene-induced hematotoxicity were reported as early as 1897, when Santesson described nine female cases of chronic benzene-induced toxicity; four of these women died from aplastic anemia. Some physicians in the 1910s and 1920s, inspired by benzene's leukopenic effects (reduction of white blood cells), actually prescribed benzene (three to five grams per day) for the treatment of leukemia. Patients temporarily improved, then died of aplastic anemia or related hematotoxic effects (Snyder and Kalf, 1994; Smith, 1996c). Studies of 332 benzene exposed workers in the printing industry in the 1930s reported that 130 workers had signs of benzene hematotoxicity, including 23 cases of cytopenias. Studies of 1104 rubber factory workers exposed to about 100 ppm benzene in air in1942 reported that 83 individuals had mild hematological changes and 25 showed severe pancytopenia (reviewed in ATSDR, 1997; Smith, 1996c).

Studies examining more recent benzene exposures have been conducted on cohorts in Turkey, Italy and China. In 1960 the leather and shoe industry in Turkey began using benzene-containing adhesives, which resulted in a significant number of benzene poisonings. A series of studies of the hematotoxic effects observed in these workers was reported and extensively reviewed by Aksoy (1988). In one such study, Aksoy et al. (1971) examined 217 male shoe workers exposed

to benzene. Average exposures to benzene in air were estimated to be about 15 to 30 ppm, with short-term exposure levels estimated to be as high as 210 ppm during the application of benzene-containing adhesives. Twenty-one cases of leukopenia, four cases of thrombocytopenia, ten cases of combined leukopenia and thrombocytopenia, six cases of pancytopenia, and five cases of eosinophilia were identified in this cohort. In another study, Aksoy et al. (1972) described 32 patients who had worked as shoe manufacturers. Benzene exposures for these workers were high (150 ppm to 640 ppm, based on spot measurements) and lasted from four months to 15 years. These individuals showed severe blood disorders, including pancytopenia with hypoplastic, hyperplastic, or normoplastic bone marrow. Eight of the 32 died from thrombocytopenic hemorrhage or infection. In Italy, Forni and colleagues also reported a number of cases of aplastic anemia and leukemia in benzene-exposed workers (Vigliani and Forni, 1976).

Yin et al. (1987, 1989) reported on a large retrospective cohort study of benzene-exposed workers in China as part of a joint study between the U.S. National Cancer Institute and the Institute of Occupational Medicine, Chinese Academy of Preventative Medicine. The study examined 28,460 exposed workers from 233 factories and 28,257 control workers from different industries. Estimates of benzene concentrations in air based on grab-samples taken at the time of the survey ranged from 10 to 1000 mg/m^3, with the majority of exposures in the range of 50 to 500 mg/m^3. In addition to increases in incidence of neoplasms (see Carcinogenicity section), Yin et al. (1987, 1989) also reported cases of leukopenia (white blood cell counts $<4000/mm^3$) and aplastic anemia. The Chinese Worker Cohort has since been expanded to include a total of 74,828 benzene-exposed workers, with the earliest exposures occurring in 1949, and 35,805 controls from 712 factories in 12 cities in China. Yin et al. (1996) reported finding nine cases of aplastic anemia, two cases of agranulocytosis, and seven cases of myelodysplastic syndromes in the benzene-exposed workers and none in the unexposed workers. As part of an exposure validation study, Dosemeci et al. (1996) reported 412 diagnosed cases of benzene poisoning (white blood cell counts $<4000/mm^3$ and platelet counts $<80{,}000/mm^3$ from repeated tests over several months, and greater than six months exposure to benzene) among exposed workers. Recent intensity of exposure (at 1.5 years prior to diagnosis) for <5 ppm, 5-19 ppm, 20-39 ppm, and >40 ppm was associated with relative risks of benzene poisoning of 1.0, 2.2, 4.7 and 7.2, respectively.

Additional studies of benzene-exposed workers in the U.S. (Tsai et al., 1983; Kipen et al., 1988; Cody et al., 1993; Ward et al., 1996) are described in Appendix B. Appendix B is a reproduction of OEHHA's chronic toxicity summary for benzene which describes the basis of the chronic, non-cancer Reference Exposure Level (REL), a regulatory standard for the Air Toxics "Hot Spots" Program mandated by the Air Toxics Hot Spots Information and Assessment Act of 1987, as amended.

Genetic Toxicity

There is an extensive body of literature describing the genotoxic effects of benzene in human populations. Benzene appears to elicit primarily clastogenic effects in humans, including aneuploidy, ploidy, micronuclei, and chromosomal deletions, translocations and rearrangements. Extensive reviews are available (Dean, 1978, 1985; IARC, 1982; ATSDR, 1997; Fanning, 1998). Most cytogenetic studies of benzene examined the blood lymphocytes of the exposed workers. More than 20 studies have reported increases in structural and/or numerical chromosomal aberrations in mitogen-stimulated peripheral lymphocytes from benzene-exposed workers (ATSDR, 1997; Fanning, 1998). The benzene-induced chromosome aberrations involved chromatid and/or chromosome breaks.

Significantly increased strand breaks and/or alkali-labile sites, as measured by the alkaline gel electrophoresis assay, were observed in the blood lymphocytes of workers exposed to relatively

low levels of benzene (less than one ppm) (Nilsson et al., 1996; Andreoli et al., 1997). Dose-related increases in oxidative DNA damage, as measured by 8-hydroxy-2-deoxyguanosine, were observed in benzene exposed workers relative to controls (Liu et al., 1996; Nilsson et al., 1996).

Rothman et al. (1995) examined benzene-induced DNA damage in polychromatic erythrocytes (i.e., newly formed red blood cells still containing their DNA which serve as a good measure of recent bone marrow damage). Using the glycophorin A assay, the researchers examined NN and N∅ mutant variants among benzene-exposed workers and controls. NN mutants result in loss of heterozygosity in which loss of one allele is followed by duplication of the other allele through recombination. N∅ mutants represent gene-inactivating events (resulting in hemizygous phenotypes). Rothman et al. (1995) separated workers into four exposure groups, 0, 1-100, 101-500 and >500 ppm-yrs benzene. A strong positive trend over all dose groups ($p=0.005$) was observed between cumulative exposure and the frequency of NN mutant variants. No correlation was observed for the N∅ mutants.

Specific chromosomal aberrations are commonly associated with background and therapy-related leukemias (reviewed in Smith and Zhang, 1998). Acute myelogenous leukemia and myelodysplastic syndromes often exhibit specific gains or losses in chromosomes, translocations, deletions and inversions, especially involving chromosomes 5, 7, 8, 9, 21 or 22. For example, common alterations observed in therapy-related acute myelogenous leukemia and myelodysplastic syndromes are either loss of, or long-arm deletions in, chromosomes five and seven. Human exposure to benzene has been associated with many of these same chromosomal alterations. Early studies of patients with benzene-induced myelogenous leukemia, myelodysplastic syndromes and pancytopenia observed numerical changes in chromosomes 6 through 12 and X in the blood and bone marrow of patients (Vigliani and Forni, 1976). Recent studies of lymphocytes from benzene-exposed Chinese workers relative to controls have reported increases in the frequency of several specific alterations including chromosome nine hyperdiploidy translocations between chromosomes 8 and 21, and aneusomies of chromosomes 8 and 21 (Zhang et al., 1996; Smith et al., 1998). Also, significant increases in the rates of monosomy for chromosomes five and seven but not chromosome one ($p < 0.001$, $p < 0.0001$ and $p = 0.94$, respectively), and increases in the frequencies of trisomy and tetrasomy of all three chromosomes, were observed in the blood of benzene-exposed workers (Zhang et al., 1998). Several of these chromosomal alterations associated with benzene exposure have been observed *in vitro* in human cells treated with benzene metabolites. Stillman et al. (1997) and Zhang et al. (1994) reported dose-related increases of aneuploidy of chromosomes five and seven in human hematopoietic cells treated with hydroquinone or 1,2,4-trihydroxybenzene. Zhang et al. (1994) observed that a human cell line treated with hydroquinone or 1,2,4-benzenetriol caused trisomy and tetrasomy of chromosomes seven and nine. Eastmond et al. (1994) induced hyperdiploidy in chromosome nine in human lymphocytes treated with hydroquinone.

Developmental and Reproductive Toxicity

Human studies of adverse pregnancy outcomes associated with maternal exposure to benzene are limited in their utility because of problems with exposure assessment (most due to simultaneous exposure to multiple chemicals and low statistical power). They neither support nor contradict the animal data (OEHHA, 1997a). No human studies of effects of benzene exposure on sperm are available. However, some studies have reported associations between paternal and maternal exposure to benzene and childhood leukemia, while others have not (see Benzene and Childhood Leukemia, page 61).

For female reproductive toxicity, OEHHA (1997a) summarized the available evidence in the following manner. "Consistent reports of abnormal menstruation and excessive blood loss during childbirth in women occupationally exposed to benzene were identified in three cross-sectional studies and in case series and case reports. All three of the cross-sectional studies are limited: the comparison groups were not exposed to the factory environment under study but were not otherwise described; the studies had no apparent matching and poorly described methods. These cross-sectional studies and most of the case series and case reports involve women working in leather and/or rubber factories, and many of the women had benzene-associated toxicity; concurrent exposure to other solvents is likely to have occurred in most cases. More definitive studies with accurate assessment of benzene-specific exposure are needed to further evaluate the associations suggested by these studies."

Immunotoxicity

Immunological effects have been reported from occupational exposures to benzene. Workers exposed to benzene at air concentrations of approximately three to seven ppm showed decreased levels of serum IgA and IgG, but serum levels of IgM were slightly increased. Also, workers exposed to benzene in air as low as 30 ppm had an increased susceptibility to allergies (reviewed in IPCS, 1993). A loss of leukocytes and other blood elements was reported in individuals exposed to benzene at air levels of 15 to 75 ppm. In a study of workers exposed to an average of 100 ppm benzene, no differences in mitogen-induced blastogenesis were observed compared to control workers (IPCS, 1993).

Reduced lymphocyte counts were correlated with increased exposure to benzene in workers from the Pliofilm Cohort (Ward et al., 1996). These findings suggest that exposure to benzene, even to levels lower than five ppm, may result in suppression of lymphocyte counts (Ward et al., 1996). Similarly, Rothman et al. (1996a) also reported exposure-related reductions in lymphocyte counts in Chinese workers exposed to benzene as compared to unexposed workers. For workers grouped by exposure as control (n=44), ≤ 31 ppm (n=22) or > 31 ppm benzene (n=22), mean lymphocyte counts were 1.9, 1.6 and 1.3 thousand cells/μL blood, respectively. These and other studies involving lymphocyte reduction among benzene-exposed workers are described in Appendix B.

The observed lymphocyte depression caused by exposure to benzene may be due in part to inhibition of interleukin (IL-2) dependent T cell proliferation. Prominent metabolites of benzene, namely hydroquinone, catechol and 1,4-benzoquinone, were shown to strongly inhibit proliferation of human T lymphocytes (Geiselhart et al., 1997; Li et al., 1996, 1997, 1998). The inhibition of IL-2 dependent T lymphocyte proliferation appears to be due to inhibition of ribonucleotide reductase (Li et al., 1998).

Carcinogenicity

More than 25 studies have reported increases in cancer rates from occupational exposures to benzene. Reviews of this body of evidence have been published (IARC, 1982; Austin et al., 1988; IPCS, 1993; ATSDR, 1997; Savitz and Andrews, 1997). A summary of epidemiological studies of benzene can be found in Table 8.

French Hospital Patients

Girard and Revol (1970) conducted a hospital-based case-control study to evaluate the association of leukemia and other blood disorders with benzene exposure. Sixty-one cases of chronic lymphocytic leukemia and 56 cases of myeloid leukemia were compared to 124 hospital

controls without hematological problems. Exposure to benzene and toluene were ascertained by a questionnaire in which patients were asked about the chemicals they had used in the past ten years. Relative risks for benzene and toluene were 4.1 (95 percent CI = 1.4 to 12) for chronic lymphocytic leukemia and 1.8 (95 percent CI = 0.5 to 6.6) for myeloid leukemia.

European Oil Company Workers

Thorpe (1974) investigated leukemia incidence among 38,000 workers of a large European oil company and related affiliates from 1962 to 1971. Workers were considered exposed if they had potential exposure to petroleum products or processes with at least one percent benzene. Benzene-exposed workers showed a slight, non-significant increase in leukemia compared to the general population (SMR 121, 95 percent CI = 37 to 205).

Turkish Shoe Workers

Aksoy (1994) conducted a follow-up study of 28,500 benzene-exposed Turkish shoe workers exposed in the 1950s and followed from 1967 to 1990. This study was a follow-up to previous reports of this cohort (Aksoy et al., 1974; 1976; Aksoy, 1980; 1985). As of 1990, 90 cancer cases had been identified including 59 leukemias, 13 lymphomas, five multiple myelomas, eight lung cancers, three myeloid metaplasias, and two cases of paroxysmal nocturnal hemoglobinuria (Aksoy, 1994). The incidence rate for leukemia in benzene-exposed shoe workers during an eight year period was significantly higher than the rate in the general population. The validity of this comparison is limited, however, because ascertainment of leukemia cases within the cohort was incomplete and was not conducted in the same manner as in the general population (Austin et al., 1988). Of the 59 leukemia cases, 14 developed leukemia after diagnosis with pancytopenia (24 percent).

Italian Factory Workers

Vigliani (1976) reported leukemia cases and other blood disorders among benzene-exposed workers in shoe and rotogravure industries in Italy. Eleven leukemia and seven aplastic anemia deaths were observed among 66 cases of hemopathy in workers in Milan exposed to benzene between 1942 and 1974. Thirteen leukemia and three aplastic anemia deaths were reported among 135 cases of benzene hemopathy in workers in Pavia between 1959 and 1974. Exposures were thought to range from 26 to 600 ppm for workers in shoe factories and from 200 to 400 ppm for workers in rotogravure plants. Vigliani (1976) estimated that the incidence rate of acute leukemia among workers was 20-fold greater than the rate in the general population.

Table 8. Epidemiological Studies of Workers Exposed to Benzene and Selected Reanalyses
(Adapted from Austin et al., 1988; IPCS, 1993 and extended)

Population, Study, and Description	Number of Study Subjects	Deaths or cases (exposed)	Disease	Exposure	SMR or RR[a]	95 % CI[a]
French Hospital Patients						
Girard and Revol (1970) Case-control study from 1966 to 1969 of leukemia cases versus hospital controls.	257 cases 124 controls		Acute leukemia	Occupational or household exposure to benzene	RR = 3.3	(1.2-8.9)
			Chronic lymphocytic leukemia		RR = 4.1	(1.4-12)
			Myelocytic leukemia		RR = 1.8	(0.5-6.6)
European Oil Company Workers						
Thorpe et al. (1974) Retrospective cohort study of employees of oil and petrochemical companies from 1962 to 1971.	38,000 workers Referent: general population	18	Leukemia	Employment in processes using ≥1% benzene	SMR = 121	(37-205)
Turkish Shoe Workers						
Aksoy et al. (1974) retrospective study of workers exposed from 1950-1965 during the production of shoes, handbags and related products.	28,500 workers Referent: general population	26	Aplastic anemia; Acute leukemia	150 to 210 ppm for 1 to 28 years. Peak: 210 to 640 ppm. Avg. duration among cases was 9.7 yr	SMR = 200	Not reported
Aksoy (1994) retrospective follow-up study of Turkish shoe workers. Follow-up was to 1990.	28,500 workers Referent: general population	59	Leukemia	Average exposures 150 to 210 ppm	SMR = 226	Not reported

Table 8 (continued). Epidemiological Studies of Workers Exposed to Benzene and Selected Reanalyses

Population, Study, and Description	Number of Study Subjects	Deaths or cases (exposed)	Disease	Exposure	SMR or RR	95 % CI
Pliofilm Cohort						
Infante et al. (1977); Rinsky et al. (1981)						
Retrospective cohort study– exposed between 1940 and 1949 from three plants producing rubber hydrochloride (Pliofilm®), follow-up from 1950 to 1975.	748 workers Referent: general population	7	Myeloid and monocytic leukemia	within legal limits (declining from 100 ppm to 10 ppm)	SMR = 560	p<0.02
		14	Lymphatic and hematopoietic cancers		SMR = 260	p<0.05
Rinsky et al. (1987)	1165 workers	9	Leukemia	Total	SMR = 337	(159-641)
Retrospective cohort study -- follow-up of workers exposed during the manufacture of rubber hydrochloride from 1940 to 1965 (period at risk, 1950-1981).	Referent: general population	2		1-39 ppm-yrs	SMR = 109	(12-394)
		2		40-199 ppm-yrs	SMR = 322	(36-1165)
		2		200-399 ppm-yrs	SMR = 1186	(133-4285)
		3		>400 ppm-yrs	SMR = 6637	(1334-19,393)
		15	Lymphatic and hematopoietic cancers	Total	SMR = 227	(127-376)
		4	Multiple myeloma	Total	SMR = 398	(110-1047)
		3		<40 ppm-yrs	SMR = 458	(92-1339)
		1		>40 ppm-yrs	SMR = 5347	(70-29,753)
Paxton et al. (1994a)	1212 white male workers	3[a]	Leukemia, based on the	0-5 ppm years	SMR = 197	(41-576)
Follow-up of Pliofilm Cohort through 1987, redefinition of the period at risk (beginning in 1940), analyses using three independent exposure estimates.	Referent: general population	3	Rinsky et al. (1987)	>5-50 ppm years	SMR = 229	(47-669)
		7	exposure matrix	>50-500 ppm years	SMR = 693	(278-1428)
		1		>500 ppm years	SMR = 2000	(51-11,140)
		1	Leukemia, based on the	0-5 ppm years	SMR = 88	(2-489)
		4[a]	Crump and Allen (1984)	>5-50 ppm years	SMR = 325	(88-833)
		6	exposure matrix	>50-500 ppm years	SMR = 487	(179-1063)
		3		>500 ppm years	SMR = 1034	(213-3021)

[a] if leukemia death of one female worker were included in the analysis it would have fallen in this exposure group.

Table 8 (continued). Epidemiological Studies of Workers Exposed to Benzene and Selected Reanalyses

Population, Study, and Description	Number of Study Subjects	Deaths or cases (exposed)	Disease	Exposure	SMR or RR	95 % CI
Pliofilm Cohort (continued)		1 2 4[a] 7	Leukemia, based on the Paustenbach et al. (1992) exposure matrix	0-5 ppm years >5-50 ppm years >50-500 ppm years >500 ppm years	SMR = 133 SMR = 179 SMR = 280 SMR = 1186	(3-743) (22-645) (76-716) (476-2444)
		[a] if leukemia death of one female worker were included in the analysis it would have fallen in this exposure group.				
Dow Chemical Workers						
Ott (1978) Retrospective study of benzene workers from 1940 to 1973.	594 workers Referent: general population	2	Leukemia	< 2 ppm for 18 months	SMR = 200	(24-722)
			Myelocytic leukemia		RR = 3.8	
Bond et al. (1986) Retrospective cohort study of workers exposed during manufacturing of resins and organic chemicals 1940 to 1982. This study include the 594 workers from the Ott (1978) study and 362 additional benzene-exposed workers.	956 workers Referent: general population	4	Leukemia	>0.1-35.5 ppm TWA for up to 34 years	SMR = 194	(52-488)
		4 (5 cases)	Myelogenous leukemia		Incidence ratio = 4.4	$p < 0.05$
Minnesota Workers						
Linos et al. (1980) Case-control study of adult leukemia cases in Olmsted County, MN from 1955 to 1974.	138 cases 276 controls	138	Adult leukemia	Medical record of benzene exposure	RR = 3.3	(0.6-28)
U.K. Oil Refinery Workers						
Rushton and Alderson (1981) Case-control study of oil refinery workers from 1950 to 1975.	36 cases 216 controls	36	Leukemia	Employment in low, medium or high exposure to benzene	RR = 2.0 (high or med. vs. low)	(1.0-4.0)

Table 8 (continued). Epidemiological Studies of Workers Exposed to Benzene and Selected Reanalyses

Population, Study, and Description	Number of Study Subjects	Deaths or cases (exposed)	Disease	Exposure	SMR or RR	95 % CI
Texas Refinery Workers						
Tsai et al. (1983) Retrospective cohort study of workers employed at U.S. refinery between 1952 and 1981.	454 workers Referent: general population	0	Leukemia	Median exposure was 0.53 ppm for up to 21 years		
Chemical Plant Workers						
Decouflé et al. (1983) Retrospective cohort study of chemical workers employed in a plant for more than one year during 1947 to 1960 and followed to 1977.	259 workers Referent: general population	4	Lymphatic and hematopoietic cancers	Not quantified, benzene used in large quantities	SMR = 377	(109-1024)
U.S. Chemical Workers						
Wong (1987) Retrospective cohort study of chemical workers from 7 plants in the U.S. exposed for at least 6 months between 1946 and 1975.	3536 exposed and 3074 un-exposed workers	3 5 5 5	All lymphatic and hematopoietic cancers (ICD8: 200-209)	Unexposed <15 ppm-yrs 15-60 ppm-yrs >60 ppm-yrs	RR = 1.00 RR = 2.10 RR = 2.95 RR = 3.93	
		0 2 1 3	Leukemia and aleukemia (ICD8: 204-207)	Unexposed <15 ppm-yrs 15-60 ppm-yrs >60 ppm-yrs	Undefined (SMR = 0.97) (SMR = 0.78) (SMR = 2.76)	
		2 5 4 4	Non-Hodgkin's lymphopoietic cancer (ICD8: 200, 202-207)	Unexposed <15 ppm-yrs 15-60 ppm-yrs >60 ppm-yrs	RR = 1.00 RR = 2.71 RR = 2.96 RR = 4.12	

Table 8 (continued). Epidemiological Studies of Workers Exposed to Benzene and Selected Reanalyses

Population, Study, and Description	Number of Study Subjects	Deaths or cases (exposed)	Disease	Exposure	SMR or RR	95 % CI
Chinese Worker Cohort						
Yin et al. (1987, 1989) Retrospective cohort study of exposed workers from 233 factories in China of the painting, shoe, rubber, leather and chemical industries compared to unexposed workers in 83 machine and clothing factories.	28,460 workers 28,257 controls	30	Leukemia	2 to 345 ppm (grab samples)	SMR = 574	p < 0.01
			Lung cancer		SMR = 231	
Yin et al. (1996); Dosemeci et al. (1994) Retrospective cohort follow-up study of benzene exposed workers from multiple factories in 12 Chinese cities compared with unexposed factory workers, exposures from 1949 to 1987.	74,828 workers 35,805 controls	38	Leukemia	Mean estimates ranged from 20.4 ppm in the 1950s to 11.5 ppm after 1985.	RR = 2.3	(1.1-5.0)
		17	Malignant lymphoma		RR = 4.5	(1.3-28.4)
		125	Cancers of the trachea, bronchus and lung		RR = 1.4	(1.0-2.0)
Hayes et al. (1997)[1] Retrospective cohort follow-up study of benzene exposed workers from multiple factories over 12 Chinese cities relative to unexposed workers.	74,828 workers 35,805 controls	18	All hematological cancers	<40 ppm-yrs	RR = 2.2	(1.1-4.5)
		11		40-99 ppm-yrs	RR = 2.9	(1.3-6.5)
		29		≥ 100 ppm-yrs	RR = 2.7	(1.4-5.2)
		11	Leukemia	<40 ppm-yrs	RR = 1.9	(0.8-4.7)
		8		40-99 ppm-yrs	RR = 3.1	(1.2-8.0)
		19		≥ 100 ppm-yrs	RR = 2.7	(1.2-6.0)
		6	Non-Hodgkin's lymphoma	<40 ppm-yrs	RR = 3.3	(0.8-13.1)
		1		40-99 ppm-yrs	RR = 1.1	(0.1-11.1)
		9		≥ 100 ppm-yrs	RR = 3.5	(0.9-13.2)

[1] Additional details and reports in Dosemeci et al. (1994); Li et al. (1994); Travis et al. (1994); Yin et al. (1994); Rothman et al. (1996); and Yin et al. (1996)

Table 8 (continued). Epidemiological Studies of Workers Exposed to Benzene and Selected Reanalyses

Population, Study, and Description	Number of Study Subjects	Deaths or cases (exposed)	Disease	Exposure	SMR or RR	95 % CI
Chinese Worker Cohort (continued)		5	Acute non-lymphocytic leukemia (ANLL)	<40 ppm-yrs	RR = 1.9	(0.5-7.0)
		5		40-99 ppm-yrs	RR = 4.3	(1.1-16.0)
		11		≥ 100 ppm-yrs	RR = 3.6	(1.1-11.6)
		7	ANLL and myelodysplastic syndromes[2] combined	<40 ppm-yrs	RR = 2.7	(0.8-9.5)
		7		40-99 ppm-yrs	RR = 6.0	(1.8-20.6)
		14		≥ 100 ppm-yrs	RR = 4.4	(1.4-13.5)
		10	ANLL and myelodysplastic syndromes combined	Constant exposure (see text) <10, 10-24, ≥25 ppm	RR = 3.2	(1.0-10.3)
		4			RR = 5.1	(1.3-20.6)
		8			RR = 7.1	(2.1-23.7)
European Shoe Workers						
Paci et al. (1989) Retrospective cohort shoe workers in Florence, Italy employed from 1939 to 1984. Entered into cohort if employed in January 1950.	1008 male and 1005 female workers	4	Aplastic anemia (males)	Levels not reported, exposed for up to 29 yrs	SMR = 1566	P < 0.05
		6	Leukemia (males)		SMR = 400	(146-870)
Fu et al. (1996) Retrospective cohort study which followed up 1005 males and 1003 females from the Paci et al. (1989) through 1990 and an additional 5029 male shoe and boot makers in England exposed from 1939 to 1982 and followed through 1991.	5029 English 2008 Italian Referent: general population	16	Leukemia (English)	Levels not reported, estimated 25 to 600 ppm for Italians. English cohort, exposures unknown.	SMR = 89	(51-145)
		8	Leukemia (Italian)		SMR = 214	(92-421)
			Nasal cancers (English)		SMR = 741	(383-1294)
			Nasal cancers (Italian)		SMR = 909	(23-5065)

[2] Investigated as a possible precursor to acute non-lymphocytic leukemia

Table 8 (continued). Epidemiological Studies of Workers Exposed to Benzene and Selected Reanalyses

Population, Study, and Description	Number of Study Subjects	Deaths or cases (exposed)	Disease	Exposure	SMR or RR	95 % CI
Scandinavian Service Station Workers						
Jakobsson et al. (1993) Retrospective cohort study of gas station attendants in Sweden utilizing 1970 census to identify occupation and 1971-1984 cancer registry for follow-up.	Approximately 9000 men	10	Acute myeloid leukemia	Based on literature, 0.3 to 2.4 ppm	RR = 3.6	(1.7-6.6)
Lynge et al. (1997) Retrospective cohort of gas station attendants in Denmark, Norway, Sweden and Finland, utilizing 1970 census data to identify occupation and cancer registry for 20 year follow-up.	19,000 men	28 13 12	Leukemia Acute myeloid leukemia Nasal cancer	Based on literature, 0.15 to 0.3 ppm	SIR = 0.9 SIR = 1.3 SIR = 3.5	(0.6-1.3) (0.7-2.1) (1.8-6.1)
Petroleum Distribution Workers						
Schnatter et al. (1996a) Case-control study of cancers in Canadian petroleum distribution workers.	14 cases 55 controls	3 8 3	Leukemia	0-0.22 ppm-yrs 0.23-5.49 ppm-yrs 5.50-219.8 ppm-yrs	OR = 1.0 OR = 4.37 OR = 0.92	--- (0.7-48.6) (0.1-11.2)
Rushton and Romanuik (1997) Case-control study drawn from 23,000 petroleum distribution workers in U.K.	91 cases 364 controls	22 47 20 1	Leukemia	<0.45 ppm-yrs 0.45-4.49 ppm-yrs 4.5-44.9 ppm-yrs ≥45 ppm-yrs	OR = 1.0 OR = 1.42 OR = 2.48 OR = 1.35	--- (0.8-2.6) (0.7-3.0) (0.1-12.8)
Monsanto Chemical Workers						
Ireland et al. (1997) Cohort study of U.S. chemical plant workers with low levels of benzene exposure.	4127 workers Referent: general population	5 2 0 3 3	Leukemia Multiple myeloma	0 ppm-yrs 0.5 ppm-yrs 3.5 ppm-yrs 12 ppm-yrs	SMR = 1.1 SMR = 2.5 SMR = 0.0 SMR = 4.6 SMR = 2.3	(0.4-2.6) (0.3-8.9) (0.0-5.4) (0.9-13.4) (0.7-9.4)

[a] SMR, Standardized Mortality Ratio; RR, relative risk; CI, confidence interval

The Pliofilm Cohort

Infante et al. (1977) conducted a retrospective study on a cohort of 748 white male workers occupationally exposed to benzene between 1940 and 1949 in three manufacturing facilities producing rubber hydrochloride (Pliofilm®) in two Ohio cities. Pliofilm is made from a process in which natural rubber is suspended in benzene. This manufacturing process was particularly useful for studying the effects of benzene exposure because little or no other solvents were used. Vital status was obtained for 75 percent of the workers. Cause of death was obtained from death certificates. Rinsky et al. (1981) reported a follow-up of the Infante et al. (1977) study and increased the ascertainment of vital status to 98 percent. Exposures to benzene were estimated to be within the legal limits (e.g., from 100 ppm declining over time to ten ppm). Duration of exposure was up to ten years. The standardized mortality ratio for leukemia among the benzene-exposed workers compared to the general population was 560 (95 percent CI = 225 to 1154) (Rinsky et al., 1981).

It should be noted that risk estimates based on the Pliofilm Cohort may underestimate the true risk of lymphohematopoietic cancer. Testimony at the OSHA hearings on benzene indicated that an additional five leukemias/lymphomas occurred in the cohort but were not included or reported for a variety of reasons (OSHA, 1985).

Rinsky et al. (1987) updated and expanded the retrospective study of the Pliofilm Cohort to include individuals working at least one day in the rubber hydrochloride processing plant between 1940 to 1965, with person-years at risk starting in 1950. The cohort included 1165 white males followed through 1981, representing 31,612 person-years at risk. In 1981 the vital status of the cohort was as follows: 70.3 percent were alive, 28.3 percent dead and 1.4 percent were lost to follow-up and assumed to be alive. A statistically significant increase was observed for all lymphatic and hematopoietic cancers (15 deaths) compared to that expected in the general population (SMR 227, 95 percent CI = 127 to 376). For total leukemia deaths (nine deaths) the SMR was 337 (95 percent CI =159 to 641). An increased risk of multiple myeloma (four deaths) was reported (SMR 398, 95 percent CI = 110 to 1047). Rinsky et al. (1987) in their analysis divided the cohort into four groups with increasing cumulative exposures: <40 ppm-years, 40 to 200 ppm-years, 200 to 400 ppm-years, and > 400 ppm-years. A strong positive trend in leukemia mortality was seen with increasing exposure. The number of deaths observed in the four exposure groups were two, two, two and three corresponding to SMRs of 109, 322, 118 and 6637, respectively. The latency period ranged from less than five years to 30 years, and seven of nine leukemia cases had latency periods of less than 20 years.

Paxton et al. (1994a) reported a follow-up study of the Pliofilm Cohort which extended the work of Rinsky et al. (1981, 1987). The Paxton et al. (1994a) analyses differed from Rinsky et al. (1987) in three primary respects. First, utilizing updated vital status information provided by NIOSH, the follow-up of the cohort was extended from 1981 through 1987. Second, Rinsky et al. (1987) began accumulating person-years at risk of death after January 1, 1950 or the date at which a worker achieved a cumulative exposure of one ppm-year (thereby excluding any cohort deaths before 1950). Paxton et al. (1994a) redefined the follow-up time for the cohort and started accumulating person-years beginning January 1, 1940 (having determined that benzene exposures had been adequately defined by NIOSH during this period). This change resulted in the addition of 13 lymphohematopoietic cancers to the cohort totals, including six leukemias. One of the six leukemia deaths was a female worker. Third, Paxton et al. (1994a) compared the mortality results utilizing three sets of independent exposure estimates, Rinsky et al. (1987), Crump and Allen (1984), and Paustenbach et al. (1992). The Crump and Allen (1984) estimates differ from the Rinsky et al. (1987) estimates primarily in the methods used to estimate exposures during the early years of Pliofilm manufacturing for which scant air monitoring measurements are available.

The Crump and Allen (1984) estimates are based on the assumption that benzene levels in the workplace declined over time in parallel with increasingly restrictive occupational health standards. Paustenbach et al. (1992) conducted a detailed evaluation of exposures and took into account factors such as additional historical records, information gained from worker interviews, dermal exposures, short-term high-level exposures, monitoring devices, long work hours during World War II, and respirator use. Dose-related increases in the risk of leukemia were observed for all three exposure estimates (Table 8).

Utterback and Rinsky (1995) published a critical evaluation of the exposure estimates of Crump and Allen (1984) and Paustenbach et al. (1992) for the Pliofilm Cohort. They examined the underlying premises used by Crump and Allen (1984) and Paustenbach et al. (1992) to estimate worker exposures and indicated that the exposure estimates were inconsistent with the observed hematotoxic effects in the cohort and with new information that has recently become available. Utterback and Rinsky (1995) concluded that both reanalyses overestimated the exposures of the workers and, in light of new information, the original exposure estimates (Rinsky et al., 1981; 1987) are more reasonable and consistent with existing information. As mentioned earlier, Crump and Allen (1984) made adjustments to the NIOSH exposure estimates (Rinsky et al., 1981) based on the assumption that as Threshold Limit Values (TLV) became more restrictive over time benzene exposures were lowered, presumably due to changes in work practice or engineering controls. However, there were no reports of engineering improvements or behavioral changes made during the period between 1942 and 1975 when the Pliofilm plants operated (Utterback and Rinsky, 1995). The Paustenbach et al. (1992) report was severely criticized for selected use of information, improper citations, and utilization of worst-case exposure factors and scenarios (Utterback and Rinsky, 1995).

Dow Chemical Workers

Ott et al. (1978) conducted a mortality study of 594 white male workers exposed to benzene in a Michigan chemical manufacturing facility from 1940 to 1970. Benzene was used to produce alkyl benzene, chlorobenzene, and ethyl cellulose. Other potential co-exposures included vinyl chloride, arsenic-containing compounds, and other suspected carcinogens. No statistically significant increase in total mortality was observed relative to the general population. Three cases of myelocytic leukemia (0.8 expected, $p<0.05$) and one death due to aplastic anemia were reported. Time-weighted average exposure estimates ranged from 0.1 to 6.2 ppm (1944-1973) in one production area, from 0.3 ppm to 14.7 ppm (1953-1972) in a second area, and from 4.0 to 35.5 ppm (1952-1974) in a third area. The U.S. Occupational Safety and Health Administration (OSHA) estimated that this cohort was exposed to an average of five ppm benzene for an average of nine years and concluded that this study provided direct observations of leukemogenic risk at low benzene exposures (OSHA, 1985). The relative risk for myelocytic leukemia from this study was 3.8.

Bond et al. (1986) conducted a follow-up of the 594 workers from Ott et al. (1978), included an additional 362 exposed workers, and extended the observation period through 1982. Four leukemia deaths were observed among benzene-exposed workers compared to 2.1 deaths expected (SMR = 194). Three deaths from lymphatic cancer were also reported. All four leukemias were of the myelocytic type. An additional myelogenous leukemia case was reported, although the cause of death was listed as pneumonia. For this reason, the authors reported an incidence ratio of 4.4 ($p<0.05$) for myelogenous leukemia. Cumulative exposures for the five leukemia cases were 2, 25, 29, 54 and 351 ppm-yr.

Minnesota Workers

Linos et al. (1980) reported the results of a case-control study examining the association between leukemia and benzene exposure. The study reported on 138 leukemia cases and 276 controls. The only criterion used for categorizing a person as exposed to benzene was any mention of benzene in the medical records. An odds ratio of 3.3 (95 percent CI = 0.6 to 28) was reported for benzene-exposed cases versus controls.

U.K. Oil Refinery Workers

Rushton and Alderson (1981) conducted a case-control study of leukemia deaths among workers in eight oil refineries in the U.K. Two sets of controls were chosen from the refinery population, one set matched by year of birth and the other set matched by length of service. Benzene exposures were classified as low, medium and high based on work histories, although no measurements of benzene were available. The relative risk of leukemia was 2.0 (95 percent CI = 1.0 to 4.0) for the workers in the medium- and high-dose groups combined compared to the low-dose group. Relative risks did not appear to relate to length of service.

Texas Refinery Workers

Tsai et al. (1983) reported on a retrospective mortality and medical surveillance study of 454 workers exposed to benzene in an oil refinery from 1952 to 1978. Information on vital status was obtained for 99 percent of the cohort. Extensive industrial hygiene data were available from 1972 to 1982, including 1,394 personal monitoring samples. The data indicated that 84 percent of the benzene exposures were less than one ppm, and the median exposure was 0.14 ppm for all workers and 0.53 ppm for those in benzene-related units. No abnormal blood indices or deaths from leukemia were observed in the study over the 21-year monitoring period. However, the short follow-up period for most workers limits the study's findings of no excess risk of leukemia from benzene exposure. Data from the report of Tsai et al. (1983) were used as the basis of OEHHA's chronic (non-cancer) Reference Exposure Level (see Appendix B) and are also used in this assessment to develop the health protective concentration for non-cancer effects for benzene in drinking water.

Chemical Plant Workers

Decouflé et al. (1983) conducted a mortality study of 259 men employed at a U.S. chemical plant from 1947 to 1960 in which large quantities of benzene had been used. No direct measures of benzene were available and co-exposures to other chemicals were expected. Follow-up of the workers was extended through 1977. Three leukemia deaths (one each of chronic lymphocytic, acute monocytic, and acute myelomonocytic) were reported and 0.44 deaths were expected in the general population (SMR 682, 95 percent CI = 141 to 1992).

U.S. Chemical Workers

Wong (1987) conducted a retrospective study of 4602 benzene-exposed male chemical workers and 3074 unexposed workers from the same or nearby plants in the U.S. The study group included men who were exposed to benzene for at least five months from 1946 to 1977. Jobs were categorized as having either continuous or intermittent benzene exposure. Cumulative exposures were estimated from length of employment and the estimated eight-hour TWA exposure level for each job category. For the group as a whole, exposed workers showed a non-significant excess of leukemia deaths (seven deaths) compared to the general population (SMR 117, 95 percent CI = 47 to 242). Among unexposed workers no leukemia deaths were observed although 3.4 were expected. Exposures were also categorized in the following groupings:

0, <15 ppm-years, 15-59 ppm-years, and ≥60 ppm-years. A dose-related increase in lymphatic and hematopoietic cancers (18 exposed cases) was observed with SMRs of 35, 91, 147 and 175 for the exposure groups, respectively ($p = 0.02$). SMRs for leukemia deaths were 0 (undefined), 97, 78 and 275, respectively.

Chinese Worker Cohort

Yin et al. (1987) reported on a large retrospective cohort study of benzene-exposed workers in China. The study examined 28,460 exposed workers from 233 factories and 28,257 control workers from different industries. Thirty leukemia cases were identified (23 acute, 7 chronic) in the exposed workers compared with four cases in the unexposed controls (SMR 574, $p< 0.01$). Exposure estimates from grab-samples taken at the time of the survey ranged from 3 to 313 ppm with the majority of exposures in the range of 16 to 157 ppm.

A number of detailed reports describing further study and analysis of the Chinese Worker Cohort have been published. Yin et al. (1994) reported that the cohort had been expanded to include 74,828 benzene-exposed workers (since 1949) and 35,805 controls from 712 factories located in 12 Chinese cities. Dosemeci et al. (1994) described the exposure assessment methods. Quantitative estimates of benzene exposure took into account job title and assignment to individual work units, and reflected exposures of individual workers. Li et al. (1994) investigated gender differences in hematopoietic and lymphoproliferative disorders and other cancers among the benzene-exposed cohort. No statistically significant differences in cancer mortality were observed for males versus females, although the number of cases for most endpoints was small. Travis et al. (1994) reported on the hematopoietic malignancies and other blood disorders in the benzene-exposure workers in China. Eighty-two hematopoietic neoplasms and related disorders were observed, including 32 cases of acute leukemia, seven cases of myelodysplastic syndromes, nine cases of chronic granulocytic leukemia, 20 cases of malignant lymphoma, and nine cases of aplastic anemia. In the control workers, 13 hematological malignancies were observed, including six leukemias. Yin et al. (1996) reported the overall cancer findings among the expanded benzene-exposed and control worker cohorts. An increased incidence in the benzene-exposed group compared to controls was observed for leukemia (RR 2.6, 95 percent CI = 1.3 to 5.0), malignant lymphoma (RR 3.5, 95 percent CI = 1.2 to 14.9), and lung cancer deaths (RR 1.4, 95 percent CI = 1.0 to 2.0). Among leukemia cases, incidence of acute myelogenous leukemia was increased in the benzene-exposed group (RR 3.1, 95 percent CI = 1.2 to 10.7). Significant increases were also reported for aplastic anemia and myelodysplastic syndromes.

Dosemeci et al. (1996) attempted to indirectly validate the exposure estimates of the Chinese Worker Cohort by investigating the association of diagnosed cases of benzene poisoning within the cohort with the individually assigned exposure estimates. Benzene poisoning was defined as (1) white blood cell counts $<4000/mm^3$ and platelet counts $<80,000/mm^3$ in repeated tests over several months, (2) documented benzene exposure for greater than six months, and (3) exclusion of other factors that may have altered blood cell counts. Exposure estimates were made for the 18,435 different combinations of exposure factors (e.g., job title, factory, work unit, and years of employment) in order to assign exposure indices to the 75,828 benzene-exposed workers. Some 8477 measurements of air concentrations of benzene in the workplace were utilized to assign exposure indices. An estimated 38 percent of the exposure estimates were based on monitoring data. Three measures of exposure were examined, duration of exposure, intensity of exposure (recent exposure at 1.5 years prior to diagnosis), and cumulative exposure. The authors observed a strong dose-related correlation of benzene poisoning with all three measures of exposure, especially with recent intensity of exposure to benzene.

Hayes et al. (1997) examined the exposure-response relationships for the Chinese Worker Cohort. The benzene-exposed workers were divided into categories based on estimated average exposures, duration of exposure, and cumulative exposure. Because a substantial number of individuals were historically exposed to a relatively stable level of exposure over their working life, a separate measure of "constant exposure" was developed. (Follow-up was censored 1.5 years after a worker's exposure level (i.e., <10, 10-24, and ≥25 ppm benzene) changed for the first time.) Using either average, constant or cumulative exposure indices, Hayes et al. (1997) reported statistically significant dose-related trends for all hematological neoplasms, non-Hodgkin's lymphoma, total leukemia, non-lymphocytic leukemia, and the combined incidence of non-lymphocytic leukemia and myelodysplastic syndromes (Table 8). Since myelodysplastic syndromes may be precursors to acute non-lymphocytic leukemia and were not systematically distinguished from acute non-lymphocytic leukemia in past epidemiological studies, Hayes et al. (1997) combined these two endpoints for comparative purposes. Significant trends of the exposure-response relationships for acute non-lymphocytic leukemia were also observed using average or constant exposure categories; using cumulative exposure, a marginally significant positive trend ($p = 0.06$) was observed. Hematological cancers and related disorders occurred at average exposures of less than ten ppm and cumulative exposures of less than 40 ppm-years. Increased risk of non-lymphocytic leukemia increased with increasing exposure levels, especially for exposures that had occurred within the ten years prior to diagnosis (Hayes et al., 1997). Risk of non-Hodgkin's lymphoma increased with duration of exposure and was strongest among workers exposed for more than ten years prior to diagnosis. Exposed groups had a stronger association when cases of non-lymphocytic leukemia and myelodysplastic syndromes were combined than for those of non-lymphocytic leukemia alone. The strongest association observed was with the combined incidence of non-lymphocytic leukemia and myelodysplastic syndromes and constant exposure (p-value for trend = 0.0003). Data are shown in Table 8.

European Shoe Workers

Paci et al. (1989) conducted a retrospective cohort study of 1008 males and 1005 females in Florence, Italy exposed to benzene during the manufacture of shoes. Inclusion into the cohort was based on employment on or before 1950. Vital status of the workers was obtained through 1984. No direct exposure estimates were available. However, during the highest periods of exposure (1953-1962), approximately 30 kg benzene was used per day. All cases of aplastic anemia and leukemia were observed in individuals working during this period. Standard mortality ratios for aplastic anemia and leukemia in male workers compared to the general population were 1566 and 400, respectively.

Fu et al. (1996) reported the results of a retrospective cohort study which followed 1005 males and 1003 females from the Paci et al. (1989) study through 1990 and included an additional 5029 male shoe and boot makers in England exposed to benzene and other solvents from 1939 to 1982 and followed through 1991. Exposures were not directly measured for the Italian cohort but were estimated to range between 25 and 600 ppm. It is unclear to what extent the English shoe workers were exposed to benzene. A number of aromatic and aliphatic solvents were present in the glues and cements used by the workers. An increased risk of nasal cancers was reported for the English workers (SMR 741, 95 percent CI = 383 to 1294), and a non-significant increase in risk was observed for the Italian workers (SMR 909, 95 percent CI = 23 to 5065). Standard mortality ratios for leukemia were 89 (95 percent CI = 51 to 145) for the English workers and 214 (95 percent CI = 92 to 421) for the Italian workers. Data for the male and female Italian workers were not separated.

Scandinavian Service Station Workers

Jakobsson et al. (1993) used the 1970 Swedish census to obtain occupational information and identify about 9000 petroleum service station attendants. The researchers used the Swedish cancer registry (1971 to 1984) to ascertain cases of acute myeloid leukemia. Among fuel service station attendants, ten cases of acute myeloid leukemia were reported where 2.8 were expected (RR 3.6, 95 percent CI = 1.7 to 6.6). Of the ten cases, latency periods from time of first employment as a fuel station attendant ranged from nine to 36 years. Jakobsson et al. (1993) compiled results from five studies examining airborne exposures to benzene among petrol station attendants. Estimates of air concentrations taken from the published literature ranged from 0.3 to 2.4 ppm (Jakobsson et al., 1993).

Lynge et al. (1997) studied the cancer incidence among petroleum station attendants in a cohort of 19,000 service station workers from Denmark, Norway, Sweden, and Finland identified from the 1970 censuses and followed for 20 years. Incident cancers observed in the cohort (n=1300) were compared to national incidence rates for the respective countries. Based on measurements reported in the literature, average daily exposures to benzene were expected to be low, ranging from 0.15 to 0.3 ppm. An increased risk of nasal cancer was observed (12 cases, SIR 3.5, 95 percent CI = 1.8 to 6.1). The incidence was not significantly increased for leukemia (28 cases, standardized incidence ratio (SIR) 0.9, 95 percent CI = 0.6 to 1.3), acute myeloid leukemia (13 cases, SIR 1.3, 95 percent CI = 0.7 to 2.1), or cancers of the kidney, respiratory tract or lung.

Petroleum Distribution Workers

Schnatter et al. (1996) conducted a case-control study of Canadian petroleum distribution workers and found no increases in risk of leukemia (14 exposed cases), multiple myeloma (14 exposed cases), or non-Hodgkin's lymphoma (nine exposed cases) compared to 55 controls. Exposures were low, ranging from 0.01 to 6.6 ppm benzene, and usually less than one ppm. Family history of cancer and smoking were the strongest risk factors for leukemia.

Rushton and Romanuik (1997) conducted a case-control study of 91 leukemia cases and 364 controls (four controls per case matched by company, age, and follow-up time) selected from a cohort of 23,000 men employed at petroleum distribution centers in the U.K. To be eligible for the cohort, the individual had to have worked more than one year between 1950 and 1975. Work histories were obtained through 1992. Industrial hygiene measurements of benzene concentrations were utilized where possible and were adjusted based on a number of variables influencing exposure. Odds ratios for total leukemia or for acute (myeloid and monocytic) leukemias were approximately two-fold higher in each of the top four cumulative exposure quintiles (0.26-0.59, 0.6-1.64, 1.65-4.78, or ≥ 4.79 ppm-yr) relative to the lowest quintile (<0.26 ppm-yr), although these increases were not statistically significant. Workers employed greater than ten years had odds ratios for total leukemia or for acute leukemia that were approximately three-fold higher relative to workers who had been exposed less than ten years, although these differences were not statistically significant.

Monsanto Chemical Workers

Ireland et al. (1997) conducted a retrospective cohort study of 4127 chemical plant workers in the U.S. The workers in the study were employed at one of the facilities included in the Wong (1987) study, but Ireland et al. broadened the inclusion criteria and extended the follow-up time. Exposures were fairly low and workers were divided into the following exposure categories: unexposed, < 12, 12-72, and ≥ 72 ppm-month. Cumulative exposures ranged from 0.12 to 7584 ppm-month with a median of 36 ppm-month. Rates of total leukemia were elevated but not

statistically significant (SMR 2.3; 95 percent CI = 0.7 to 5.3). The leukemias were of the acute non-lymphocytic and chronic lymphocytic types and occurred predominantly in workers hired before 1950. Incidences of leukemias in each exposure category are provided in Table 8. Rates of multiple myeloma were also increased relative to the general population, but did not reach statistical significance (SMR 2.3, 95 percent CI = 0.7 to 9.4).

Human Cancers Associated with Benzene Exposure

There is considerable debate in the scientific literature regarding the specificity of cancers in humans elicited by exposure to benzene (Infante, 1995; Wong, 1995; Wong and Raabe, 1995, 1997; Eastmond et al., 1997; Savitz and Andrews, 1997; U.S. EPA, 1998). In this section, we will briefly review the arguments for and against causal associations with different cancer types. We conclude that total leukemia is an appropriate endpoint for the dose-response assessment carried out in a later section of this document. It is well established that benzene can cause acute myelogenous leukemia and myelodysplastic syndromes. Strong evidence exists to implicate benzene in causing other forms of leukemia as well. Whether benzene causes lymphoma and multiple myeloma is less clear.

In animals, benzene is clearly a multi-site carcinogen (Table 6, Table 7). However, there is not consensus at this time in the scientific community that benzene is associated with non-hematopoietic cancers in humans. Elevated risks of lung cancer were reported in two large occupational cohorts exposed to benzene (Yin et al., 1996; Lynge et al., 1997), but these findings have not been confirmed in other studies. Smoking status has not been adequately controlled and this may have obscured any association between benzene and lung cancer. For the purposes of this assessment, the primary target sites of concern for benzene-induced cancers in humans are the bone marrow and lymphatic tissues.

Classifications of lymphohematopoietic diseases have changed over time. Table 9 shows the classifications of lymphomas and leukemias by the 7th, 8th and 9th Revisions of the International Classification of Diseases (ICD) and from the French-American-British (FAB) classification system (WHO, 1957, 1967; U.S. DHHS, 1979; Eastmond, 1997).

Acute myelogenous leukemia (AML) (ICD9: 205.0) and Acute non-lymphocytic leukemia (ANLL) (ICD9: 205.0, 206.0, 207.0, 208.0). There is little controversy in the scientific literature that benzene causes AML or ANLL. Increases in AML and ANLL are the most frequently observed cancers in the early case reports (Aksoy, 1988) and occupational studies of benzene exposure (ATSDR, 1997). Several cohort studies have reported significant dose-response relationships for these cancers (Ott et al., 1978; Rinsky et al., 1987; Paxton et al., 1994a; Hayes et al., 1997). Additional evidence comes from mechanistic studies which have observed specific changes in bone marrow cells (reviewed in Smith and Fanning, 1997).

Myelodysplastic syndromes (MDS) (ICD9: 238.7) as a neoplasm or precursor to ANLL. A group of hematological disorders called refractory anemias have been recognized for over 50 years and are characterized by the primary clinical feature of not responding to any known treatment (Lee et al., 1993). Terms such as preleukemia or smoldering leukemia were used in the past to describe these conditions; however, more recently MDS has been proposed as a more accurate description (Lee et al., 1993). If these disorders do not cause death, then they are associated with a high frequency of progression to ANLL. Thus, MDS might be viewed as precursors to ANLL, although not a required step in the pathogenesis of benzene-induced leukemia. The appropriateness of the term MDS has been questioned, since it is not clear whether the abnormal

marrow cells are dysplastic or neoplastic, benign or malignant (Lee et al., 1993). The epidemiological studies on benzene have not been consistent in ascertaining cases of MDS. In the reports of the Chinese Worker Cohort, the subtypes of MDS were not analyzed separately. The National Cancer Institute combined MDS and ANLL for several analyses (Hayes et al., 1997). They noted that MDS have not been systematically distinguished from ANLL in past epidemiological studies.

Table 9. Classifications of Lymphohematopoietic Neoplastic Diseases

Disease/cause of death	ICD-7 (1957-1966)	ICD-8 (1967-1978)	ICD-9 (1979-1998)	FAB codes
Lymphomas	200-202	200-202	200-202	
Hodgkin's disease	201	201	201.0-201.9	
Non-Hodgkin's lymphomas	200.0-200.2 202.0-202.1	200.0-200.1 202.0-202.2	200.0-200.8, 202.0-202.2, 202.8-202.9	NHL
Ill-defined and unspecified sites			159.1, 196.0-196.9, 202.3, 202.5-202.6	
Multiple myeloma	203	203	203.0,203.2-203.8	
Leukemias	204	204-207	204-208	
Lymphocytic	204.0	204	204	
Acute lymphocytic	*	204.0	204.0	ALL, L1, L2, L3
Chronic lymphocytic		204.1	204.1	CLL
Other lymphocytic		204.9	204.2-204.9	
Myeloid (granulocytic, myelocytic)	204.1	205	205	
Acute myeloid	*	205.0	205.0	AML
Myeloblastic leukemia, with minimal differentiation				M0
Myeloblastic leukemia, no maturation				M1
Myeloblastic leukemia with maturation				M2
Promyelocytic leukemia				M3
Myelomonocytic leukemia				M4
Monocytic leukemia				M5
Erythroleukemia				M6
Megakaryoblastic leukemia				M7
Chronic myeloid		205.1	205.1	CML
Other myeloid		205.9	205.2-205.9	
Monocytic	204.2	206	206	
Acute monocytic	*	206.0	206.0	M5
Chronic monocytic		206.1	206.1	
Other monocytic		206.9	206.2-206.9	
Other	204.4	207	207	
Other acute	*	207.0	207.0	
Other chronic		207.1	207.1	CML
Aleukemic, subleukemic, ill-defined or unspecified sites		207.9	202.4, 203.1, 207.2, 207.8, 208.0-208.2-208.9	
Myelodysplastic syndromes			238.7	MDS
Refractory anemia (RA)				RA
RA with ring sideroblasts				RARS
RA with excess blasts				RAEB
Chronic myelomonocytic leukemia				CMML
RA with excess blasts in transformation				RAEB
Chronic myeloproliferative disorders			238.7	

* Acute leukemias (ICD7: 204.3) were not separated by lineage.

Total leukemia (ICD9: 204-208). It has been common practice in epidemiological studies to combine different subtypes of leukemia and treat them as a group of related diseases. There is a rational biological basis for this practice since all blood cells arise from the same small population of pluripotent stem cells located in the bone marrow. Primitive pluripotent cells (roughly 1 in 100,000 marrow cells) and multipotent and early progenitor cells (about 2 to 5 in 1000 marrow cells) proliferate and differentiate to the many mature blood cells, a process controlled by numerous growth factors, cytokines and other cellular regulators (Eastmond, 1997). Multipotent cells are committed to either the myeloid or lymphoid lineage.

However, early progenitor cells committed to the T-cell lineage migrate from the bone marrow to the thymus for further maturation. Pre-B cells mature in the marrow but then migrate to the lymph nodes and the circulatory system. Therefore, the target tissue for lymphomas and some types of lymphocytic leukemias is likely to be the lymphatic system, although early lymphoid progenitor cells may have been damaged in the bone marrow prior to migration to thymus or lymph nodes. The bone marrow is the target tissue for AML, CML, MDS and those lymphocytic leukemias that result from transformation of a bone marrow progenitor cell. Also, different growth factors and cytokines are required for maturation of blood-cells within specific lineages; these factors may be selectively affected by benzene exposure. This could theoretically affect observed dose-response relationships for the different leukemia subtypes. Some investigators have argued that leukemia subtypes are distinct entities and recommend that they be analyzed separately (Wong, 1995; Wong and Raabe, 1995); some have noted that AML and CML may arise from separate stem cells (Maher et al., 1993). Other researchers have argued that the clinical, epidemiological and experimental evidence of benzene-induced hematopoietic damage indicates that benzene induces biochemical damage and alteration of cell proliferation at all levels of the stem cell hierarchy (Gill et al., 1980; Jacobs, 1989; Infante, 1995).

This assessment contends that it is reasonable to use total leukemia as a group for assessment of the cancer risks posed by benzene exposure. Benzene has been shown to alter both stem and progenitor cells in the marrow, and thus can affect precursor cells that give rise to both lymphoid and myeloid cell types. Chronic and acute leukemias appear to result from different genetic alterations in target stem cells and not from differences in tissue of origin. Numerous epidemiological studies have reported statistically significant increases in incidences of total leukemia and benzene exposure (Table 8). Many of these epidemiological studies indicated significant dose-response relationships between benzene exposure and total leukemia (Ott et al., 1978; Rinsky et al., 1987; Wong, 1987; Paxton et al., 1994a; Hayes et al., 1997). Savitz and Andrews (1997) reviewed the results of epidemiological studies of benzene that reported different subtypes of leukemia. They found that relative risks for AML were generally no higher than other myeloid or lymphoid leukemias. The two epidemiological cohorts identified as providing the most useful data for this assessment, exhibited increased relative risks for non-AMML leukemias (combined acute monocytic and myelocytic leukemias) (RR = 1.3 to 1.9, Pliofilm Cohort) (Crump, 1994) and non-ANLL leukemias (RR = 2.0, Chinese Worker Cohort) (Hayes et al., 1997). For the Chinese Worker Cohort, this increase was consistent across all industries (Hayes et al., 1997). It should be noted that the ICD classification system used between 1957 and 1966 (ICD-7) did not separate acute leukemias by cellular lineage; thus, acute lymphocytic, myeloid, and monocytic leukemias had the same classification code (204.3) (Table 9). These practical considerations also support the use of the more general category of total leukemia for risk assessment purposes.

All lymphohematopoietic cancers (ICD9: 200-208). It has also been common practice among many epidemiological investigators to report all leukemias and lymphomas together as a related group of diseases. However, most lymphomas (usually of the B- or T-lymphocytes) originate in

lymph nodes or related extranodal sites, not the bone marrow (Rappaport, 1987). With respect to assessing the risks posed by exposure to benzene, two questions must be answered. (1) Does benzene cause lymphoma? And (2) if so, should risks of benzene-induced lymphoma be addressed separate from leukemia or should they be addressed as part of a related group (i.e., all lymphohematopoietic cancers).

The evidence that benzene causes lymphoma is less clear than for leukemias. In mice, benzene causes increased incidences of lymphoma in several studies (Table 6, Table 7). In humans, benzene is immunosuppressive, as evident by reduced lymphocyte count in benzene-exposed workers (see Immunotoxicity, page 40). Several occupational studies reported increased relative risks of "all lymphohematopoietic cancers" associated with benzene exposure (Wong, 1987; Rinsky et al., 1987; AIPHSP, 1993; Paxton et al., 1994a; Hayes et al., 1997); all were statistically significant. However, only one epidemiological study reported statistically significant increases in relative risk of lymphoma with benzene exposure. Hayes et al. (1997) reported that benzene-exposed workers in China had significantly elevated relative risks of non-Hodgkin's lymphoma compared to unexposed workers. However, the increased risk was not consistent among different industries, an observation that reduces the strength of this association. The association was significant among workers whose exposure occurred greater than ten years in the past but not in workers with exposure less than ten years in the past. Some might argue that the high rate of infections among the Chinese population may be a significant factor contributing to the increased risks of lymphoma. However, Eastmond (1997) examined large benzene studies of workers in Turkey (Aksoy, 1988), the U.S. (Paxton et al., 1994a), and China (Yin et al., 1996) and noted that in all three studies the proportion of lymphoma to the total hematopoietic neoplasia was relatively constant (~20 percent). Thus, the ratio of the lymphoma rate relative to the total hematopoietic neoplasia rate does not appear to differ appreciably between the Chinese, Turkish, or American cohorts examined by Eastmond (1997).

The second issue, whether lymphoma cases should be combined with leukemias into an overall category of lymphohematopoietic cancer or analyzed separately, is also difficult. It is possible that lymphatic tissues have different pharmacokinetic or susceptibility properties than does the bone marrow, and that such differences could affect dose response relationships. It is true that if lymphoma metastases in the marrow grow to become a uniform proliferation, then the appearance is indistinguishable from leukemia (Rappaport, 1987). However, the rate of misclassification between lymphoma and leukemia is expected to be much lower than among specific types of leukemia. Also, since lymphomas and leukemias generally arise from different target tissues, they probably should be analyzed separately.

Multiple myeloma (ICD9: 203). Multiple myeloma is a neoplasm of the plasma cells. Recent cytogenetic studies of individuals with multiple myeloma have reported common genetic abnormalities in plasma cells, myeloid cells and lymphoid cells, suggesting that multiple myeloma arises from an alteration of hematopoietic stem cells (Ng et al., 1999). Benzene exposure has been associated with increases of multiple myeloma in the Pliofilm Cohort (see below) and in other studies (Wong, 1987; Ireland et al., 1997). The magnitude of the relative risks ranged from about two to four; however, due to low number of cases most were not statistically significant. No increased rates of multiple myeloma were observed among benzene-exposed workers in the Chinese Worker Cohort. However, since multiple myeloma occurs primarily late in life, the relatively young age of the Chinese workers reduces the chance of detecting such an association. A meta-analysis of more than 250,000 petroleum workers in four nations did not find increases in mortality ratios of multiple myeloma relative to the general population (Ott and Raabe, 1997).

Conclusions. There is strong support for basing the PHG on a metric of total leukemia. Inclusion of MDS would be desirable, but limitations in reporting MDS mortality rates in the general

population limit this option at present. Also, there is evidence to suggest that exposure to benzene may be associated with lymphoma and multiple myeloma. Currently, these associations have not been as well established as for leukemia in order to justify adding the risks associated with lymphoma and multiple myeloma to the risks estimated for leukemia. Because data are available for the Chinese Worker Cohort, the risks of benzene-induced non-Hodgkin's lymphoma are assessed in this report; however, the results were not used in setting the PHG. They are discussed as a significant source of uncertainty (e.g., potential underestimation) of the true risk.

Benzene and Childhood Leukemia

Benzene has been implicated as a potential risk factor for the development of childhood leukemia (OEHHA, 1997; Ries et al., 1997; Smith and Zhang, 1998; U.S. EPA, 1998). Some large epidemiological studies have reported increases in childhood leukemia associated with *in utero* exposures to benzene, and paternal exposure prior to conception. However, other studies do not suggest an association. The studies and their strengths and limitations are discussed below.

Childhood cancer is the second biggest killer of children (the first being accidents), and the most common form of childhood cancer is leukemia (Sandler and Ross, 1997). Moreover, childhood cases through 14 years of age account for 12 percent of all leukemia cases in the U.S. (Sandler and Ross, 1997). The incidence of leukemia among children younger than 15 years of age has remained fairly constant over the past 20 years except for a sharp increase from 1983 to 1984, which likely reflects a change in diagnosis or reporting (Linet et al., 1999). Deaths rates from childhood leukemia have declined steadily since 1975, which is believed to be due to increased survival from medical advances in treatment (Linet et al., 1999). Exposures to carcinogens during *in utero* development and in early childhood have been suggested as possible causal factors responsible for some of the leukemia cases (Reis et al., 1999). In adults the most common leukemia types are myeloid and lymphatic, whereas the predominant type of leukemia in children is lymphatic. Benzene exposure in adults is most strongly associated with acute myelogenous leukemias (AML), although increased risks of non-AML leukemias are also reported (Crump, 1994; Hayes et al., 1997). Likewise, some epidemiological studies that have examined childhood leukemias by subtype with respect to paternal or maternal exposures to benzene have also found the strongest associations with acute myelogenous leukemias (Shu et al., 1988; Buckley et al., 1989).

Paternal or maternal exposure to benzene and childhood leukemia

A large case control study reported finding a statistically significant association, including a trend in exposure duration, between paternal benzene exposure and childhood acute non-lymphocytic leukemia among progeny (Buckley et al., 1989), while one study with a smaller number of cases did not (Kaatsch et al., 1998). Additionally, a study examining paternal benzene exposure and childhood leukemia (not separated by subtype) reported a positive association (McKinney et al., 1991). Two studies of paternal benzene exposure prior to conception and childhood leukemia (not separated by subtype) (Shaw et al., 1984; Feychting et al., 2001) or acute lymphocytic cases only (Shu et al., 1999) did not find an association. With respect to maternal exposure to benzene, high relative risk estimates have been reported for benzene exposure and childhood acute non-lymphocytic leukemia among progeny in one report (Shu et al., 1988), while a separate report did not find an association (Kaatsch et al., 1998). Studies of childhood acute lymphocytic leukemia, the most common childhood leukemia subtype, did not find an association with maternal benzene exposure (Kaatsch et al., 1998; Shu et al., 1999). Other studies of parental exposures to childhood leukemia and benzene (among other agents)

were also identified (Lowengart et al., 1987; Feingold et al., 1992) but the numbers of cases (or numbers of benzene-exposed parents) were too small to provide any meaningful information.

Shaw et al. (1984) examined the association between disease and risk factors including maternal age, birth order, socioeconomic status, and paternal occupation in a matched case-control study evaluating 255 cases of childhood leukemia reported to the California Tumor Registry. Controls (N=510) were matched by sex and county, by selecting the birth certificate immediately preceding and following the case's birth certificate. Exposure was determined from the occupation of the father as listed on the birth certificate. Occupational classifications determined by the NIOSH National Occupational Hazard Survey 1971-74 were used to classify fathers in the study as "potentially exposed" or "not exposed". This study found no association between paternal benzene exposure and childhood leukemia. As noted by the authors, it is possible that the failure to detect an association in this study is due to misclassification of exposure status. A limitation of the study is the likelihood of multiple chemical exposures confounding the data.

Shu et al. (1988) examined the association between maternal and paternal occupational exposures during pregnancy and childhood leukemia in a well-designed matched case-control interview study in Shanghai, China. Using a population registry, 309 childhood leukemia cases in China were compared to 618 control children. Exposures were ascertained through personal interviews with the parents which inquired about occupational exposures and history of x-rays, drug use, diseases and other potential risk factors. Paternal occupations during pregnancy did was not associated with childhood leukemia, and exposures prior to conception were apparently not ascertained. These investigators found an association between childhood leukemia and maternal occupation in the chemical industry (chemical processors and related workers, rubber and plastic products makers, leather workers, painters, and chemical analysts) (OR 3.3, 95 percent CI = 1.6 to 6.8). They found increased risks associated with self-reported occupational exposure to benzene (OR 2.0, 95 percent CI = 0.9 to 4.3) and gasoline (OR 1.6, 95 percent CI = 0.8 to 3.1). When childhood leukemia cases were separated by histopathological cell type, maternal benzene exposure was found to be associated with statistically significant increased risks of acute non-lymphocytic leukemia (OR 4.0, 95 percent CI = 1.8 to 9.3) but not with acute lymphocytic leukemia. Maternal gasoline exposure was associated with an increased risk of acute lymphocytic leukemia (OR 1.7, 95 percent CI = 1.0 to 3.0).

Buckley et al. (1989) conducted a case-control study of paternal and maternal occupational exposure to benzene of 204 children, aged 18 or less, in the U.S. with acute non-lymphocytic leukemia. The study included 204 controls who were identified by random digit dialing and were matched by date of birth and race. Exposures were assessed through a one hour questionnaire administered to the mother and father. An elevated association between acute non-lymphocytic leukemia and the father's occupational exposure to solvents (including benzene) was observed. Odds ratios (OR) for childhood leukemia and paternal exposure to solvents relative to fathers with no solvent exposure were OR=2.6 (95 percent CI 1.3-5.5) for 1-1000 days exposed and OR=2.0 (95 percent CI 1.2-3.8) for fathers exposed for more than 1000 days. Similar associations were observed for childhood leukemia and paternal exposure to petroleum products (OR 2.4 for prolonged exposure, 95 percent CI = 1.3 to 4.1, p-value for trend = 0.002). This study is limited by the possibility of recall bias, although the authors believed that this was not likely to be occurring. Also, exposure groups included multiple chemicals of which benzene was only a part. One strength of the study for the question at hand is that it focused exclusively on acute non-lymphocytic leukemia, the subtype that is most strongly associated with adult exposures to benzene. No positive associations with maternal exposure to solvents were reported.

In another matched case-control study, McKinney et al. (1991) evaluated the associations between self-reported parental exposures to specific agents and childhood leukemia and non-

Hodgkin's lymphoma in three areas of England with previously documented high rates of these diseases. Children diagnosed with leukemia or non-Hodgkin's lymphoma in the study area between 1974 and 1988 were included in the study. Cases occurring during this period included 113 cases of acute lymphoblastic leukemia (75 percent), 21 other cases of leukemia (14 percent), and 17 cases of non-Hodgkin's lymphoma (11 percent). Each case was matched to two controls by sex, date of birth, and health district of birth. Cases were included in the analysis if data were available for the case and at least one control. Exposure data were collected through face-to-face home interviews that asked questions specifically about parental exposure at work or through hobbies to a variety of suspected toxicants. Twelve of 101 cases (12 percent) compared to six of 178 controls (three percent) had fathers who reported preconception exposure to benzene (OR 5.81, 95 percent CI = 1.67 to 26.44). Of all the specific agents examined, the only independent contributions to risk in the preconceptional period were exposure to wood dust, radiation, and benzene . This study is limited by the possibility of recall bias.

Kaatsch et al. (1998), in a case-control study, examined the associations of various risk factors for 1037 cases of acute lymphocytic leukemia, 147 cases of non-lymphocytic leukemias and 234 cases of non-Hodgkin's lymphoma in Germany. Cases were identified through the German Childhood Cancer Registry. One control for each case was identified and recruited through local registration offices. Controls were matched by age, sex, and place of residence at the time of diagnosis. Exposure information (including benzene exposure) was obtained through self-administered questionnaires and subsequent telephone interviews by trained interviewers. Response rates for both the questionnaire and the telephone interview were different for cases and controls. For example, questionnaire response rates were 81.1 percent for cases and 66.6 percent for controls. Although no data were provided, the authors noted that they did not find any association between parental benzene exposure and childhood leukemias. The authors stated in their methods section that they analyzed the data by leukemia subtype using conditional logistic regression, but no benzene-related results by subtype were presented. Kaatsch et al. (1998) found no associations between exposure to ionizing radiation and childhood leukemia, and reported a significant negative association between maternal smoking and childhood leukemia. The study may be limited by response bias, which the authors did not address in the discussion of the findings. As with the other studies, this study is limited in that multiple chemical exposures of the parents are potential confounders. A strength of the study is the large number of subjects examined.

In a case-control study, Shu et al. (1999) examined 1984 case of child acute lymphocytic leukemia in the U.S. and Canada, identified through the Children's Cancer Group. Controls (N=1986) were selected by random digit dialing, matched by age, race and area code. Exposure information was obtained through a questionnaire and telephone interviews with the mother and also with the father if available. Paternal or maternal exposure to benzene or "petroleum products," either prior to conception, during pregnancy or postnatal was not associated with childhood acute lymphocytic leukemia. As with the studies described above, this study is limited in that multiple chemical exposures of the parents are potential confounders. The numbers of cases assessed in the study is high; however, the study was limited to acute lymphocytic leukemias. Benzene is most commonly associated with acute non-lymphocytic leukemias from occupational exposures as adults (ATSDR, 1997). Also, Shu et al. (1988) suggested that benzene exposure *in utero* was more strongly associated with childhood non-lymphocytic leukemia than childhood lymphocytic leukemias. Thus, limiting the focus of the study to acute lymphocytic leukemias, as was done by Shu et al. (1999), may reduce the ability to observe an association with benzene exposure.

Feychting et al. (2001) examined 161 leukemia cases as part of a larger cohort study of Swedish children born to married couples in 1976-77 or 1981-82 (N=235,635 births). All children were

followed through 15 years of age, and their vital status was determined through the Swedish Cause of Death Registry. Exposures to the father were obtained from the father's occupation as listed on the 1975 census (for births occurring in 1976 or 1977) or on the 1980 census (for births occurring in 1981 or 1982). The father's occupation was linked to a job-exposure matrix constructed as part of the study by industrial hygienists. Two occupational hygienists assessed the probability of exposure to different agents based on the type of industry and job title. Benzene was one of many specific compounds considered. Benzene was not significantly associated with paternal exposure prior to conception (RR = 1.23, 95 percent CI 0.39-3.85). The study strengths included the cohort study design which does not have the potential for recall bias and large numbers of children considered, although the total number of leukemia cases was moderate (N=161). Potential limitations of the study include significant possibility for exposure misclassification. Also, the study did not examine associations of benzene exposure with leukemia subtype.

Benzene is a component of gasoline and diesel fuels and engine exhaust; thus, workers in occupations closely related to motor vehicles are expected to be exposed to benzene. Researchers from the National Cancer Institute published a review of the epidemiological studies of childhood leukemia and paternal exposures via occupations involved with motor vehicles or exhaust gases (Colt and Blair, 1998). They summarized the evidence as follows:

> "There have been 12 studies of childhood leukemia and paternal employment in occupations related to motor vehicles or involving exposure to exhaust gases. Elevated risk was found in most of these studies, with statistically significant findings in six. Significant associations were found among diverse occupations such as motor vehicle or lorry drivers..., mechanics and gas station attendants..., and broader groups of motor vehicle-related occupations.... In their review of leukemia, Linet and Cartwright ... suggested that the link between motor vehicle occupations and adult leukemia may be due to benzene and other components in engine exhausts.".

As with the case-control studies described above, this database provides suggestive evidence of an association between parental exposure to benzene and childhood leukemias.

Sperm DNA damage

Associations of paternal exposures and childhood leukemia are consistent with observations in animals that benzene induces DNA damage in sperm. Mice administered benzene via i.p. injection at seven doses ranging from 0.1 to 1.0 mL/kg-day on five successive days exhibited statistically significant dose-related increases in sperm head abnormalities in dose groups 0.4 mL/kg or higher with a peak effect at 0.6 mL/kg-day (Topham, 1980). Dose-related increases in chromosomal aberrations (breaks, fragments, exchanges) in the sperm were also observed in mice following administration of single oral doses of benzene at 0.25, 0.5 or 1.0 mL/kg relative to controls (Ciranni et al., 1991). The doses used in these studies were high, and it is not known if the effects would be detected at lower doses.

Transplacental genotoxicity and carcinogenicity

Associations of maternal exposures and childhood leukemia in humans are supported by observations in animal studies that indicate that benzene crosses the placenta and induces DNA damage in the fetus. In mice, hematopoiesis is initiated in the fetal liver on gestational day 10, and peaks on gestational day 12 or 13, which is soon followed by the initiation of hematopoiesis in the bone marrow (OEHHA, 1997a). Thus, gestational days 13 to 15 are considered a sensitive period for induction of hematopoietic genotoxicity in the fetal liver. For this reason, most of the relevant studies administered benzene on gestational days 13 to 15.

Increases in benzene-induced micronuclei were observed in fetal liver erythrocytes (polychromatic erythrocytes, PCE) in three studies (Ciranni et al., 1988; Ning et al., 1991; Xing et al., 1992) following exposure of the dams to benzene. A significant increase in fetal liver PCE micronuclei was found in mice given benzene by gavage on gestational day 13 (Ciranni et al., 1988) and on gestational day 14 or 15 (Ning et al., 1991; Xing et al., 1992), but not when given on gestational days 16 to 17 (Harper et al., 1989). Also, two studies reported increases in sister chromatid exchange in fetal cells after dams were administered benzene i.p. (Sharma et al., 1985; Xing et al., 1992).

Most studies of transplacental genotoxicity compared effects in the fetus to those in the dam. Two studies found effects in the fetus (liver) and the dam (bone marrow) at similar doses (Sharma et al., 1985; Xing et al., 1992). Two other studies, both using i.p. administration (Ning et al., 1991; Xing et al., 1992) reported an effect in the fetus at a lower dose than in the dam. Thus, mouse fetuses appear to be susceptible to the genotoxic effects of benzene, but sensitivity relative to dams is unclear. Additionally, two studies using oral administration compared benzene-induced genotoxicity in the fetus, dam, non-pregnant female, and adult male. Cirrani et al. (1988) found similar increases in micronuclei of PCEs in virgin females as in pregnant dams and fetuses, but a larger effect in males. Harper et al. (1989) also reported a larger effect in males, a smaller effect in virgin females and, as mentioned above, no effect in pregnant dams or their fetuses.

Some information on transplacental carcinogenicity comes from studies in which rats were exposed to benzene (200 ppm) throughout gestation and lactation, and for an additional eight weeks after weaning (Maltoni et al., 1985, 1989). Cancer rates in the offspring were compared to control animals and to their dams, who were exposed to the same concentration of benzene for the same period. Although no statistical analyses was reported, the authors stated that "an enhanced carcinogenic effect of benzene was observed in animals on which treatment was started during embryonal life" and that animals whose exposure began *in utero* appeared to have a higher incidence of some tumor types (Maltoni et al., 1985). No carcinogenicity studies of benzene where exposure was limited to pregnancy were located.

Transplacental alteration of hematopoiesis

As discussed in the Carcinogenic Mode of Action section, epigenetic mechanisms are likely involved in benzene-induced leukemia and include the alteration of hematopoiesis and clonal selection. Evidence in animals suggests that exposure to benzene *in utero* alters maturation of lymphocytes, erythrocytes and granulocytes (OEHHA, 1997a). The consequences of *in utero* exposure to benzene can be detected as alterations in cell population numbers and functional properties that in several cases persist into adulthood (Keller and Snyder, 1986, 1988; Corti and Snyder, 1996). Damage during the initial *in utero* stages of hematopoiesis could have lasting effects as has been demonstrated for a number of other toxicants (OEHHA, 1997a). Studies of developmental hematopoietic toxicity observed effects in rodents following inhalation exposures to benzene as low as 5 to 20 ppm (Keller and Snyder, 1986, 1988; Corti and Snyder, 1996).

Conclusions

There are little data on the effects of direct exposure of children to benzene. Because of their small size, increased activity, and increased ventilation rates compared to adults, children may have greater exposure to benzene in the air, on a unit body weight basis (U.S. EPA, 1998).

Although no human cancer studies of benzene-exposed children are available, there is good reason to believe that childhood exposure to benzene would also contribute to adult-onset leukemias. Also, there is some evidence to suggest that exposure to benzene is associated with childhood leukemia. Paternal exposure to benzene prior to conception in humans has been associated in some studies with increased childhood leukemia, especially of the acute non-lymphocytic type, findings that are supported by observations in animals of benzene-induced DNA damage to sperm. Maternal exposure to benzene in humans also has been associated with increased incidences of childhood leukemia. These findings are supported by observations in animals of benzene-induced transplacental genotoxicity, altered hematopoiesis, and of carcinogenicity, following exposure *in utero* and continuing until weaning. It should be noted that other epidemiological studies that represented a large number of cases of various subtypes of leukemia (Kaatsch et al., 1998) or acute lymphocytic leukemia only (Shu et al., 1999) did not find an association with paternal benzene exposure. Thus a casual relationship would be difficult to establish.

Carcinogenic Mode of Action

Benzene and benzene metabolites elicit myriad potentially adverse effects. Coupled with benzene's complex metabolism, it is understandable that, despite extensive research, the carcinogenic mechanism of action of benzene remains elusive. As described in the sections above, there is extensive evidence of benzene's potential to cause DNA damage and alter hematopoiesis. In workers, benzene exposure has been associated with clastogenic effects (including chromosomal aberrations, aneuploidy, micronuclei, translocations and rearrangements), oxidative DNA damage, and mutations (see Genetic Toxicity, page 38). The purpose of this section is to describe the molecular mechanisms that may give rise to these observed effects, as well as epigenetic mechanisms that may be involved in benzene-induced leukemogenesis.

A number of different mechanistic factors that may be playing a role in benzene-induced leukemias are listed in Table 10. These factors that may contribute to carcinogenic mechanisms include direct DNA damage, altered DNA replication, and altered cell proliferation and differentiation. In all cases, the metabolites of benzene are likely responsible for these effects. It should be stressed that these actions are not mutually exclusive and in many cases are interrelated. For example, hydroquinone can not only alter bone marrow stem cell recruitment but also can be activated by bone marrow peroxidases to DNA-reactive quinone species. Several recent reviews describing benzene's mode of action have been published (Snyder and Kalf, 1994; Irons and Stillman, 1996; Smith, 1996b; Smith and Fanning, 1997; Eastmond, 1997; Smith and Zhang, 1998; U.S. EPA, 1998).

DNA Damage

Benzene has the potential to elicit DNA damage either directly, such as through DNA binding of metabolites resulting in mutagenic or clastogenic effects, or indirectly, for example through interaction with cellular constituents involved in DNA replication. Specific chromosomal alterations are commonly associated with background, therapy-related, and benzene-related leukemias (Pedersen-Bjergaard and Rowley, 1994; Smith and Fanning, 1997; Smith and Zhang,

1998). Thus, those mechanisms that lead to DNA strand breakage, rearrangement, or aneuploidy are likely to be the most important in benzene pathogenesis.

There are several possible mechanisms by which benzene can cause direct DNA damage. In animals, radiolabeled benzene administered to rodents resulted in significant binding to DNA and other cellular macromolecules; however, the magnitude of the binding is relatively low compared to other genotoxic agents (Arfellini et al., 1985; Muzzullo et al., 1989). There are numerous potentially reactive metabolites of benzene (Table 4, page 16) which include various epoxides, quinones, aldehydes and free radical species. Several DNA adducts of 1,4-benzoquinone (Pongracz and Bodell, 1991; Bodell et al., 1993; Reddy et al., 1989, 1990; Chenna et al., 1995) and benzene oxide (Krewet et al., 1993) have been characterized. Interestingly, recent work has suggested that NQO1 protects the marrow against hydroquinone- and 1,2,4-benzenetriol-induced toxicity through an unexpected mechanism, inhibition of high molecular weight DNA adducts (Wiemels et al., 1999). Adducts from other metabolites such as phenol, catechol, 1,2,4-benzenetriol, and t,t-muconaldehyde have been observed *in vitro* (Eastmond, 1997). Dose-related DNA adduction from *in vivo* exposure of rodents to benzene has been reported in studies employing standard radiobinding techniques (Arfellini et al., 1985; Muzzullo et al., 1989), ^{32}P-postlabeling assay (Levay et al., 1996), and accelerator mass spectrometry (Creek et al., 1997). DNA adducts of benzene oxide (i.e., phenylguanine) have been observed in the urine of workers exposed to benzene (Norpoth et al., 1988). Dose-related increases in protein adducts of benzene oxide (which serve as a surrogate for DNA binding) have been observed in the blood of workers exposed to benzene (Yoewell-O'Connell et al., 1998). Also, protein adducts of benzene oxide, 1,2- and 1,4-benzoquinone were measured in the blood and bone marrow of rats and mice following exposure to benzene (McDonald et al., 1994). High background levels of benzoquinone DNA adducts in rodents (Reddy et al., 1994) and benzoquinone protein adducts in rodents and humans (McDonald et al., 1993; Waidyanatha et al., 1998) have been observed. The role that these background adducts play, if any, in benzene toxicity is unknown. It seems likely that benzene exposure could result in protein-DNA or other macromolecular crosslinks because its metabolite 1,4-benzoquinone readily forms crosslinked conjugates with many diverse substrates (Tijssen, 1985) and has commonly been used for this purpose as an immunochemistry reagent.

Table 10. Factors potentially involved in the carcinogenic modes of action of benzene

Factors	Characteristics
Multiple sources of potential DNA damage (direct or indirect) • Quinones • Benzene oxide • *t,t*-Muconaldehyde and other open-ring metabolites • Oxygen and other free radicals	Chromosomal damage (including, aneuploidy, aberrations, SCE, micronuclei, strand breaks, non-disjunction, rearrangements and translocations) and mutation. Adducts, abasic sites. Spindle poison (i.e., quinone-binding to tubulin and histones). Inhibition of topoisomerase II.
Multiple possible epigenetic mechanisms • Alteration of cytokine-dependent cell proliferation and differentiation • Alteration of apoptosis • Selective pressure for clonal selection • Leukemia as a secondary effect of severe (high-dose) marrow toxicity	Altered stem cell recruitment and development, involving inflammatory mediators, growth factors, and second messengers. Abnormal bone marrow cell kinetics. Benzene-resistant cells, apoptosis-resistant cells, progression of aberrant cells. Association of aplastic anemia and similar conditions with leukemia.

The phenolic metabolites of benzene, hydroquinone, catechol and benzenetriol, have been shown to induce chromosome breakage and interfere with chromosome segregation (Eastmond, 1993; Chen et al., 1994). Hang et al. (1996) and Singer and Hang (1999) studied the mechanism of cleavage of 1,4-benzoquinone-derived adducts by human apurinic/apyrimidinic (AP) endonuclease to form abasic sites. Sagi et al. (1998) demonstrated that DNA adducts of 1,4-benzoquinone also reduce DNA duplex stability.

Additionally, benzene may act indirectly to cause DNA damage. Peroxidase metabolism of phenol, hydroquinone, and other phenolic metabolites is accompanied by production of oxygen free radicals, lipid peroxidation products, and glutathione free radicals (Subramanyam et al., 1991). Oxidative DNA damage, as measured by formation of 8-hydroxy-2'-deoxyguanosine, was observed in marrow-derived cells in culture treated with benzene metabolites. Increased 8-OHdG formation in marrow cells was reported in mice administered benzene *in vivo* (reviewed in Smith, 1996b) and in workers exposed to benzene (Liu et al., 1996; Nilsson et al., 1996). Increased levels of 8-OHdG have been shown to cause point mutations and strand breakage (Smith, 1996b). Various phenolic metabolites of benzene also act to inhibit topoisomerase II (Chen and Eastmond, 1995). Inhibition of this enzyme may function to cause strand breaks resulting in chromosomal aberrations and translocations (Smith, 1996b).

In addition, quinone species, such as 1,4-benzoquinone, have a high binding affinity for sulfur groups such as glutathione and cysteinyl residues of proteins (McDonald et al., 1993; 1994). Quinol thioethers result from reaction of glutathione and hydroquinone (enzymatically) or 1,4-benzoquinone (non-enzymatically). These quinol thioethers have been implicated in benzene-induced hematotoxicity (Bratton et al., 1997) via generation of oxygen free radicals (Rao et al., 1996). The high affinity of 1,4-benzoquinone for sulfur groups may also cause chromosomal damage through other potential mechanisms. Benzene metabolites were found to bind selectively to the histone fraction of mouse bone marrow cells (Smith, 1996b), thus potentially altering DNA winding in the nucleus and making the DNA more susceptible to subsequent damage. Histones contain a high proportion of sulfur-containing cysteine. Also, 1,4-benzoquinone has been hypothesized to interact with mitotic spindle formation, giving rise to aneuploidy. Tubulin, the polymeric protein which forms the mitotic spindle, is rich in cysteine. Interestingly, Neun et al. (1994) observed that mouse erythroid progenitor cells that survive exposure to benzene develop into bone marrow cells that are resistant to the toxic (quinone) metabolites of benzene. The sulfhydryl content of the surviving (resistant) marrow cells was found to be twice as high as cells from untreated mice.

Epigenetic mechanisms

Several epigenetic mechanisms may be involved in benzene leukemogenesis. These include alteration of marrow cell proliferation and differentiation, alteration of apoptosis, and alteration of clonal expansion of blood progenitor cells. A great deal of research has investigated benzene's ability to alter bone marrow cell development. These perturbations may proceed through a variety of means including alteration of inflammatory mediators, growth factors and other cellular messengers (MacEacher and Laskin, 1994; Snyder and Kalf, 1994). IL-1 has been shown to prevent benzene-induced bone marrow toxicity, and hydroquinone and 1,4-benzoquinone have been shown to inhibit the activation of IL-1 (Renz and Kalf, 1991; Miller et al., 1994; Niculescu et al., 1995). Later work indicated that granulopoiesis is stimulated through inhibition of IL-1 (Niculescu and Kalf, 1995). Irons and coworkers demonstrated that treatment of mouse or human marrow progenitor cells of the granulocyte-macrophage lineage with hydroquinone increased the number of colonies dependent on granulocyte-macrophage-colony-stimulating-factor (GM-CSF) (reviewed in Irons and Stillman, 1996). In theory, this increase provides more targets for the genotoxic effects of benzene (Smith, 1996b). Hazel and

Kalf (1996) reported that induction of granulocyte differentiation by hydroquinone involves the leukotriene D_4 receptor.

In addition to the hematopoietic progenitor cells that give rise to circulating blood cells, bone marrow contains a highly developed stroma made up of several different cell types. The bone marrow stroma supports the survival and differentiation of hematopoietic cells via production of a range of growth factors and cytokines. In a study of bone marrow samples taken from workers with symptoms of benzene poisoning, abnormalities of the stroma were observed in 37 percent of 152 patients (Ruiz et al., 1994). Several investigators have reported that, in mice, the bone marrow macrophages are particularly subject to cytotoxicity (Gaido and Wierda, 1984; Thomas et al., 1989; Twerdok and Trush, 1990; Chertkov et al., 1992; Ganousis et al., 1992); toxicity appears to be modulated by NQO1 status. One potential mechanism for reduced stromal function after benzene exposure may be disruption of IL-1 production (Thomas et al., 1989; Renz and Kalf, 1991; Miller et al., 1994; Niculescu et al., 1995). The role of IL-1 in early hematopoiesis is not completely defined, but this cytokine causes fibroblasts and endothelial cells to secrete other factors that act on developing hematopoietic cells. Therefore, disruption of IL-1 production and/or other stromal changes affecting hematopoiesis results in selective pressure for the development of abnormal clones.

Other epigenetic mechanisms that may be involved in benzene tumorigenesis include inhibition of apoptosis (Hazel et al., 1996; Hiraku and Kawanishi, 1996), hyperphosphorylation of pRb105 and p53 (growth promoter effects) (Dees and Travis, 1994; Dees et al., 1996), and stimulation of mature marrow leukocytes to produce elevated levels of growth regulatory cytokines such as TNFα and IL-1 (MacEachern and Laskin, 1992). Bone marrow leukocytes from hydroquinone- and benzenetriol-treated mice showed increased sensitivity to the inflammatory mediators lipopolysaccharide and interferon-γ which resulted in increased production of nitric oxide (Laskin et al., 1995).

Clonal selection is an important event in leukemogenesis (Smith and Fanning, 1997). Benzene and its metabolites may exert a selective pressure on blood cell maturation that facilitates the growth of aberrant clones. The degree to which the epigenetic mechanisms described above may impact clonal selection is not well understood at this time (Smith and Fanning, 1997).

Some scientists have suggested that a preleukemic state arising out of (high dose) marrow toxicity is a necessary step in benzene-induced leukemogenesis. This contention probably arose because the early case reports of benzene-induced blood disorders such as aplastic anemia were identified in workers with very high levels of benzene exposure. A high proportion of these cases progressed to leukemia. High dose toxicity and compensatory mitogenesis are likely to play a role in some of the documented cases of benzene-induced leukemia. However, extensive evidence of from epidemiological studies of leukemia and MDS arising in groups of individuals with relatively low exposures to benzene (see Carcinogenicity, page 41) would argue against the notion that a high-dose bone marrow toxicity preleukemic state is a required step in leukemogenesis.

Thus, it appears that benzene could elicit DNA damage and initiate the leukemogenic process through several different mechanisms. Additionally, various epigenetic mechanisms may function to make genetic damage more likely in certain populations of bone marrow cells by altering proliferation and development, and may play a role in clonal selection and expansion of aberrant clones. The diverse range of adverse effects that stem from the different metabolites of benzene indicate that no single pathway is likely responsible for all benzene-induced leukemias.

Inter-individual Variability and Benzene-induced Hematotoxicity

Scientific evidence from early case reports and from recent molecular epidemiological research indicates numerous sources of inter-individual variability in susceptibility to the toxic effects of benzene. These susceptibilities stem from both environmental and host factors. Significant sources of variability in the population stem from genetic polymorphisms in key enzymes involved in the metabolism of benzene, namely, cytochrome P450 2E1, NADPH-dependent quinone oxidoreductase, myeloperoxidase, glutathione S-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. Other factors such as pre-existing inflammation, radiation exposure, and ethanol consumption also can potentiate the toxic effects of exposure to benzene. Taken together, the range of susceptibility to benzene toxicity within the general population could be very large.

Genetic Susceptibility

The impact of inter-individual variability in the key enzymes involved in benzene metabolism and toxicity has been investigated in a few studies. These enzymes include cytochrome P450 2E1 (CYP2E1 gene product), NADPH-dependent quinone oxidoreductase (NQO1 gene product, also called DT-diaphorase), glutathione S-transferase, and myeloperoxidase. Others may be implicated in the future. Significant portions of the general population carry genetic variants (polymorphisms) of these enzymes. These polymorphisms may increase or decrease an individual's susceptibility to the toxic effects of benzene as described below.

To review, benzene is metabolized by liver cytochrome P450 2E1 to benzene oxide, which spontaneously forms phenol (Figure 2, page 14). Phenol is in turn further oxidized by P450 2E1 to di- and tri-hydroxybenzenes, such as hydroquinone and 1,2,4-benzenentriol. Thus, P450 2E1 plays a central role in activating benzene to toxic metabolites (Valentine et al., 1996). Benzene oxide can be detoxified by glutathione transferase leading to the formation of phenylmercapturic acid. Benzene oxide can also be converted to benzene dihydrodiol by epoxide hydrolase in the liver. Benzene dihydrodiol, in turn, can be converted to catechol or can spontaneously form an open ring product, *t,t*-muconaldehyde (a hematotoxic and genotoxic metabolite) leading to *t,t*-muconic acid. The phenolic metabolites of benzene (hydroquinone, catechol and 1,2,4-trihydroxybenzene) can be further converted by myeloperoxidase in the bone marrow to benzoquinone and semiquinone species (potent hematotoxic and genotoxic metabolites). Benzoquinones can be reduced back to less toxic hydroxybenzenes by the activity of NQO1.

CYP2E1 and NQO1

CYP2E1 is polymorphic and inducible, and NQO1 is polymorphic. A 50-fold difference in the relative rates of CYP2E1 enzyme activity has been measured between individuals. A single mutation in NQO1 has been found in a significant portion of the population, which varies widely among ethnic groups. The activity of NQO1 decreases by about three-fold in individuals with one variant allele (wild type/variant) and results in a loss of activity for homozygous (variant/variant) individuals (Kuehl et al., 1995; Wiencke et al., 1997).

Rothman et al. (1997) evaluated the impact of genetic variants of CYP2E1 and NQO1 and their association with the development of benzene-induced hematotoxicity in Chinese workers. Fifty cases of benzene-induced toxicity and matched controls were assayed for CYP2E1 activity (phenotype) and NQO1 genotype. Study subjects were categorized as rapid or slow metabolizers, based on the rate of metabolism of chlorzoxazone (a drug metabolized primarily by P450 2E1) over the eight-hour period. To assess NQO1 polymorphisms, a PCR assay was utilized to test for a homozygous $^{609}C \rightarrow T$ mutation in NQO1, which causes loss of enzyme

activity. The joint effects of CYP2E1 and NQO1 status, shown in Table 11, indicate that rapid CYP2E1 activity or a non-functional NQO1 increase an individual's risk of benzene hematotoxicity.

Table 11. Joint effects of CYP2E1 activity and NQO1 genotype on benzene-induced hematotoxicity in Chinese workers (from Rothman et al., 1997)

CYP2E1 activity	NQO1 genotype	Cases	Odds Ratio (95 %CI) benzene hematotoxicity[1]
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)

[1] Adjusted for age and sex.

Traver et al. (1997) estimated the NQO1 genotype ($^{609}C \rightarrow T$) on 96 human lung tissue samples, utilizing the same PCR assay as described in Rothman et al. (1997). Samples were from Caucasian, African-American, and Hispanic donors. Seven percent of the individuals were homozygous for the mutation (i.e., a loss of activity for NQO1), 42 percent were heterozygous and 51 percent were homozygous for the wild type. Wide variability was observed between males and females and across different ethnicities. The incidences of homozygosity for the mutation were three percent for males, 25 percent for females, 18 percent for Caucasians, zero percent for Hispanics, and zero percent for African-Americans.

Kelsey et al. (1997) investigated the frequency of wild type and variant (V) genotype of NQO1 in 114 non-Hispanic whites, 161 Mexican-American Hispanics, 136 African-Americans, 69 Korean-Americans and 49 Chinese-Americans (Table 12). As much as a five-fold difference in the frequency of homozygous variants (V/V) was observed among ethnic populations.

Table 12. Frequencies of NQO1 genotype among different ethnic groups (from Kelsey et al., 1997)

Ethnic Group	Wild type/Wild type	Wild type/Variant	Variant/Variant
Non-Hispanic white	56.1 %	39.5 %	4.4 %
Mexican-American Hispanic	32.2 %	52.2 %	15.5 %
African-American	61.0 %	33.8 %	5.2 %
Asian-Americans	31.4 %	48.3 %	20.3 %
Korean	33.3 %	47.8 %	18.8 %
Chinese	28.6 %	50.5 %	22.4 %

Seaton et al. (1994, 1995) examined inter-individual variability of benzene metabolism in activation reactions (CYP2E1) and detoxification reactions (phenol sulfation and hydroquinone glucuronidation). Human liver samples donated by ten individuals were assayed for CYP2E1 activity by measuring the rate of hydroxylation of *p*-nitrophenol. CYP2E1 activity varied by about 13-fold (1.3 nmol/mg/min ± 0.85 SD) among these ten samples.

Stephens et al. (1994) noted that there is a 50-fold variation in CYP2E1 activity among humans, and it is unclear whether this variation stems from genetic or environmental factors. The researchers investigated the frequency of two polymorphisms in the CYP2E1 gene among different ethnic groups. The first polymorphism, detectable by *Rsa*I restriction enzyme digestion,

is in a regulatory region involved with transcriptional activation of the gene. The second polymorphism, detectable by *Dra*I restriction enzyme digestion, is located in an intron near the *Rsa*I site. Rare alleles at each of these loci have been associated with increased lung cancer in some populations. Among the 695 individuals examined, statistically significant differences in allelic frequencies were observed between Taiwanese and African-Americans or European-Americans for each polymorphism. Allelic frequencies for variants in the *Rsa*I and *Dra*I sites, respectively, were 28 percent and 24 percent in Taiwanese, one percent and eight percent in African-Americans, and four percent and 11 percent in European-Americans.

Myeloperoxidase

Myeloperoxidase activates the phenolic metabolites of benzene, forming many reactive species including semiquinones, benzoquinones, and other free radical byproducts (Subrahmanyam et al., 1991). Myeloperoxidase is found at high concentrations in the bone marrow and is present primarily in the lysosomes of neutrophils. Austin et al. (1993) described a genetic polymorphism in the myeloperoxidase gene involving a single base substitution (G to A) in the promoter region of the gene. For those individuals who have two copies of the variant gene (homozygous, A/A), expression of the gene is decreased by about two-thirds (Piedrafita et al., 1996). Thus, individuals who are homozygous variants would be predicted to be at lower risk for hematotoxic effects of benzene. Rates of the polymorphism did not appear to differ substantially among ethnic populations (London et al., 1997) (Table 13).

Table 13. Frequency of myeloperoxidase polymorphism in control individuals (from London et al., 1997)

Population	Myeloperoxidase genotype		
	G/G	G/A	A/A
African-American	50 %	41 %	9 %
Caucasians	61 %	31 %	8 %

Interestingly, G/G genotype, associated with higher expression of myeloperoxidase, appears to be over-represented in acute myeloid leukemia cases compared to controls (Reynolds et al., 1997). These observations suggest that higher levels of myeloperoxidase may be associated with increased risk of some leukemias.

Glutathione S-transferases (GST)

Large inter-individual differences in activity of GST exist within the population. For example, Seidegard and Pero (1985) tested the activity of GST (towards a model epoxide, trans-stilbene oxide) in a population of 248 individuals. They observed 100- to 200-fold inter-individual differences in overall GST activity.

The role of polymorphisms of glutathione S-transferases in benzene hematotoxicity is unclear. The subclasses μ (GSTM1) and θ (GSTT1) are effective catalysts for conjugation of glutathione with epoxides such as benzene oxide, leading ultimately to the formation of relatively non-toxic phenylmercapturic acid (Snyder and Hedli, 1996). However, GST also conjugates quinone metabolites of benzene resulting in quinol thioethers which are hematotoxic (Bratton et al., 1997) and generate free radical byproducts (Snyder and Hedli, 1996). Studies examining associations between GST polymorphisms and benzene-induced hematotoxicity have reported mixed results. Chen et al. (1996) reported that the homozygous gene deletion in GSTT1 was associated with increased risk of myelodysplastic syndrome, however, a larger study by Preudhomme et al. (1997) did not find an association.

Prostaglandin H synthase

Experimental evidence in animals has implicated prostaglandin H synthase (PHS) in benzene-induced genotoxicity and myelotoxicity. PHS has the ability to catalyze two-electron oxidations (cyclooxygenase moiety) as well as one-electron oxidations (peroxidase function) and may function to convert hydroquinone and other phenolic metabolites of benzene to benzoquinone or benzosemiquinone, or may generate other free radical species. Co-administration of an anti-inflammatory drug, indomethacin (which inhibits PHS) and high doses of benzene (100 to 1000 mg/kg), prevented or significantly ameliorated bone marrow cytotoxicity and genotoxicity (micronucleus formation in polychromatic erythrocytes) compared to administration of benzene alone (Kalf et al., 1989, Pirozzi et al., 1989). Indomethacin also reduced the inhibition of bone marrow stromal cell-supported hematopoiesis induced by benzene or hydroquinone (Gaido and Wierda, 1987).

Inter-individual PHS activity varies widely. Although there are polymorphisms in the genes coding for PHS (Jones et al., 1993), a greater source of variability is the response of PHS to physiological conditions and signals, including inflammation. For some individuals, tissue peroxidase/PHS activity is 20- to 100-fold higher than typical levels (Kadlubar et al., 1992). This may confer added susceptibility to the hematotoxic effects of benzene exposure.

Data from one study of benzene exposed workers are suggestive of a polymorphism in the dihydrodiol dehydrogenase gene

Gobba et al. (1997) examined the metabolism of benzene in 80 bus drivers in a large city in Italy. *Trans,trans*-muconic acid and unmetabolized benzene were measured in the urine following the first-half of a workday shift. The authors estimate that the average air concentrations of benzene were low, ranging from about 0.003 ppm to 0.3 ppm. The distribution of benzene concentrations in urine appeared to be lognormal (mean 1155 ng/L +/- 494 SD (range 85 to 1980 ng/L)). Concentrations of *trans,trans*-muconic acid ranged from 20-1295 μg/g creatinine. However, the distribution of *trans,trans*-muconic acid was clearly bimodal, indicating two distinct populations with differing metabolic capability and potential susceptibility to benzene toxicity. Based on our current understanding of the metabolism of benzene to *trans,trans*-muconic acid (Figure 2), a polymorphism in the dehydrogenase enzyme that converts benzene dihydrodiol to catechol may account for the findings of Gobba et al. (1997).

Several dehydrogenase enzymes have been shown to be polymorphic. Polymorphisms have been identified in two families of dehydrogenase enzymes (alcohol dehydrogenase and aldehyde dehydrogenase) which process many endogenous and xenobiotic substrates (Daly et al., 1993). Of the polymorphisms identified and studied, all seem to confer significant differences in catalytic activity. The frequency of these polymorphisms varies widely among ethnic groups.

Other polymorphic enzymes involved in benzene metabolism

Additional important enzymes in the activation or detoxification of benzene are known to be polymorphic. These include epoxide hydrolase, UDP-glucuronide transferase, and sulfotransferase. A number of studies demonstrated considerable inter-individual variation in cytoplasmic and microsomal epoxide hydrolase activity, although the frequency distribution appears to be unimodal (Daly et al., 1993). Sulfotransferases have been implicated in tissue specificity of benzene-induced solid tumors in rats (Low et al., 1995). Multiple polymorphisms have been observed within the gene families coding for these enzymes in humans (Daly et al., 1993). Seaton et al. (1994, 1995) examined inter-individual variability of benzene metabolism for detoxification reactions (phenol sulfation and hydroquinone glucuronidation). Subcellular fractions from ten human liver samples were also assayed for sulfation of [^{14}C]phenol (cytosol fraction) and glucuronidation of [^{14}C]hydroquinone (microsomal fraction). Rates of phenol

sulfation and hydroquinone glucuronidation both varied about three-fold in the human samples. Based on a physiological compartmental model, estimates of steady-state concentrations of phenol varied about six-fold, and estimates of steady-state concentrations of hydroquinone varied about five-fold.

Evidence of genetic susceptibility from case studies of benzene toxicity

Aksoy (1988) reviewed the numerous early case studies of benzene toxicity and suggested that family susceptibility plays a role in the frequency of disease, noting a number of benzene-related deaths among related family members. He also noted that diabetes mellitus and obesity, afflictions that have genetic components, appeared to be associated with an increased risk of benzene-induced toxicity.

Toxicokinetic modeling

Large inter-individual variations within the human population in the rate of metabolism of benzene have been predicted by toxicokinetic modeling (Bois et al., 1996; Medinsky et al., 1989). For example, for low exposures to benzene, the fraction of benzene metabolized per day in humans was estimated to range from about 35 percent to as much as 75 percent (Bois et al., 1996).

Dietary and Environmental Factors

In addition to genetic susceptibilities, dietary and environmental factors also add potentially large sources of inter-individual variability for benzene hematotoxicity. Dietary and endogenous sources of phenol, hydroquinone, catechol and 1,2,4-benzenetriol (the phenolic metabolites of benzene) are found in the blood and urine of unexposed individuals. The concentrations vary widely and are quite high for many individuals. The roles that these background sources play in benzene toxicity are unknown, although some researchers have hypothesized that they are involved in background rates of leukemia (McDonald et al., 2001). Other environmental factors that may confer additional variability include ethanol consumption, infection, and co-exposures to radiation and other leukemogens.

Background levels of benzene metabolites

A major potential source of inter-individual variability stems from endogenous and environmental sources of phenol, catechol, hydroquinone and 1,2,4-trihydroxybenzene (the primary phenolic metabolites of benzene). High levels of phenol, catechol, and hydroquinone are found in the urine of individuals without known exposure to benzene (Table 14). The inter-individual variability in urinary concentrations is large: phenol >5-fold, catechol >50-fold, and hydroquinone >30-fold (Inoue et al., 1986, 1988). The distributions of catechol and hydroquinone concentrations in urine of unexposed individuals overlap with those of workers exposed to benzene at levels between 9 to 14 ppm (seven hour, TWA) (Inoue et al., 1988). Regardless of the source, once formed these phenolic metabolites can be transported to the bone marrow where they are activated to reactive species capable of covalent binding to cellular macromolecules, as measured by covalent background adducts of benzoquinone with marrow proteins of unexposed rats and mice (McDonald et al., 1994). [^{13}C]-labeled benzene administered to rats and mice at fairly high concentrations either by inhalation (Bechtold et al., 1996) or by oral administration (McDonald et al., 1994; Waidyanatha et al., 1998) produced concentrations of labeled phenolic metabolites (or related covalent adducts) in the blood and bone marrow that were lower than equivalent [^{12}C]background levels. Based on these studies, Bechtold et al. (1996) estimated that single (workday) exposures to benzene at concentrations of ten ppm or less would add marginally to the background blood and bone marrow burdens of phenolic metabolites.

PBPK modeling by Medinsky et al. (1996) predicts that dietary and endogenous sources of phenol are more readily conjugated and removed by the liver on a first pass effect than phenol generated from benzene metabolism. This difference is due to the heterogeneous distribution of hepatic enzymes and may help explain why phenol, the primary metabolite of benzene, was essentially negative in the NCI cancer bioassay (NCI, 1980). However, high concentrations of phenol and hydroquinone measured in blood (Bechtold et al., 1996) and high levels of phenolic-derived covalent adducts measured in the blood and marrow (McDonald et al., 1994) clearly indicate that a significant proportion of the dietary and endogenous phenolics escape the first pass effect and distribute throughout the body.

The background sources of phenol, catechol and hydroquinone are dietary, environmental, and endogenous (McDonald et al., 2001). A significant source of background phenolic metabolites is likely to be production of phenol by the gut flora during the breakdown of tyrosine residues of protein (Bone et al., 1976; Smith and MacFarlane, 1996). The urinary phenol concentrations of ten patients fed a diet containing no tyrosine declined by roughly half after two weeks (Bures et al., 1990a). Subsequent studies have shown that high inter-individual variability in urinary phenol concentrations may not depend so much on protein (tyrosine) intake but rather on the variability in the metabolic capability of the intestinal bacterial flora (Bures et al., 1990b; Smith and MacFarlane, 1996).

Table 14. Background concentrations of major benzene metabolites in unexposed individuals

Metabolite	Levels of Phenolic Metabolites in Urine	Study
Phenol	2 - 21 mg/L	Docter and Zielhus (1967)
	7.0 mg/day (2.7-10.7) men	Bone et al. (1976)
	11.7 mg/day (6.8-20.3) women	Bone et al. (1976)
	5 - 100 mg/L	Roush and Ott (1977)
	3.5 - 30 mg/L	Lauwerys (1983) (review)
	6.91 mg/L ± 2.61 SD	Inoue et al. (1986)
	7 - 10 mg/L	Adlkofer et al. (1990)
	<10 to >400 μmol/L (lognormal distr.)	Pekari et al. (1992)
Catechol	10.0 mg/L ± 7.3 SD (N=11)	Carmella et al. (1982)
	10.63 mg/L ± 1.92 GSD (N=131) men	Inoue et al. (1988)
	9.77 mg/L ± 2.01 GSD (N=43) men	Inoue et al. (1988)
	11.08 mg/L ± 1.87 GSD (N=88) women	Inoue et al. (1988)
Hydroquinone	4.19 mg/L ± 4.66 GSD (N=131) men	Inoue et al. (1988)
	4.24 mg/L ± 5.42 GSD (N=43) men	Inoue et al. (1988)
	4.17 mg/L ± 1.92 GSD (N=88) women	Inoue et al. (1988)

Direct dietary and environmental sources of the phenolic metabolites of benzene make up the balance of the background levels of these compounds and also are expected to vary widely among individuals (McDonald et al., 2001). Catechol and hydroquinone are components of cigarette and wood smoke (Carmella et al., 1982; Hoffmann and Wynder, 1986). Phenol and simple phenolic compounds are found in a wide range of foods such as vegetable-based products (Maga, 1978; Carmella et al., 1982) and honey (Takeba et al., 1990). Hydroquinone is found in fruits such as cranberries, blueberries and pears (Harbison and Belly, 1982) and herbal teas, especially comfrey and cranberry teas. Catechol and benzenetriol are found in coffee (Rahn and König, 1978).

Associations with infection and disease

Aksoy (1988) reviewed the numerous early case studies of benzene toxicity and suggested that infection and other disorders may increase individual susceptibility to benzene, noting associations of tuberculosis, heart disease, nervous disorders, and nephritis with benzene-induced toxicity. Cooper et al. (1996) reported that acute myeloid leukemia and acute lymphoblastic leukemia were significantly associated with childhood viral diseases including chickenpox and measles.

Ethanol consumption

Evidence from both animal (Baarson and Snyder, 1991) and human studies (Aksoy, 1988) indicate that ethanol consumption increases the severity of the toxic effects of benzene. This synergistic effect is due to induction of CYP2E1, which is the first step in benzene metabolism and is central to benzene hematotoxicity (Valentine et al., 1996).

Conclusions

Taken together, the many sources of inter-individual variability and susceptibility to the adverse effects of benzene suggest the need for added concern with respect to regulating this chemical. The potential magnitude or range of susceptibilities cannot be accurately quantified at this time; however, current evidence suggests individual differences in susceptibility may be two or three orders of magnitude. In the dose-response section of this assessment, we justify the use of the upper 95 percent confidence bound on the cancer potency slope factor in order to address this large inter-individual variability.

DOSE-RESPONSE ASSESSMENT

Noncarcinogenic Effects

Recently OEHHA reviewed the literature regarding chronic, non-cancer effects of benzene and developed a chronic Reference Exposure Level (REL) for inhalation (OEHHA, 1999a). OEHHA (1999a) selected the Tsai (1983) study of benzene-exposed workers as the basis of the chronic REL. In this study, no abnormal hematological changes were observed among 303 male refinery workers with a median exposure of 0.53 ppm (i.e., NOAEL=0.53 ppm). As stated above, no adequate studies of oral exposure to benzene in humans were located. Thus, the data used to form the basis of the chronic REL developed by OEHHA will be used to estimate a health protective concentration for non-cancer endpoints from exposure to benzene in drinking water. The draft chronic REL document (OEHHA, 1999a) has been reproduced as Appendix B.

Carcinogenic Effects

Approaches Taken in Previous Assessments

Numerous analyses have been conducted to assess the carcinogenic risks from exposures to benzene (U.S. EPA, 1979b, 1983, 1985, 1998; White, 1982; IARC, 1982; ARB, 1984; Crump and Allen, 1984; DHS, 1987; Rinsky et al., 1987; Austin et al., 1988, Thorslund et al., 1988; Brett et al., 1989; Crump, 1994, 1996; Paxton et al., 1994a,b). Although both animal and human cancer studies have been utilized to estimate cancer risks of benzene (U.S. EPA, 1979b, 1985; ARB, 1984), most regulatory agencies have relied upon evidence from occupational exposures to

form the basis of regulatory standards for benzene. The approaches taken to assess the leukemia risk from benzene exposure have been varied and have resulted in risk estimates that range considerably in magnitude. Reviews of the previously published risk assessments of benzene-induced leukemia can be found elsewhere (Brett et al., 1989; Crump, 1992). The following section will briefly summarize in turn some of the approaches taken by previous investigators.

(1) U.S. EPA (1979b)

The Carcinogen Assessment Group (CAG) of U.S. EPA evaluated the data from the Pliofilm cohort (Infante et al., 1977), Dow cohort (Ott et al., 1978), and Turkish workers (Aksoy et al., 1974, 1976, 1977) (U.S. EPA, 1979b). Different leukemia subtypes were assessed for each of the three cohorts: total leukemia (Infante et al., 1977), myelogenous leukemia (Ott et al., 1978), and non-lymphocytic leukemia (Aksoy et al.). A basic relative risk model was used to estimate extra cases expected in an exposed population per unit exposure (Equation 1),

$$\text{Lifetime probability of leukemia per unit exposure} = P_l \times (RR-1)/\text{exposure} \qquad \text{(Equation 1)}$$

where P_l is the probability of dying from leukemia in the referent population, and RR is the relative risk in the exposed cohort. Based on 1973 U.S. vital statistics, P_l for total leukemia, myelogenous leukemia, and non-lymphocytic leukemia were 0.006732, 0.002884 and 0.004517, respectively. After converting occupational exposures to those expected from continuous exposure of the general population, CAG estimated lifetime probabilities of leukemia per unit exposure (i.e., risk) to be 0.015/ppm based on the Pliofilm data, 0.020/ppm based on the Turkish workers, and 0.046/ppm based on the Dow cohort data, respectively. The geometric mean of the results from the three studies was 0.024/ppm or 24 deaths per 1000 people exposed to one ppm benzene for life.

(2) IARC (1982)

IARC took two approaches to evaluating the leukemia data from the Pliofilm Cohort as reported by Rinsky et al. (1981). First, assuming that risk is greatest for those with longest exposure, the cases were separated into those with less than five years exposure and those with more than five years exposure. For those with greater than five years exposure, IARC (1982) applied a relative risk model to estimate extra cases expected in an exposed population per unit exposure (Equation 2),

$$\text{Extra cases per unit exposure} = (RR-1) \times I_{Referent}/\text{exposure} \qquad \text{(Equation 2)}$$

where $I_{Referent}$ is the incidence of leukemia in the referent population, and RR is the relative risk in the exposed cohort. Five cases of leukemia were observed compared to 0.237 expected, giving a relative risk of 21.1. The expected male lifetime probability of dying from leukemia in the general population was approximately seven per 1000. Assuming an upper end exposure estimate of 100 ppm, IARC estimated a lifetime risk of (21.1 - 1.0) x (7/1000)/100 ppm or 1.4×10^{-3}/ppm or 1.4 deaths per 1000 individuals exposed to one ppm for life.

In an alternate approach, IARC (1982) assumed leukemia risk increases linearly with continuous exposure. The overall relative risk of leukemia for the entire cohort was 5.6 with an average duration of exposure of 8.5 years. Thus, based on the model assumptions of the IARC analysis, over a working lifetime (45 years) relative risk increases linearly from 0 at year zero to (5.6 - 1.0)

x 45/8.5 = 24.4 at year 45. The age-specific relative risk of 24.4 was inserted into Equation 2, and, assuming 100 ppm exposure, a risk estimate of 1.7 x 10^{-3}/ppm was obtained (e.g., 1.7 deaths per 1000 individuals exposed to one ppm for 45 years).

(3) ARB (1984)

As part of the Toxic Air Contaminants Program in California, the Air Resources Board (ARB, 1984) produced an assessment of benzene risks via air exposures. For the analysis of available human epidemiological studies, ARB (1984) relied on an analysis conducted by U.S. EPA in 1983. The follow-up of the Pliofilm cohort had been extended and the results were reported by Rinsky et al. (1981). U.S. EPA updated its 1979 assessment (U.S. EPA 1979b) with the new data on the Pliofilm Cohort. ARB (1984) noted that the updated potency estimate for the Pliofilm Cohort was 0.052 $(mg/kg\text{-}d)^{-1}$, from which they estimated a risk of 0.048 for lifetime exposure to one ppm. ARB noted that the data from the Rinsky re-evaluation was compatible with lifetime risk estimates of 0.032 to 0.12 ppm^{-1}, based on 95 percent confidence intervals. The geometric mean of results from Pliofilm (Infante et al., 1977), Dow (Ott et al., 1978), and Turkish workers (Aksoy et al., 1974, 1976, 1977) was 0.022/ppm or 22 deaths per 1000 persons exposed to one ppm.

Results from the epidemiological studies were compared to cancer potency estimates derived from long-term animal bioassays (see Table 29). ARB (1984) reported a range of scientifically defensible lifetime risk estimates from human and animal evidence of 0.024 to 0.17 ppm^{-1} (e.g., 24 to 170 deaths per 1000 individuals exposed to one ppm for life).

(4) Crump and Allen (1984)

In a detailed assessment conducted for OSHA, Crump and Allen (1984) analyzed cancer data from the Pliofilm cohort (Rinsky et al., 1981), the Dow cohort (Ott et al., 1978), and the U.S. chemical worker cohort (unpublished data, later reported as Wong, 1987). Crump and Allen (1984) employed two models, a relative risk model (Equation 3) and an absolute risk model (Equation 4);

Relative risk model: $E(O_i) = \alpha E_i(1 + \beta d_i)$ (Equation 3)

Absolute risk model: $E(O_i) = E_i + (\alpha + \beta d_i)Y_i$ (Equation 4)

where,

$E(O_i)$ is the expected number of leukemia deaths in the i^{th} dose category;

E_i is the expected number of leukemia deaths in the i^{th} dose group based upon age-, sex- and race-specific U.S. mortality rates (i.e., E_i is an age-specific mortality rate for leukemia in the comparison population);

α is the parameter accounting for any differences that may exist between the comparison population and the study population (some investigators have included this term to account for a healthy worker effect (DHS, 1986));

β is the potency (additional risk of leukemia) associated with unit increase in d_i; and

Y_i is the number of person-years in the i^{th} group.

Both models assume that O_i are distributed as Poisson random variables with means $E(O_i)$. The absolute risk model assumes that the added risk associated with a dose d_i is the same for all age groups. Cancer potency estimates from the worker data were used to estimate risk to the target population (i.e., general U.S. population) using life table methods.

Crump and Allen (1984) investigated the range of cancer risks using four different measures of exposure: (1) cumulative dose, (2) weighted cumulative dose, (3) window dose, and (4) peak exposure dose. Cumulative dose integrated all previous exposures of benzene into a summary metric such as ppm-years. The weighted cumulative dose metric attempted to incorporate observations of leukemia risk over time as observed following exposures to ionizing radiation (e.g., atomic bomb survivors). Crump and Allen (1984) used a weighting function that gave no weight to recent exposures (< 2.5 years) and progressively less weight to exposures after 7.5 years. The window dose metric considered only exposures that occurred within a ten-year window defined by 2.5 and 12.5 years prior to tumor formation (death). This dose metric tested the idea that exposures greater than 15 years in the past had no effect on current leukemia incidence. This hypothesis was not supported by the data, as potency estimates using window dose were greater than three-fold lower than those using cumulative dose. Peak exposure dose measured all cumulative exposures that exceeded 100 ppm. This exposure metric was used to investigate the hypothesis that only very high levels of exposure (>76 ppm) carry any risk of leukemia. The hypothesis also was not supported by the data, since estimates of leukemia risk were higher among workers with peak exposure levels <100 ppm as compared with workers with levels >100 ppm. Risk estimates from the Crump and Allen (1984) analysis using the cumulative, weighted and window exposure estimates as summarized in Crump (1992) are presented in Table 15.

Crump and Allen (1984) found that because of the small number of observed leukemia cases, linear models could not be distinguished from many non-linear models. In one case they applied the relative risk quadratic model (defined by replacing d_i in the relative risk model with d_i^2) to the combined data from the Rinsky et al., Ott et al. and Wong et al. studies (see Table 15). The model predicted an additional risk of about 2.4 per 1000 for occupational exposure to one ppm for 40 years starting at age 20, a value roughly one-third of the estimate from the linear model. Crump et al. (1984) applied a three stage polynomial to the Pliofilm dataset used by Crump and Allen (1984). Estimates of additional leukemia deaths per 1000 workers exposed to one ppm benzene in an occupational scenario were 3.3 for the first stage and 3.0 for the second stage.

Table 15. Leukemia risk estimates from Crump and Allen (1984) analysis
(based on one ppm, occupational exposure scenario[1]) (Crump and Allen, 1984; OSHA, 1987)

Exposure Matrix/Dataset	Relative Risk Model Risk Estimate, ppm^{-1} (upper 95 % CI)	Absolute Risk Model Risk Estimate (ppm^{-1})
Cumulative exposure		
Rinsky et al., Ott et al., and Wong et al.	$9.5x10^{-3}$ ($2.2x10^{-2}$)	
Rinsky et al., and Ott et al.	$3.7x10^{-3}$	$2.0x10^{-3}$
Wong et al.	$1.3x10^{-2}$ ($3.1x10^{-2}$)	
Rinsky et al.	$6.6x10^{-3}$ ($1.5x10^{-2}$)	$2.1x10^{-3}$
Weighted cumulative exposure		
Rinsky et al., and Ott et al.	$3.0x10^{-3}$	$1.5x10^{-3}$
Rinsky et al.	$4.9x10^{-3}$	$1.9x10^{-3}$
Window exposure		
Rinsky et al., and Ott et al.	$1.2x10^{-3}$	$1.1x10^{-3}$
Rinsky et al.	$1.7x10^{-3}$	$1.3x10^{-3}$

[1] Occupational exposure scenario was defined as eight hours per day, five days per week, 50 weeks per year, for 40 years beginning at age 20.

(6) Rinsky et al. (1987)

Rinsky et al. (1987) reported mortality experience of the Pliofilm Cohort through 1981 and estimated the risks of benzene exposure from the updated information. The authors employed a conditional logistic regression model (Equation 5) which assumes that the odds ratio of leukemia is related exponentially to exposure. X_i is the exposure estimate for each exposure category (i), and β_i is the coefficient of dose, where i = 1 to n.

$$\text{Odds ratio} = e^{(\beta iXi + \ldots + \beta nXn)} \qquad \text{(Equation 5)}$$

Rinsky et al. (1987) employed a matched, case-control analysis. Cases were matched with ten cohort members who were alive at the time of the leukemia death. β_1 was estimated to be 0.0126 (upper 95 percent CI = 0.0224) which corresponded to an occupational risk, defined as exposure eight hours per day, five days per week, 50 weeks per year, for 40 years, of 12 deaths per 1000 exposed to one ppm (upper 95 percent CI = 18 deaths per 1000 exposed to one ppm).

(7) DHS (1988)

DHS (1988) recommended a cancer potency of 0.1 $(mg/kg\text{-}d)^{-1}$ be used to estimate risk specific intake levels of benzene for the Proposition 65 program (Safe Drinking Water and Toxic Enforcement Act of 1986). This value was later adopted as the consensus potency value for use in regulatory programs within California. DHS selected the 'most credible' cancer potency estimate, which was derived from the Pliofilm Cohort (Rinsky et al., 1981) using the weighted cumulative dose relative risk model. The maximum likelihood potency estimate was 0.041 ppm^{-1} and the upper 95 percent confidence bound was 0.088 ppm^{-1} (i.e., 88 deaths per 1000 persons exposed to one ppm for life). These risk estimates were converted to population-based cancer potency estimates of 0.044 $(mg/kg\text{-}d)^{-1}$ (mean) and 0.095 $(mg/kg\text{-}d)^{-1}$ (95 percent upper CI), which was rounded to 0.1 $(mg/kg\text{-}d)^{-1}$.

(8) Thorslund et al. (1988)

Thorslund et al. (1988) also utilized the Pliofilm Cohort data and the absolute risk model, the model the authors believed was most consistent with epidemiological data on radiation-induced leukemia. Thorslund et al. (1988) employed a weighted dose measure based on leukemia responses observed in patients treated with ionizing radiation for ankylosing spondylitis (ossification of the joints and ligaments in the spine). Acute leukemia and myelodysplastic syndromes were the only endpoints related to benzene exposure assessed by the authors. Statistical analysis was conducted on individual worker data. The results of the Thorslund et al. (1988) analysis are presented in Table 16.

Table 16. Excess risk estimates from continuous lifetime exposure to one ppm benzene (Thorslund et al., 1988, as reported by Crump, 1992)

Model or Modification	**Risk Estimate** (ppm^{-1})
Basic model (combined U.S. EPA, 1985 estimate)	$2.6x10^{-2}$
Model restricted to absolute risk, weighted cumulative exposure, Rinsky data	$1.8x10^{-2}$
Three years of cohort follow-up added	$1.7x10^{-2}$
Job code errors corrected	$1.8x10^{-2}$
New weighted cumulative dose, new statistical method for estimating transition rate parameters	$3.2x10^{-3}$
New definition of diseases induced by benzene; new estimates of background rates in U.S. population	$3.5x10^{-3}$
Quadratic model, two molecules of benzene required to initiate carcinogenesis	$1.4x10^{-4}$
Linear-quadratic model, upper bound estimate	$1.0x10^{-3}$

(9) Austin et al. (1988)

Austin et al. (1998) proposed a different approach which assumes that the proportional excess of leukemia mortality observed during the follow-up period will continue until all the cohort members have died (Equation 6):

$$Excess_L = ((O_L-E_L)/E_{total}) \times (exp_{target}/exp_{obs}) \qquad \text{(Equation 6)}$$

In this equation $Excess_L$ represents excess leukemia deaths, O_L is the observed number of leukemia deaths in cohort, E_L is the expected number of leukemia deaths in cohort, E_{total} is the total number of expected deaths in cohort, exp_{target} is the target cumulative exposure of interest, and exp_{obs} is the cumulative exposure for the cohort. Austin et al. (1988) summed the observed and expected leukemia deaths, total expected deaths and cumulative exposure, respectively, from the Rinsky et al. (1987) study (nine deaths, 2.7 expected, 332 total deaths, 69 ppm-yr), the Bond et al. (1986) study (four deaths, 2.1 expected, 269 total deaths, 46 ppm-yr), the Tsai et al. (1983) study (zero observed, 0.42 expected, 59 total deaths, 4 ppm-yr), and the Wong et al. (1983) study (six observed, 4.4 expected, 613 total deaths, 30 ppm-yr). Combined results were 19 observed leukemia deaths, 9.6 expected leukemia deaths, 1273 total deaths, and a weighted average of 42 ppm-yr, which yielded an excess risk estimate of 5.3 deaths per 1000 exposed to one ppm for 30 years (95 percent CI, 1.0 to 11 per 1000 exposed to one ppm).

(10) Brett et al. (1989)

Brett et al. (1989) analyzed the Pliofilm Cohort data updated through 1981 (Rinsky et al., 1987). Like Rinsky et al. (1987), Brett and colleagues employed conditional logistic regression (Equation 5) to examine the data in a case-control analysis. However, three sets of matched controls were developed: one set of controls was the same as selected by Rinsky et al., a second set was matched on date of birth and date of entering Pliofilm work, and the third set matched on date of birth, date of entering Pliofilm work, and plant location. Brett et al. (1989) applied the three control sets separately using the exposure matrices of Rinsky et al. (1987) and Crump and Allen (1984), for a total of six comparative analyses. Utilizing the Rinsky exposure estimates, occupational risk (defined as exposure to benzene eight hours per day, 50 weeks per year, for 45 years) was estimated to be 5.1×10^{-3} ppm^{-1} (i.e., 5.1 deaths per 1000 persons exposed to one ppm) (95 upper percent CI = 1.2×10^{-2} ppm^{-1}) for the first control set, 6.4×10^{-3} ppm^{-1} (95 upper percent CI = 1.5×10^{-2} ppm^{-1}) for the second control set, and 4.2×10^{-3} ppm^{-1} (95 upper percent CI = 8.7×10^{-3}) for the third control set. Occupational risk estimates utilizing the Crump and Allen exposure estimates were approximately ten-fold lower than values utilizing the Rinsky exposure estimates.

(11) Paxton et al. (1994a,b)

Updated mortality information on the Pliofilm Cohort provided by NIOSH through 1987 was reported by Paxton et al. (1994a). Paxton et al. (1994b) analyzed the new data using a Cox proportional hazards model (Equation 7):

$$h_X(t) = h_0(t)\, e^{(\beta iXi + \ldots + \beta nXn)} \qquad \text{(Equation 7)}$$

In Equation 7, $h_X(t)$ is the hazard as a function of exposure, X, and time, t; $h_0(t)$ is the baseline hazard as a function of time. Under the model, $h_X(t)$ varies with time, but the relative hazard, $h_X(t)/h_0(t)$, will not (i.e., proportional hazard). The matching criteria of Rinsky et al. (1987) were used by Paxton et al. (1994b) to define the strata in the proportional hazards model (i.e., sex, race, date of birth, and date of first employment). Predictions of additional leukemia deaths in the general population were obtained by applying the model outputs and assuming a background lifetime mortality rate of 0.00707. Paxton et al. (1994a,b) compared results from the proportional hazards analysis using exposure estimates of Rinsky et al. (1987), Crump and Allen (1984), and Paustenbach et al. (1992) (Table 17).

Table 17. Risk estimates of the Pliofilm Cohort (based on model output equivalent to one ppm exposure) Paxton et al. (1994b)

Exposure matrix	β (upper 95 % CI)[1]	Occupational[1] Risk (upper 95 % CI), ppm^{-1}	70-year Risk[2] (upper 95 % CI), ppm^{-1}
Rinsky	0.0038 (0.0065)	1.3×10^{-3} (2.4×10^{-3})	8.8×10^{-3} (1.6×10^{-2})
Crump/Allen	0.0008 (0.0016)	2.6×10^{-4} (5.3×10^{-4})	1.8×10^{-3} (3.6×10^{-3})
Paustenbach	0.0015 (0.0005)	4.9×10^{-4} (8.4×10^{-4})	3.3×10^{-3} (5.7×10^{-3})

[1] Occupational exposures were defined as 8 hr/d, 5 d/wk, 50 wk/yr, for 45 years.
[2] Risk adjusted for 24 hr/d, 7 d/wk, 52 wk/yr for 70 years.

(12) Crump (1994, 1996)

Additional analyses of the Pliofilm Cohort data were conducted by Crump (1994, 1996) which utilized additional information from the follow-up through 1987 (Paxton et al., 1994a) and the exposure reanalysis conducted by Paustenbach et al. (1992). Work history and mortality information from 1212 benzene-exposed, non-black male workers from 'wetside' operations (follow-up through 1987) was combined with data for non-black male workers from 'dryside' operations (505 workers whose last follow-up was through 1981). The linear multiplicative risk model (Equation 9) and the linear additive risk model (Equation 8) were applied to the data. The multiplicative risk model assumes that benzene increases the background mortality rate by a multiplicative factor that depends on prior benzene exposure. The linear additive model assumes that benzene increases the background mortality rate by an additive amount that depends on prior benzene exposure, and assumes that added risk associated with a dose is the same for all age groups. The age-dependent mortality rate, h(t), for the cancer of interest is a function of the background mortality rate at age t, a(t), which was estimated from appropriate mortality rates of U.S. white males. X(t) is a summary measure of benzene exposure before age t, and β is an estimate of carcinogenic potency of benzene.

Linear additive risk model: $h(t) = a(t) + \beta X(t)$ (Equation 8)
Linear multiplicative risk model: $h(t) = a(t)\,[1+\beta X(t)]$ (Equation 9)

For non-linear forms of the model, βX(t) was replaced by the polynomial, $\beta X(t) + \beta X^2(t) + \beta X^3(t)$. Two exposure metrics were examined, cumulative exposure and weighted exposure. Cumulative exposure was calculated with lag times of zero, three or five years (i.e., exposures in the last period of exposure prior to disease were assumed not to affect mortality). The author utilized a lag time of five years because it provided better fits to the cancer data compared to using a lag of zero or three years. A weighted exposure was estimated by employing a weighting function at age t (in years) (Equation 10) that accounted for latency and a changing risk profile for leukemia over time that was based on observations of leukemia following exposure to ionizing radiation (Crump and Allen, 1984, Thorslund et al., 1988).

$$X_w(t) = \int_0^{t-L} x(v)w(t-v)dv \qquad \text{(Equation 10)}$$

x(v) is the instantaneous benzene exposure at age v, and w(t) is the weighting function defined by $w(t) = (t/K)^2 \exp(-t/K)$, where K is the mode of the latency distribution. Thus, w(t) increases to a maximum at age t = K years and is given maximal weight Crump (1994) described the use of a weighting function for cumulative exposure that includes a latency (or "lag") period, L.

Crump (1994, 1996) used exposure matrices developed by Crump and Allen (1984) and Paustenbach et al. (1992), but not that of Rinsky et al. (1981, 1987). Crump (1994, 1996) obtained risk estimates for acute myeloid leukemia or total leukemia for different combinations of linear versus non-linear forms of the multiplicative or additive models, and for each exposure matrix (Table 18). Fits for non-linear forms of the model were not statistically significant, except in one case where the Paustenbach exposure estimates were fit to a non-linear (exposure) intensity-dependent model (p=0.08, *a priori* significance criteria of p>0.1).

Crump (1994) estimated additional lifetime risk for general population exposures to benzene through modified life table methods. Crump (1994) constructed a life table encompassing each year of life from age 0 to 100. Since rates are reported in five-year intervals, linear regression was used to estimate rates for years between the intervals. The probability of dying of leukemia with and without exposure to benzene was calculated. Lifetime additional risk was obtained from the difference of these two estimates. Results are shown in Table 18 and indicate mean lifetime risk estimates that range from 2 to 24 deaths per 1000 persons exposed to 1 ppm benzene for life.

Table 18. Risk estimates derived from the Pliofilm Cohort by Crump (1994, 1996)

Endpoint / Model[3]	Exposure matrix	Exposure metric	β (SE)	Occupational[1] Risk ppm^{-1}	Lifetime[2] Risk ppm^{-1}
Acute Myeloid or Monocytic Leukemia (AMML)					
Add.	Crump	weighted	$6.6x10^{-5}$ ($2.7 x10^{-5}$)	$2.2x10^{-3}$	$1.1x10^{-2}$
Mult.			$2.3x10^{-0}$ ($8.9 x10^{-1}$)	$3.2x10^{-3}$	$1.6x10^{-2}$
Add.	Paustenbach	weighted	$4.9x10^{-5}$ ($1.8 x10^{-5}$)	$1.5x10^{-3}$	$7.1x10^{-3}$
Mult.			$1.1x10^{-0}$ ($4.1 x10^{-1}$)	$1.6x10^{-3}$	$7.7x10^{-3}$
Add.	Crump	cumulative	$2.0x10^{-6}$ ($8.2 x10^{-7}$)	$2.6x10^{-3}$	$1.5x10^{-3}$
Mult.			$4.5x10^{-2}$ ($1.8 x10^{-2}$)	$4.3x10^{-3}$	$2.0x10^{-3}$
Add.	Paustenbach	cumulative	$1.3x10^{-6}$ ($5.1 x10^{-7}$)	$1.8x10^{-3}$	$9.9x10^{-3}$
Mult.			$2.7x10^{-2}$ ($1.0 x10^{-2}$)	$2.6x10^{-3}$	$1.2x10^{-2}$
All Leukemia					
Add.	Crump	weighted	$7.1x10^{-5}$ ($2.9 x10^{-5}$)	$2.4x10^{-3}$	$1.1x10^{-2}$
Mult.			$8.4x10^{-1}$ ($3.4 x10^{-1}$)	$3.0x10^{-3}$	$1.8x10^{-2}$
Add.	Paustenbach	weighted	$9.0x10^{-5}$ ($3.4 x10^{-5}$)	$1.9x10^{-3}$	$9.9x10^{-3}$
Mult.			$4.0x10^{-1}$ ($1.6 x10^{-1}$)	$1.7x10^{-3}$	$8.3x10^{-3}$
Add.	Crump	cumulative	$1.9x10^{-6}$ ($8.8 x10^{-7}$)	$2.6x10^{-3}$	$1.4x10^{-2}$
Mult.			$1.7x10^{-2}$ ($6.8 x10^{-3}$)	$5.1x10^{-3}$	$2.4x10^{-2}$
Add.	Paustenbach	cumulative	$1.3x10^{-6}$ ($5.6 x10^{-7}$)	$1.8x10^{-3}$	$9.9x10^{-3}$
Mult.			$1.1x10^{-2}$ ($3.9 x10^{-3}$)	$3.1x10^{-3}$	$1.5x10^{-2}$

[1] Risk based on occupational exposures from age 20 to 65.
[2] Risk adjusted for lifetime exposure 24 hr/d, 7 d/wk, 52 wk/yr.
[3] Linear additive and linear multiplicative risk models, Equations 8 and 9.

(13) U.S. EPA (1998)

U.S. EPA culminated a multiyear review of the carcinogenic effects of benzene by issuing a document entitled "Carcinogenic Effects of Benzene: An Update" (U.S. EPA, 1998). U.S. EPA reviewed the available evidence including epidemiological data, animal studies and information on the carcinogenic mechanism of action. Their review also examined the evidence for low-dose linearity and concluded that insufficient evidence was available to deviate from using a linear dose-response curve. U.S. EPA did not conduct a re-assessment of the lifetime cancer risks from exposure to benzene, but selected a range of estimates from the literature. The range of lifetime risk estimates was 7.1×10^{-3} to 2.5×10^{-2} ppm^{-1} (i.e., 7.1 to 25 deaths per 1000 exposed to one ppm for life), and was based on linear risk models applied to the Pliofilm cohort (Crump, 1994, see Table 18 above). The 7.1×10^{-3} ppm^{-1} risk estimate is based on acute myeloid and monocytic leukemia combined, while the 2.5×10^{-2} ppm^{-1} (actually reported as 2.4×10^{-2} ppm^{-1} in Crump, 1994) is based on total leukemia. The U.S. EPA range of selected estimates are mean estimates and do not include the upper 95% confidence bound.

Comparison of approaches

Over time the risk assessments of benzene have increased in complexity as new information and improved mathematical techniques have been used. The wide range of risk estimates as reported in studies (1) through (12) reflect the different approaches and datasets used. Most studies employed linear relative or absolute risk models, some using simple computations (U.S. EPA, 1979b; IARC, 1982), others using more computationally rigorous estimation (Thorsland et al., 1988; Crump and Allen, 1984; Crump, 1994). Lifetime risk estimates obtained from linear relative risk models were slightly higher (more health protective) than those obtained using absolute risk models (Crump and Allen; 1984; Crump, 1994). Three assessments (Rinsky et al., 1987; Brett et al., 1989; Paxton et al., 1994) selected models that assume increases in leukemia rates are exponentially related to exposure. Rinsky et al. and Brett et al. used a conditional logistic regression model in a case-control analysis whereas Paxton et al. used a proportional hazards model. Risk estimates for comparable datasets indicated that the studies using exponential models (Brett et al., 1989; Paxton et al., 1994b) produced potency estimates that were five- to ten-fold lower than estimates using linear risk models (Crump and Allen, 1984; Crump, 1994). Investigations by Crump (1994) indicated that linear models fit the Pliofilm data, using the Crump and Allen (1984) exposure estimates, better than non-linear ones. With respect to assessments examining the Pliofilm data, a major source of variability in the risk estimates seemed to stem from differences in the exposure estimation (e.g., Rinsky, Crump and Allen, or Paustenbach exposure estimates).

Low Dose Linearity

There has been extensive debate in the literature regarding the true shape of the dose-response curve at low doses for benzene-induced leukemia (Crump and Allen, 1984; Crump, 1994; Cox, 1996; Raabe and Wong, 1996; Smith, 1996a; Schnatter et al., 1996b; U.S. EPA, 1998). Some researchers have suggested that a threshold of response may exist based on observations of benzene-exposed workers (Raabe and Wong, 1996; Schnatter et al., 1996b) or based on complex mathematical models of hematotoxicity (Cox, 1996). However, there is also evidence, stemming from both epidemiological investigations and mechanistic studies, to suggest that the relationship is linear at low doses. This section examines the scientific evidence with respect to the question of benzene-induced low dose linearity and applies this evidence to the criteria for selecting a linear or non-linear approach as outlined in the guidelines for carcinogen assessment (DHS, 1985; U.S. EPA, 1996).

The California guidelines (DHS, 1985) and the U.S. EPA proposed guidelines (U.S. EPA, 1996) provide assistance in selecting between linear or non-linear approaches for carcinogen assessment. A linear assumption is taken when the evidence supports a mode of action that is genotoxic or other mode of action expected to function linearly with dose. The assumption of non-linearity is appropriate when there is no evidence for linearity and sufficient evidence to support an assumption of non-linearity (U.S. EPA, 1996). Alternately, a non-linear approach might be considered if the mode of action was clearly understood and proceeded through a threshold process (e.g., cancer as a effect secondary to toxicity) (U.S. EPA, 1996).

The guidelines note that whenever data are sufficient, a biologically based dose-response model should be developed which better describes empirical observations (U.S. EPA, 1996). However, our understanding of how benzene causes cancer is unclear. As reviewed in Carcinogenic Mode of Action (page 67), benzene likely functions to cause cancer through multiple modes of action. At a workshop co-sponsored by OEHHA, leading researchers in the fields of benzene toxicity and

leukemia research clearly indicated that our understanding of leukemia biology and benzene's carcinogenic mode of action are insufficient at this time to develop a biologically based model of benzene-induced cancer (Smith and Fanning, 1997).

Benzene and its metabolites are clearly genotoxic, causing chromosomal damage and mutations in short term systems *in vitro*, in animals *in vivo*, and in humans occupationally exposed (see sections on Genetic Toxicity, pages 25 and 38). Historically, this fact alone would argue for employing a linear approach. A closer examination of the available genetic toxicity data indicates that limited information is available to help understand the dose-response relationships of these effects at low exposure levels (e.g., less than 10 ppm). For example, few genotoxicity studies have carried out detailed dose-response analysis, especially in humans. Increases in the frequency of DNA strand breaks and chromosomal aberrations have been found in groups of workers exposed to concentrations of benzene in the low ppm range (TWA), while others have failed to find an association. However, most studies compared exposed versus unexposed workers, and, thus, do not provide insight into dose-response trends. Two recent studies have reported dose-related trends in aneusomy and deletions in chromosomes five and seven (Zhang et al., 1998) and aneusomy and translocations in chromosomes eight and 21 (Smith et al., 1998) in benzene-exposed workers in China. At present such biomarkers of effect have not been successfully utilized to extend the dose-response range to exposure levels lower than have been associated with leukemia in epidemiological studies of benzene nor have they suggested a threshold of response. Data from *in vitro* genotoxicity studies of benzene metabolites are likewise not very informative regarding the shape of the dose-response at low doses. We estimate that most *in vitro* assays used doses that correspond to cellular concentrations that would likely arise only from high exposures in humans.

Benzene's toxicity is intimately tied to its metabolism. The metabolism of benzene is linear at low doses and non-linear at higher doses due to saturable or competitive metabolism. Production of the suspected toxic metabolites by benzene in rodents and humans (e.g., hydroquinone and muconic acid) is linear at low doses and less than linear at saturable doses (e.g., supralinear) (Henderson, 1996; Mathews et al., 1998; Rothman et al., 1998). The formation of hydroquinone and muconic acid decreased as a function of the total metabolites produced in the urine of benzene-exposed workers, whereas production of phenol and catechol increased as a function of total metabolites (Rothman et al., 1998). These observations suggest that benzene and its phenolic metabolites are competing for P4502E1 and other critical enzymes at higher exposures. Macromolecular adducts, which can serve as a measure of the production of reactive metabolites, increased linearly over a wide range of administered doses (700 pg/kg to 16 mg/kg) of benzene administered to rodents (Creek et al., 1997). Likewise, linear production of 1,4-benzoquinone and benzene oxide protein adducts was observed in the bone marrow of rats and mice administered [$^{14}C/^{13}C_6$]benzene (McDonald et al., 1994). Recent studies have examined DNA and protein binding of very low doses of benzene (five μg/kg) administered to different strains of mice and rats (Mani et al., 1999). The authors noted that their results were consistent with the hypothesis that the capability to metabolize benzene to toxic metabolites contributes to the difference in benzene's ability to elicit a carcinogenic response in different species.

As detailed in the section entitled Inter-individual Variability and Benzene-induced Hematotoxicity (page 71), high background concentrations of phenol, hydroquinone, catechol, and 1,2,4-benzenetriol are found in the blood and urine of unexposed individuals. These phenolic compounds stem from endogenous, dietary and environmental sources (McDonald et al., 2001). Exposures at moderate to low levels of benzene (less than ten ppm) are expected to add only marginally to the existing burdens of these phenolic species (Bechtold et al., 1996). The role that these background 'metabolites' play in benzene-induced leukemogenesis is unknown. One explanation is that these phenolic species do not play as critical a role as many researchers

have contended, so that benzene may cause adverse effects only at high exposure where background levels are significantly exceeded. Another possibility is that the background concentrations of these phenolic compounds are involved in background disease processes (McDonald et al., 2001). If this were the case, then any exposure to benzene would add (linearly) to these background processes.

Benzene alters the proliferation and differentiation of blood cells in the bone marrow. These effects are believed to play an important role in benzene-induced leukemia. In studies of human hematopoietic progenitor cells in culture, it has been found that extremely low concentrations of hydroquinone, likely relevant to environmental exposures, are able to disrupt developmental response to growth factors (Irons and Stillman, 1996). The reported dose-response curve appeared to be roughly linear with the log of dose, at low doses. On a linear scale, that relation would be strongly supralinear. The quantitative relationship between this effect and leukemia risk is not known.

As mentioned above, some researchers have suggested that a threshold of response may exist based on observations of no excesses in leukemia rates among benzene workers with low exposures to benzene (Raabe and Wong, 1996; Schnatter et al., 1996b). However, in most epidemiological studies examining dose-response relationships, the data appear linear to low doses of benzene. For example, visual inspection of the dose-response relationships of the two studies selected as the basis of this assessment, the Pliofilm Cohort and the Chinese Worker Cohort, suggest a linear relationship at low doses (Figures 3, 5 and 6). Indeed, in the Chinese Worker Cohort elevated excess risk of leukemia was observed at an average concentration of 1.2 ppm (Table 23).

Some researchers have argued that benzene-induced leukemias derive from preceding syndromes characterized by hematotoxicity and bone marrow suppression. Since overt toxicity is likely to be associated with higher doses of benzene, this subset of benzene-induced leukemias may fit a threshold dose-response relationship. However, extensive evidence from epidemiological studies of leukemia arising in groups of individuals with relatively low exposures to benzene (see Carcinogenicity, page 41) would argue against the notion that a high-dose toxicity preleukemic state is a required step in leukemogenesis. At low exposure levels, it is likely that the genotoxic effects and the subtler epigenetic effects on hematopoietic cell development are more important than overt toxicity; different dose-response relationships would be expected for leukemias arising from different mechanistic pathways.

In conclusion, benzene is genotoxic, and a clear threshold has not been established. Insufficient information and understanding of the carcinogenic mode of action prevent the development of a biologically based model at this time. Other information such as metabolism to toxic species, altered hematopoiesis, and observations of dose-related increases in leukemia rates in workers to low exposure levels suggest that a linear approach is justifiable. Applying the available scientific evidence on benzene to the criteria for selecting linear or non-linear approaches as described in the guidelines for carcinogen assessment results in the selection of a linear approach as appropriate.

Analysis of Leukemia Risks Associated with Human Exposures to Benzene

Study selection

As summarized in Table 8 above, over 20 epidemiological studies have investigated associations of cancer and exposure to benzene. In selecting studies for risk evaluation, the following criteria

were employed. A cohort study design was preferred over case-control or other study designs. Studies examining large numbers of individuals at risk and good exposure estimates were deemed critical. Preference was also given to studies containing dose-response information and a separate unexposed control group.

Two cohorts, the Chinese Worker Cohort (Hayes et al., 1997) and the Pliofilm Cohort (Rinsky et al., 1981, 1987; Paxton et al., 1994a) were judged to be the best studies available and will form the basis of the PHG for cancer risk. Other cohort studies were also analyzed for comparison purposes. These include the U.S. Chemical Workers (Wong, 1987), the Dow Chemical Workers (Ott et al., 1978; Bond et al., 1986), Turkish Shoe Workers (Aksoy, 1994), European Shoe Workers (Fu et al., 1996), Scandinavian Service Station Workers (Jakobsson et al., 1995, Lynge et al., 1997) and Monsanto Chemical Workers (Ireland et al., 1997).

The Pliofilm Cohort (Rinsky et al., 1981, 1987; Paxton et al., 1994a) has the advantages of insignificant co-exposures, good dose-response relationships, and high relative risks. It has been extensively studied and has undergone significant scientific debate. Its disadvantages lie in that it is a high-dose study and for some workers the potential for saturable metabolism exists. The Pliofilm Cohort has relatively small numbers (1291 exposed workers, 21 lymphohematopoietic cancers followed through 1987), and a large proportion of exposures occurred before 1960 where few actual measurements were made.

The Chinese Worker Cohort (Hayes et al., 1997; Dosemeci et al., 1994; Yin et al., 1996) has many advantages including large numbers of exposed and control workers (74,828 exposed workers, 35,805 controls), dose-response information, good exposure information (especially for most recent ten years of follow-up), a published exposure validation study, and exposures remained relatively constant for a large portion of the cohort. The study reported consistent clinical diagnoses and recording; diagnoses were verified by U.S. pathologists. The disadvantages of this study include the potential for co-exposures, the issue of whether the Chinese population is representative of the U.S. population, and the level of scientific analysis and debate has not been as extensive as for the Pliofilm Cohort.

Analysis of Pliofilm Cohort data

Although several analyses (Paxton et al., 1994b; Crump, 1994; Schnatter et al., 1996b) have been published using the most recent update of the Pliofilm Cohort, OEHHA conducted separate analyses, reported here, to investigate additional questions not addressed by the previous publications. These extensions include (1) the application of linear relative risk models to the 'wetside'[3] workers only, (2) the application of relative risk models utilizing Rinsky exposure estimates, (3) a re-examination of the potential risks of multiple myeloma, (4) an examination of the impact of the starting date of the observation time, and (5) a re-examination of the shape of the dose-response curve. The analyses conducted here also provide independent confirmation of the results reported by Crump (1994).

The mortality, work history and exposure data for each individual in the Pliofilm Cohort (1868 workers total) were kindly provided to OEHHA by the National Institute of Occupational Safety and Health (NIOSH). These data included follow-up information through 1987 on 1291 'wetside' workers, e.g., those workers employed in the rubber hydrochloride processing sections

[3] The terms 'wetside' and 'dryside' were originally coined by the Pliofilm workers. These terms have been retained out of convenience by other researchers (Crump, 1994; Paxton et al., 1994a) and are utilized here for the same reasons.

of the plant that involved direct benzene exposures. Among 1212 non-black male 'wetside' workers[4], 14 leukemia, four multiple myeloma and three lymphoma deaths were recorded. One leukemia case was reported among 66 female workers. Additionally, information was provided on 577 'dryside' workers whose job was not involved in direct rubber hydrochloride processing but may have been exposed to benzene as well. One additional lymphoma death and no additional leukemia cases were observed among the 'dryside' workers. NIOSH did not include the 'dryside' workers in its SMR estimates (Rinsky et al., 1987) and did not follow-up the mortality experience of these workers beyond 1981. Paxton et al. (1994b) used only the 'wetside' workers in their analysis; however, Crump (1994) combined both 'wetside' and 'dryside' workers (1717 non-black, male workers) in his analysis.

As described in the section above on Human Cancer Studies, three separate estimates of exposure are available: (1) the Rinsky exposure matrix (Rinsky et al., 1981, 1987); (2) the Crump exposure matrix (Crump and Allen, 1984), and (3) the Paustenbach exposure matrix (Paustenbach et al., 1992). Paustenbach et al. (1992) attempted to improve on the Rinsky and Crump exposure matrices by including additional information such as extended work weeks, dermal exposure, and several other factors. However, the exposure estimates of Paustenbach et al. (1992) are likely to be unreasonably high and the methods used to generate them have been criticized (Utterback and Rinsky, 1995). The Crump matrix adjusts the exposures where measurements are scarce by relating them to reductions in the Threshold Limit Values (TLV) over time. However, Utterback and Rinsky (1995) noted that available information, including information that has recently become available, suggests that overhead ventilation systems were in place before 1942. There is no evidence of significant improvements in these control systems through 1975 at which point the process was terminated. Although refinements are possible, the Rinsky exposure matrix remains the most consistent with the available information of the Pliofilm production processes and conditions. For comparison purposes, all analyses were repeated using each of the three exposure matrices.

One issue that was addressed in the analysis was to examine the impact on the start date for calculating the person-years at risk for the cohort. Rinsky et al. (1987) defined the cohort as individuals working at least one day in the rubber hydrochloride processing plant between 1940 and 1965 but began accumulating person-years at risk starting in 1950. Paxton et al. (1994a) began accumulating person-years at risk starting in 1940 since they believed the cohort had already been well established by NIOSH. The redefinition added 13 additional lymphoid or hematopoietic cancers (12 male, one female deaths) and added a significant amount of person-years of follow-up. The reason that Rinsky et al. began follow-up in 1950 was that there was a policy at the company in the 1940s that if a worker left the job his or her records were pulled and placed in storage (microfiched). Because of the way in which cohort members were identified and tracked, this meant that all workers who left the plant before 1950 were effectively excluded from the analysis (personal communication Dr. Rinsky, December 1998). Thus, inclusion of follow-up from 1940 could potentially underestimate risk if the rate of leaving one's job at the plant between 1940 and 1950 was related to benzene-induced susceptibility or symptoms. NIOSH has learned via local physician reports of at least two additional leukemia cases in individuals who were workers at the rubber hydrochloride plant, but left before 1950. It is very difficult, if not impossible, for NIOSH to systematically determine how many individuals were excluded through this mechanism, thus, these two cases cannot be included in the cancer count. To address this issue, all analyses were conducted separately using 1940 or 1950 as the beginning of the observation time.

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[4] Thirteen 'wetside' workers were coded as black and for many race was not known. For six workers, the sex was unspecified. Thus, the 1212 workers used in the analysis were treated as white males.

Data on the individual worker histories were read by the data transformation and tabulation program DATAB, which is part of the EPICURE software package (Preston et al., 1993). After boundaries for categorical variables are defined, the program designates cells by a set of integers, one for each categorical variable to identify the category number of that variable. DATAB calculates cases, person-years, and estimates expected deaths based on U.S. age-sex-race-year-cause-specific mortality rate tables. Summary information and relative risks (observed/expected) for categories of cumulative exposure for Pliofilm 'wetside' are provided in Table 19 and are shown graphically in Figure 3. Similar summary information was developed for the 1717 non-black males of both 'wetside' and 'dryside' operations (Table 19). DATAB was programmed to generate time-dependent variables covering each calendar year at risk (e.g., 1940-1987) which were saved for use by the regression program, AMFIT. Exposure categories, as originally reported by Rinsky et al. (1987), were used here because they provided a roughly even distribution of cases across exposure categories for the Rinsky and Crump exposure estimates. These cutpoints were originally chosen because they are equivalent to 1, 5, 10 or >10 ppm benzene for 40 years. Results using different exposure category cutpoints were reported by Paxton et al. (1994a) and are shown in Table 8 above.

Table 19. Summary information and relative risk estimates for leukemia among white male Pliofilm workers[1] by exposure category

Exposure Matrix	Categories of cumulative exposure (ppm-years)	Mean	Person-years	Cases	Expected number of cases	Relative risk	95 % CI
'WETSIDE' WORKERS (N=1212)							
Rinsky	0-39	15.9	29648	5	2.228	2.24	0.73-5.24
	40-199	93.3	7209	4	0.677	5.91	1.61-15.13
	200-399	260.8	2476	2	0.232	8.64	1.03-31.12
	≥ 400	530.1	1059	3	0.086	34.76	7.21-101.98
Crump	0-39	15.5	24907	4	1.842	2.17	0.59-5.56
	40-199	108.0	9358	3	0.801	3.74	0.77-10.95
	200-399	279.0	3100	4	0.280	14.29	3.89-36.57
	≥ 400	808.4	3026	3	0.320	9.39	1.94-27.41
Paustenbach	0-39	16.7	20354	3	1.405	2.14	0.44-6.24
	40-199	108.9	9827	2	0.816	2.45	0.29-8.85
	200-399	290.9	3587	2	0.349	5.73	0.69-20.69
	≥ 400	745.3	6623	7	0.673	10.41	4.18-21.43
'WETSIDE' AND 'DRYSIDE' WORKERS (N=1717)							
Rinsky	0-39	13.3	41688	5	2.874	1.74	0.56-4.06
	40-199	92.9	7256	4	0.699	5.72	1.56-14.65
	200-399	260.8	2476	2	0.232	8.64	1.03-31.12
	≥ 400	530.1	1059	3	0.086	34.76	7.21-101.98
Crump	0-39	14.3	36261	4	2.441	1.64	0.45-4.20
	40-199	108.0	10041	3	0.847	3.54	0.73-10.35
	200-399	276.9	3151	4	0.283	14.11	3.85-36.18
	≥ 400	808.4	3026	3	0.320	9.39	1.94-27.41
Paustenbach	0-39	16.0	28461	3	1.810	1.66	0.34-4.85
	40-199	108.6	12072	2	0.938	2.13	0.26-7.70
	200-399	293.4	4042	2	0.373	5.36	0.64-19.36
	≥ 400	747.1	7905	7	0.770	9.09	3.65-18.73

[1] Non-black male 'wetside' workers (n=1212); person-years of risk from 1940 to 1987. Data on individual worker exposure and vital status were provided to OEHHA by NIOSH.

Comparing the summary measures in Table 19, combining the data from the 'dryside' workers with that of the 'wetside' workers effectively adds person-years of exposure to the lower exposure categories without adding new cases, compared to the workers from the 'wetside' only.

Figure 3. Excess relative risk (RR-1) of total leukemia versus cumulative exposure for white male 'wetside' rubber hydrochloride workers comparing three sets of exposure estimates

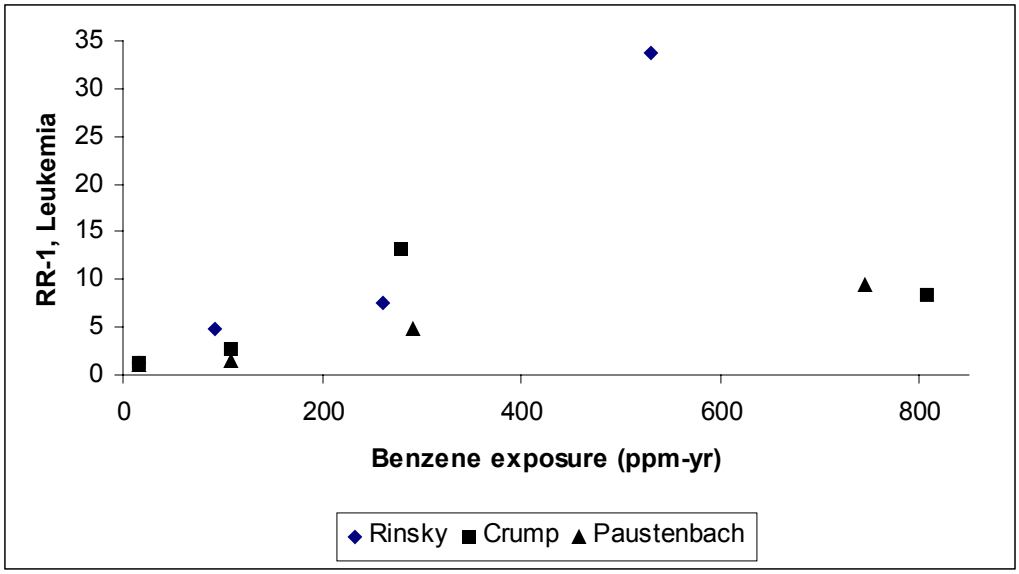

Figure 3 suggests an increasing dose-response relationship with all three exposure measures. The excess risk estimates using the Rinsky and Crump exposure matrices are similar in magnitude for the first three dose categories, but differ in the highest dose category. The mean excess relative risk estimates using the Crump matrix appear less than linear relative to the lower exposure categories. The Rinsky estimates appear linear or possibly greater than linear relative to the lower dose groups; however, confidence bounds on these estimates are large, which limits any conclusions regarding the shape of the dose response relationships.

Poisson regression analysis (individual survival data)

Absolute and relative risk models were applied to the available data using Poisson regression. In the relative risk model (Equation 11), the rate of expected deaths λ(dose, t) is a linear function of the cumulative exposure (dose), where λ_0* is the application of the background rate of expected deaths based on age-dependent U.S. cancer mortality rate tables. This model is equivalent to the linear multiplicative model employed by Crump (1994) (Equation 9). The model was also applied without external rates (Equation 12) such that λ_0 represents the crude background cancer rate estimated from the data. Equation 13 represents the absolute (additive) risk model. The cancer potency, β_1 , was estimated by Poisson regression, a maximum likelihood procedure.

$\lambda(dose, t) = \lambda_0^*[1 + \beta_1(dose)]$ (Equation 11, Relative risk model)
$\lambda(dose, t) = \lambda_0[1 + \beta_1(dose)]$ (Equation 12)
$\lambda(dose, t) = \lambda_0^* + \beta_1(dose)$ (Equation 13, Absolute risk model)

Analyses were performed using the program AMFIT, which is part of the EPICURE computer software package (Preston et al., 1993). AMFIT, written by Dale Preston and Donald Pierce, utilizes the event-time tables generated by the DATAB program from the individual survival data to do Poisson regression analysis. AMFIT computes maximum likelihood estimates of parameters in a general class of hazard function models, including excess and relative risk models. This program was used by both the BEIR IV and BEIR V Committees (NRC, 1990).

Poisson regression analysis was performed on the 1212 non-black male 'wetside' workers and the 1717 non-black male 'dryside' and 'wetside' workers (combined) for each of the three sets of exposure estimates (i.e., Rinsky, Crump and Paustenbach). Inclusion of the 'dryside' workers in the analysis had little impact on the potency estimate (Table 20). However, results using the Rinsky exposure matrix were roughly two-fold higher than results using the Crump matrix and roughly four-fold higher than those using the Paustenbach matrix.

Additionally, for the 'wetside' workers three separate DATAB programs were written to remove individuals with cumulative exposures of 400 ppm-yrs or greater for each of the Rinsky, Crump or Paustenbach exposure estimates. This step effectively removes the top dose group from the analysis (Table 19). Poisson regression using AMFIT was conducted on the remaining individuals: Rinsky (n=1182), Crump (n=1129) and Paustenbach (n=1021). The reasons for this approach are as follows. A primary difference in the Rinsky and Crump exposure matrices is how the researchers handled the early high-dose exposures where few actual measurements were made. Greater uncertainty may exist with respect to these exposure estimates. Also, the true shape of the dose-response curve may be supralinear (i.e., the trend in relative risk falls below a linear trend with dose) as is seen for the Chinese Worker Cohort (Figure 4, Figure 5) and for the Pliofilm Cohort using the Crump exposure estimates (Figure 3). The possible reasons for the supralinearity are not clear, but could be due to competitive metabolism among benzene and its metabolites (Rothman et al., 1998) or may also be related to susceptible subpopulations and the use of the cumulative dose metric. Thus, if the true relationship is supralinear, then inclusion of individuals with high exposures would underestimate the risks at low exposures. It should be noted that in the analysis of the Chinese Worker Cohort below, the top dose group was also removed. The average exposure level in the top dose group was 60 ppm benzene for the Chinese Worker Cohort, and 19 ppm and 60 ppm for the Rinsky and Crump exposure matrices, respectively, for the Pliofilm Cohort. Thus, removal of the top dose group from the respective datasets is comparable. Results, shown in Table 20, indicate that cancer potency estimates based on the Crump and Rinsky exposure matrices are essentially the same with the top dose-group removed.

Previous investigators conducted their analyses using different latency or "lag" times (e.g., zero, three or five years) (Crump and Allen, 1984; Thorslund, 1988; Crump, 1994). Lag times are employed to adjust exposure and risk estimates if one has reason to believe that recent exposures before death are not related to the disease process. Crump (1994) tested zero-, three- or five-year lag times and selected a lag time of five years because of improvements to the goodness of the curve fits. For the analyses conducted here, a lag time of zero was used for ease of computation. Based on radiation data, the BEIR V committee utilized a minimum latency of two years for radiation-induced leukemia following a single or short-term exposure (NRC, 1990). Based on data from use of chemotherapeutic agents, increased risks of therapy-related leukemia have been observed after one year for alkylating agents (Eastmond, 1997) and six months for topoisomerase II inhibitors (Larson et al., 1996). The National Cancer Institute selected a lag time of 1.5 years for estimating benzene exposures of the Chinese Worker Cohort (Hayes et al., 1997). In case studies of benzene-exposed workers, leukemia developed in certain individuals in as little as one year after first exposure (Eastmond, 1997). Thus, the overall error induced by defining a lag of zero instead of one or 1.5 years is expected to be small. Indeed, our analyses employing a lag of

zero obtained similar potency and standard error estimates as those reported by Crump (1994) using a lag of five for equivalent datasets.

Table 20. Cancer potency estimates for leukemia among Pliofilm workers from Poisson regression using the relative risk model (Equation 11)

Exposure matrix	Cohort subset[1] (wetside/dryside)	Potency, β_1 $(ppm\text{-}yr)^{-1}$	95 % CI
All data points			
Rinsky	wetside	$4.5x10^{-2}$	$(1.3x10^{-3} - 7.7x10^{-2})$
Rinsky	wetside+dryside	$4.4x10^{-2}$	$(1.2x10^{-3} - 7.6x10^{-2})$
Crump	wetside	$2.0x10^{-2}$	$(6.0x10^{-3} - 3.5x10^{-2})$
Crump	wetside+dryside	$2.0x10^{-2}$	$(5.6x10^{-3} - 3.4x10^{-2})$
Paustenbach	wetside	$1.3x10^{-2}$	$(4.1x10^{-3} - 2.3x10^{-2})$
Paustenbach	wetside+dryside	$1.2x10^{-2}$	$(3.5x10^{-3} - 2.0x10^{-2})$
Exposures < 400 ppm-yr			
Rinsky	wetside	$3.9x10^{-2}$	$(3.5x10^{-3} - 7.4x10^{-2})$
Crump	wetside	$4.1x10^{-2}$	$(8.0x10^{-3} - 7.4x10^{-2})$
Paustenbach	wetside	$1.9x10^{-2}$	$(4.7x10^{-3} - 4.2x10^{-2})$

[1] Utilizes person-years at risk beginning in January 1, 1940 for non-black, male workers. Cancer potency estimates are based on individual worker data provided by NIOSH (see text).

Defining the follow-up observation period to begin in 1940 versus 1950 had little impact on the magnitude of the potency estimates, β_1, obtained from Poisson regression analyses. When the follow-up period was defined as beginning in 1950, potency estimates from the relative risk model (Equation 11) were 0.045, 0.023, and 0.015 $ppm\text{-}yr^{-1}$, respectively, for the Rinsky, Crump and Paustenbach exposure estimates (all data points). These results are close to those obtained when the follow-up was defined as starting in 1940 (Table 20). Thus, for purposes of this assessment we will define follow-up to begin January 1, 1940 as did Paxton et al. (1994a) and Crump (1994).

Potency estimates from modeling runs where background rates were estimated from the cohort data (Equation 12) were lower than estimates for which external rates were applied. Estimates of β_1 for the 1212 'wetside' workers were 0.023, 0.012 and 0.010 $ppm\text{-}yr^{-1}$ for the Rinsky, Crump and Paustenbach matrices, respectively, and were not significantly different from zero.

The absolute risk model (Equation 13) was also applied to the data. Risk estimates for continuous lifetime exposures, shown in Table 25, were lower (about two-fold) than those obtained from the relative risk model. Model fit as measured by the deviance[5] indicated that the relative risk and absolute models fit the datasets equally well.

Risk estimates for AML or other leukemia subtypes from the Pliofilm Cohort were not evaluated here. Crump (1994) observed that risk estimates for AMML were equivalent or slightly lower than those derived for total leukemia (Table 18).

Addressing the pattern of excess leukemia risk over time following exposure

[5] AMFIT estimates model parameters by minimizing the deviance, which is equivalent to maximizing the likelihood function for the Poisson probability of the hazard of the cancer of interest. Deviance is defined as two times the difference between the log-likelihood contribution of the term and the log-likelihood contribution if the term fit perfectly.

The pattern of risk for leukemia and bone cancer over the years following exposure to radiation is different from most other cancer sites. The BEIR V committee (NRC, 1990) made the following statement with respect to exposure to radiation:

> Following an instantaneous exposure to radiation, the rates of leukemia and bone cancer appear to follow a wave like pattern, rising within 5 years after exposure and then returning to near baseline rates within 30 years. For populations that have been followed for at least this long, no problems of projection arise. One simply models the risk of leukemia over the study period as a function of dose, F(D), and treats that as a lifetime excess risk estimate.

Observations of secondary leukemias following exposure to several classes of chemotherapeutic agents also appear consistent with the same wave-like pattern of risk (Larson et al., 1996; Brusamolino et al., 1998). Using the Pliofilm Cohort data, we investigated whether the changing risk pattern of benzene-induced leukemia was consistent with the observations from individuals exposed to radiation and chemotherapeutic drugs. To test this hypothesis, we reanalyzed the individual cohort data in the following manner. Following the hypothesis that, like radiation, benzene-induced leukemia risks rise and then return to background levels after some period of time (e.g., 30 years) following exposure, we redefined the person-years-at-risk as the duration an individual worked[6] (exposed) plus 5, 10, 15 or 30-years following his last exposure. Person-years-at-risk was conservatively defined in this manner because one does not know when the carcinogenic process for each case began (e.g., first day of exposure to last day of exposure). Cases, expected cases, person-years and cancer potency estimates were calculated by the same methods described above. Results are listed in Table 21. For comparison, the analysis was repeated in which person-years-at-risk was defined as 5, 15, 30, or 40 years following the entry date into the cohort (e.g., date of first exposure) until death or 1987, the end of follow-up. The observed cases, relative risk of leukemia, and person-years for each time point are as follows: 5 years (2, 12.5, 6004); 15 years (4, 6.7, 17608); 30 years (8, 4.4, 32970); 40 years (12, 4.1, 39614).

As seen in Table 21, the pattern of risk for benzene-induced leukemia in the Pliofilm Cohort appears consistent with the wave-like pattern of excess risk observed with radiation and chemotherapy. Clearly the risks of leukemia are highest within the first 15 years of exposure and the rate of excess risk declines considerably by 30 years. This is also reflected in the decreasing potency estimates over time. There is some question as to whether the total benzene-specific leukemia risk has been accounted for by 30 years, since there were two leukemia deaths observed following the 30-year period where only 0.99 deaths were expected. However, the number of individuals and person-years that make up this comparison is small, making any conclusions tenuous. These observations support the contention that, like radiation and other agents, benzene exposure increases risk of leukemia which rises quickly and then declines to (or near) background levels by 30 years. Recently, another researcher has analyzed the temporal patterns of leukemia formation among the Pliofilm workers (Finkelstein, 2000) using a matched case control approach and conditional logistic regression. Finkelstein (2000) also concluded that the pattern of benzene-induced leukemia followed a wave-like pattern similar to ionizing radiation. These observations are also consistent with the findings in the Chinese Worker Cohort in which recent exposures (within ten years of diagnosis) were highly correlated with risks of ANLL whereas distant exposures (greater than ten years) were not (Hayes et al., 1997). Since we do not have the data to accurately define the pattern of benzene-induced leukemia risk over time, this assessment

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[6] The actual analysis defined duration of work beginning at January 1, 1940 unless the start date of employment was after this date. (Results were not appreciably different when the start date was defined as the actual start date of employment for all workers.)

contends that a reasonable approach is to calculate excess risk where only exposures within the last 30 years are calculated as contributing significantly to leukemia risk. The life table calculations of additional risk for general population exposure to benzene (Figure 6) incorporate this concept.

Table 21. Effect of defining the person-years-at-risk for total leukemia as duration exposed plus 5, 10, 15 or 30-years following last exposure compared to total cohort follow-up (1940-1987)

	Person-years-at-risk (duration of employment plus X years following last exposure)				
X years	5	10	15	30	Follow-up 1940 to 1987
Leukemia cases	6	7	7	12	14
Expected cases	0.45	0.73	1.07	2.25	3.24
Relative risk	13.3	9.59	6.54	5.36	4.32
Person-years	11297	16960	22260	35199	40391
Rate of change in excess risk[a]		-0.74	-0.61	-0.08	
Potency, β_1 (Rinsky)[b]	0.068	0.055	0.041	0.048	0.045
Potency, β_1 (Crump)[b]	0.038	0.031	0.023	0.024	0.020

[a] Average rate of change in excess risk per year from the previous run, $((RR-1)_{X2}-(RR-1)_{X1}/X_2-X_1))$

[b] Poisson regression employing the relative risk model, 1212 'wetside' white male workers, Rinsky and Crump exposure estimates. Data on individual worker histories were provided by NIOSH.

Multiple myeloma

Among the 1212 white male 'wetside' workers, four cases of multiple myeloma were observed where 1.17 cases were expected based on U.S. mortality rates; RR = 3.416 (p=0.03, Fisher Exact, Poisson Test). However, one of the cases had worked at the Pliofilm plant for only four days with daily exposure estimates of approximately ten ppm (Rinsky matrix) or 60 ppm (Crump matrix). Poisson regression of the multiple myeloma cases provided a positive, but non-significant potency estimate. As discussed above, the body of evidence suggesting that benzene causes multiple myeloma is not as well established as it is for leukemia. Potential risks from multiple myeloma were not evaluated further.

Poisson Regression Analysis (grouped data)

The impact of using group mean data, as presented in Table 19, compared to more complicated analyses utilizing individual survival data was explored. Potency values were estimated from the mean estimates for the cumulative exposure, observed cases, expected cases (based on U.S. mortality rate tables) and person-years, for each of four exposure categories (Table 19). The mean estimates were fit to the relative risk model: $\lambda = \alpha\lambda_0(1 + \beta d_i)$ where λ is the expected rate of observed deaths, λ_0 is the rate of the expected deaths in each exposure category based on U.S. mortality rates, and d_i is the cumulative exposure (dose) in each exposure category. α is a parameter to account for the differences between the U.S. population and the background rate in the cohort. This was found not to be different from one and, thus, was set equal to one for the subsequent analyses. This methodology is equivalent to that utilized by Crump and Allen (1984).

The resulting potency estimates using grouped mean data, as might be obtained from published summary tables of standard mortality ratios, differed only slightly from estimates utilizing individual survival data (Table 22). Analyses were also conducted on data grouped by different exposure categories, such as those used by Paxton et al. (1994a) and Crump (1994), resulting in similar estimates.

Table 22. Potency estimates for benzene-induced leukemia: comparison of results from grouped data[1] to results from individual survival data

Exposure matrix	Cohort subset (wetside/dryside)	Potency, β_1 $(ppm\text{-}yr)^{-1}$	95 % CI	Difference from estimates using survival data[2]
Rinsky	wetside	$5.0x10^{-2}$	$(1.6x10^{-2} - 8.4x10^{-2})$	10 %
Rinsky	wetside + dryside	$4.7x10^{-2}$	$(1.4x10^{-2} - 8.0x10^{-2})$	6 %
Crump	wetside	$2.1x10^{-2}$	$(6.0x10^{-3} - 3.5x10^{-2})$	5 %
Crump	wetside + dryside	$2.0x10^{-2}$	$(5.6x10^{-3} - 3.4x10^{-2})$	0 %
Paustenbach	wetside	$1.4x10^{-2}$	$(4.2x10^{-3} - 2.3x10^{-2})$	7 %
Paustenbach	wetside + dryside	$1.2x10^{-2}$	$(3.3x10^{-3} - 2.0x10^{-2})$	0 %

[1] Data utilizes person-years at risk beginning in January 1, 1940 for non-black, male workers.
[2] Comparisons to Poisson regression of relative risk model (Equation 14), results as shown in Table 20.

The Rinsky and Crump exposure estimates differ primarily in the manner in which early high exposures were estimated. If one performs Poisson regression on the group mean data with the top dose group removed, then the potency estimates for total leukemia among the 1212 'wetside' white male workers are as follows: 0.045 $ppm\text{-}yr^{-1}$ (0.019 SE) using the Rinsky exposure estimates, 0.039 $ppm\text{-}yr^{-1}$ (0.016 SE) using the Crump exposure estimates, and 0.017 $ppm\text{-}yr^{-1}$ (0.011 SE) using the Paustenbach exposure estimates. Thus, as seen with the potency estimates using the individual data (Table 20), the cancer potencies for the Crump and Rinsky exposure estimates are nearly the same when the top dose-group is removed.

Analysis of Chinese Worker Cohort data

The individual data from the Chinese Worker Cohort were not available to OEHHA. Summary data grouped by four exposure categories from Hayes et al. (1997) were utilized. Hayes et al. (1997) summarized the cases, person-years and relative risks for various hematological neoplasms using several different metrics for exposure. Exposure metrics included cumulative exposure (ppm-yr), average exposure (ppm), 'constant' exposure (ppm) and duration of exposure (yr). The 'constant' exposure group represents a subset of the full cohort (representing about 76 percent of the total person-years at risk) in which exposures remained relatively constant over their work experience. Thus, there is increased confidence in the exposure assignment for this subset of workers. Mean exposure estimates were kindly provided by NCI (Dr. Hayes, personal communication), and included mean estimates for the cumulative exposure group (ppm-yr), and means for the 'constant' exposure groups (ppm) and means of cumulative exposure within the 'constant' exposure group (ppm-yr). Data are shown in Table 23.

Potency estimates were derived for the following hematological neoplasms: ANLL/MDS, ANLL, total leukemia, and non-Hodgkin's lymphoma. Since MDS may be precursors to ANLL and have

not been systematically distinguished from ANLL in past epidemiological studies, NCI provided combined relative risk estimates for ANLL and MDS (Hayes et al., 1997).

Table 23. Summary data used for risk calculations of the Chinese Worker Cohort (Hayes et al., 1997)[a]

	Referent group	Constant, ppm (ppm-yr)			Cumulative exposure, ppm-yr		
		<10	10-24	≥ 25	< 40	40-99	≥ 100
		1.2	15	60	4.3	63.4	347.2
		(6.7)	(67)	(299)			
Neoplasm[b]							
ANLL/MDS							
cases	4	10	4	8	7	7	14
RR		3.2	5.1	7.1	2.7	6.0	4.4
ANLL							
cases	4	6	4	5	5	5	11
RR		1.9	4.9	4.4	1.9	4.3	3.6
Leukemia							
cases	9	12	7	7	11	8	19
RR		1.7	4.0	2.8	1.9	3.1	2.7
NHL							
cases	3	7	0	3	6	1	9
RR		3.0	--	3.5	3.3	1.1	3.5
Person-years	405,000	324,000	88,000	121,000	300,000	119,000	279,000

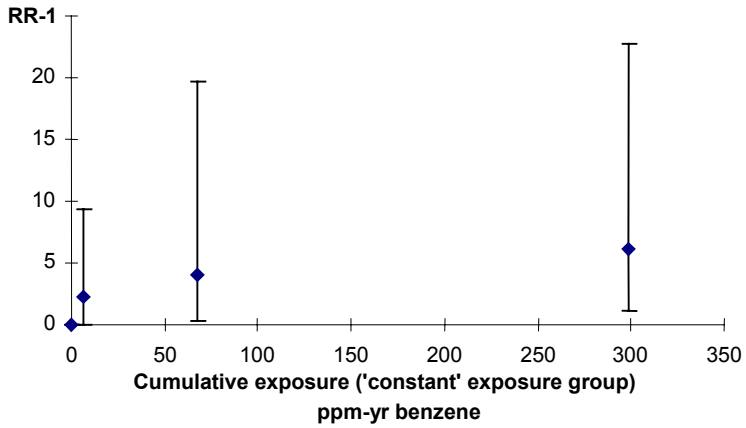
[a]Mean exposure estimate for each exposure category were provided by NCI.
[b] Abbreviations: ANLL, acute non-lymphocytic leukemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin's lymphoma; RR, relative risk.

Modeling of the Chinese worker data presents several special concerns. The first is the apparent supralinear shape of the dose-response curve, as exemplified in Figure 4 for ANLL/MDS in the 'constant' exposure group. Application of linear risk models is problematic and application of exponential risk models is contraindicated; both may underestimate the true risk. The second concern is whether the Chinese population is representative of the U.S. population, especially in the application of background cancer rates. The use of appropriate, unexposed workers as controls provides confidence in the relative risk estimates.

Several approaches were postulated for modeling the Chinese worker data. One approach is to follow the methods suggested by the Proposed Carcinogen Guidelines (U.S. EPA, 1996). That is, assuming the cancer risk is linear at low doses, one would model the (supralinear) data with a best fit curve and select a point-of-departure (e.g., a benchmark dose) from which a straight line is drawn to the origin. The cancer potency is the slope of the extrapolated straight line. The problem with this approach is selecting the appropriate point-of-departure. U.S. EPA (1996) does not provide guidance for selecting this value in cases where human data are available. One could consider using the risk estimate from the lowest dose group as the point-of-departure, or one could select some other point that utilizes more of the data, such as the exposure at which the relative risk is two or three. Alternately, one could apply a non-linear model and use the linear parameter (i.e., q_1*) as the cancer potency estimate. A separate modeling approach is to simply apply a linear dose-response model. Since the error bars surrounding the relative risk estimates are large, the data are not inconsistent with a linear fit, although this may lead to an

underestimation of the true risk (Figure 5). The reasons for the supralinearity are not clear, but may be due to competitive metabolism among benzene and its metabolites at higher exposures (Rothman et al., 1998) or may also be related to susceptible subpopulations and the use of the cumulative dose metric. For these reasons, the data also were analyzed with the top dose group removed.

Figure 4. Excess relative risk (RR-1) of acute non-lymphocytic leukemia and myelodysplastic syndrome (combined) (ANNL/MDS) among benzene-exposed Chinese workers (Hayes et al., 1997)

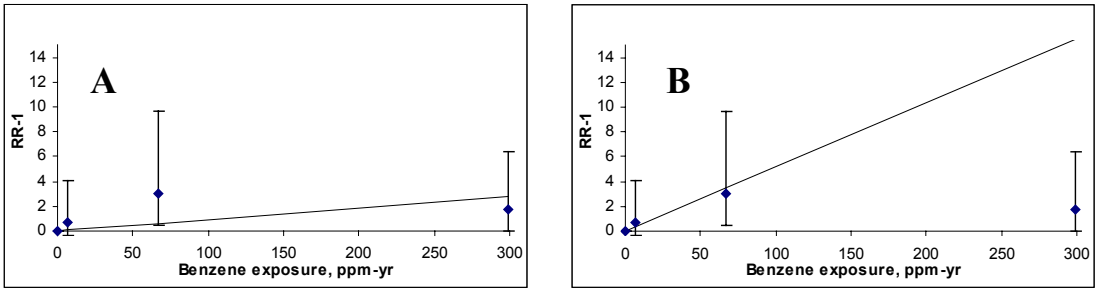


Poisson regression of grouped data

A relative risk model, $\lambda_i = \alpha\lambda_{0i}(1 + \beta d_i)$, and an absolute risk model, $\lambda_i = \alpha\lambda_{0i} + \beta d_i$, were applied to the grouped data for the different cancer endpoints of concern. λ_i is the expected rate of observed deaths, λ_{0i} is the rate of the expected deaths in each exposure category based on rates observed in the unexposed control workers, and d_i is the cumulative exposure (dose) in each exposure category. β is the cancer potency estimate. α, the parameter to account for the differences between the target population and the background rate in the cohort, was set equal to one for the relative risk model and to zero for the absolute model, since the expected rates were estimated from a separate and large group of worker controls.

Cancer potency estimates from Poisson regression fitting of the relative risk and absolute risk models to the data from the 'constant' exposure group are shown in Table 24. The analysis here has focused primarily on the use of the 'constant' exposure group of benzene-exposed Chinese workers. We believe that these provide the most reliable exposure estimates. They may also provide some degree of control for time-dependency of the exposures since these workers were less likely to be employed in jobs at different points in their work life that had significantly higher exposures. More detailed analysis of the individual worker data is needed to study the issues of time-dependency.

Figure 5. Excess total leukemia risk (RR-1) versus cumulative benzene exposure among Chinese workers in the 'constant' exposure group: linear fit of all points (A) and without top dose group (B)



Cancer potency estimates using methodology adapted from U.S. EPA (1996) Proposed Cancer Guidelines

The U.S. EPA Proposed Carcinogen Guidelines (U.S. EPA, 1996) describe methods for assessing non-linear cancer data. Assuming the cancer risk is linear at low doses, the data is fit with a non-linear curve function and a point-of-departure (e.g., a benchmark dose) is selected from which a straight line is drawn to the origin. The cancer potency is the slope of the extrapolated straight line. For animal data, a default point-of-departure is the lower bound of a ten percent response (LED_{10}). However, U.S. EPA (1996) does not provide guidance for selecting this value for human data in which the observed incidences are often on the order of one case in 1000 at risk. One could consider using the risk estimate from the lowest dose group as the point-of-departure. Alternately, one could select some other point that utilizes more of the data, such as the exposure at which the relative risk is two or three. However, without guidance on selecting a point-of-departure, this method seems arbitrary in that the assessor can essentially 'pick a slope' (potency) based on whatever point-of-departure he or she chooses. Thus, this approach was not favored for the Chinese data. For comparison purposes, the potency estimated for total leukemia in the 'constant' exposure group using mean, not upper-bound, estimates for the lowest dose group as the point-of-departure would be 0.7/6.7 (e.g., RR-1/ppm-yr) or 0.104 $(ppm\text{-}yr)^{-1}$ (Table 24).

Table 24. Potency estimates of various lymphohematopoietic cancers derived from Chinese workers in the "constant" exposure group

Neoplasm/Dose categories[a]	Model/Method[b]	β (units)[c]	SE
Total Leukemia			
All dose groups	RR	9.41E-03	3.96E-03
Top dose removed	RR	5.18E-02	2.24E-02
All dose groups	AR	1.19E-07	8.17E-08
Top dose removed	AR	1.03E-06	4.50E-07
Lowest dose	U.S. EPA	1.04E-01	
ANLL/MDS			
All dose groups	RR	2.75E-02	9.02E-03
Top dose removed	RR	1.03E-01	4.35E-02
All dose groups	AR	2.54E-07	8.36E-08
Top dose removed	AR	9.14E-07	3.91E-07
Lowest dose	U.S. EPA	3.28E-01	
ANLL			
All dose groups	RR	1.65E-02	7.25E-03
Top dose removed	RR	6.80E-02	3.63E-02
All dose groups	AR	1.55E-07	6.81E-08
Top dose removed	AR	6.32E-07	3.39E-07
NHL			
All dose groups	RR	7.23E-03	3.32E-03
All dose groups	AR	6.69E-08	3.05E-08

[a] Abbreviations: ANLL, acute non-lymphocytic leukemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin's lymphoma

[b] RR, relative risk model; AR, absolute risk model; U.S. EPA, risk estimate using methods described in U.S. EPA *Proposed Carcinogen Guidelines (U.S. EPA, 1996)* where the point-of-departure is the lowest dose group. Utilizes summary data as shown in Table 23.

[c] β has different units for the AR and RR model forms: $(\text{ppm-yr})^{-1}$ for the RR and $(\text{deaths/person-yr})(\text{ppm-yr})^{-1}$ for the AR model.

Estimation of additional lifetime risk

Once a potency estimate, β_1, has been calculated from the cohort data, the final stage of the dose-response assessment involves the calculation of additional lifetime risk for a pattern of exposure in the population of interest (e.g., continuous exposure of the general population). This is done with standard life table techniques (NRC, 1990). Several important decisions were made regarding the appropriate inputs for the life table. As demonstrated below (Table 26), these choices significantly affect the magnitude of the final risk estimate. These decision points include the following.

(1) *Defining the target population*. The target population was defined as all races, both sexes. This choice differs from nearly all previous assessments that used white males as the target population, primarily because background leukemia rates for white males are higher than other subpopulations. This assessment uses the entire general population as the target group

and treats white males as a potential sensitive subpopulation in the analyses, since inter-individual variability is likely to be a much larger factor than race or sex.

(2) *Use of incidence or mortality rates*. Although potency estimates were derived from mortality data, the additional risk estimates can utilize either incidence or mortality rates in the target population. The question is whether the assessor feels it is appropriate to protect against 'getting' leukemia or dying from leukemia. This assessment has chosen to use incidence rates for the basis of the risk estimates, an approach supported by current guidelines.

(3) *Wave-like pattern of leukemia risk following exposure.* Calculations of lifetime risk here take into account the wave-like pattern of excess risk of leukemia over time following exposure to benzene (as with radiation and chemotherapeutic agents). That is, exposures prior to some period in the past (i.e., 30 years) do not add significantly to current-year leukemia risk; their inclusion would likely overestimate the true risk. This approach differs from previous assessments of benzene risk, which utilized cumulative exposure over a lifetime. Previous assessors, however, have tried to address this issue by weighting the exposure estimates (Crump and Allen, 1984; Thorslund et al., 1988, Crump, 1994).

(4) *Lag time*. A lag time of 1.5 years for leukemia was utilized to account for minimum latency between exposure and disease (Hayes et al., 1997).

(5) *California versus U.S. background rates*. For leukemia and leukemia subtypes, California and U.S. incidence and mortality rates are very similar. As demonstrated below, risk estimates based on either source are nearly identical.

Potency estimates obtained from the occupationally exposed cohort were converted to those expected for exposure of the general population. Potency estimates were divided by a factor of 0.33 to account for the difference in days per year exposed and for volume of exposed air consumed in a standard work-day relative to 24-hour estimates; e.g., (240 days/365 days)*10 m^3/20 m^3) = 0.33.

Central and 95 percent upper bound potency estimates, adjusted to a continuous exposure scenario, were used to estimate lifetime risks of cancer mortality using life table methods described by DHS (1986). Age-specific mortality rates of the U.S. or California general populations were used to estimate the lifetime cancer risk (in units of ppm^{-1}). An example life table, shown in Figure 6, calculates additional risk for total leukemia in the general population, based on a potency estimate from the Pliofilm Cohort using the Rinsky exposure estimates.

California-specific death rates and leukemia rates were also obtained and compared to the U.S. mortality rates. Rates of total leukemia in California from 1990 through 1994 were reported by Perkins et al. (1997). Deaths for all causes for California for the years 1990 through 1994, reported in five-year age intervals, were kindly provided by the California Department of Health Services, Center for Health Statistics. Age-specific population estimates were obtained from the California Department of Finance, Demographic Research Unit (http://www.dof.ca.gov/html/Demograp/data.htm). Average annual death rates for 1990-1994 were computed from the age-specific death and population statistics. The California Department of Health Services did not publish mortality or incidence rates for specific subtypes of leukemia (e.g., acute myeloid) (Perkins et al., 1997); however, average annual rates for 1991 through 1995 were kindly provided to OEHHA upon request (personal communication, C. Perkins, California Cancer Registry). The California and U.S. mortality rates for total and various subtypes of

Figure 6. Life table for calculating lifetime additional cancer risks from general population exposure to benzene

LIFE TABLE FOR CALIFORNIANS, ALL RACES, BOTH SEXES
CUMULATIVE LEUKEMIA (Incidence) RISK AT CONSTANT EXPOSURE TO 1 PPM BENZENE
Multiplicative relative risk model
Lag time, 1.5 years
Risks from exposures within the last 30 years

Potency from cohort (1/ppm-yr), adjusted for continuous exposure: 0.16823143
(Estimate using Poisson regression, Rinsky exposure estimates)

		<---------- BACKROUND ---------->							<----------WITH EXPOSURE---------->						
period	age interval (i to i+4)	California 1990-94 annual death rate per [10^5] in age interval (i,i+4)	p(i) P{survival to age i + 4, given survival to age i} (a)	cumu-lative P{survival to age i} (b)	1990-4 Calif. leukemia incidence rate per [10^5] in (i, i+4) background	P{leukemia death, given survival to age i} (c)	Uncon-ditional P{leukemia death in (i, i+4)} (d)	cumulative P{leukemia by age i+4} background (e)	Adjusted 1990-94 annual death rate per [10^5] in age interval (i,i+4) (f)	p(i) P{survival to age i + 4, given survival to age i}	cumu-lative P{survival to age i}	Leukemia cancer incidence rate per [10^5] in (i, i+4) with exposure (g)	P{leukemia death, given survival to age i}	Uncon-ditional P{leukemia death in (i, i+4)}	Cumulative P{leukemia death by age i+4} with exposure
1	0-4	189.1	0.991	1.000	7.5	3.73E-04	3.73E-04	0.000373	182.86	0.991	1.000	8.76	4.36E-04	4.36E-04	0.000436
2	5-9	19.0	0.999	0.991	4.3	2.15E-04	2.13E-04	0.000586	19.04	0.999	0.991	8.64	4.32E-04	4.28E-04	0.000864
3	10-14	24.4	0.999	0.990	3	1.50E-04	1.48E-04	0.000734	26.95	0.999	0.990	8.55	4.27E-04	4.23E-04	0.001287
4	15-19	90.2	0.996	0.988	3.1	1.55E-04	1.53E-04	0.000887	95.44	0.995	0.989	11.44	5.71E-04	5.64E-04	0.001851
5	20-24	108.9	0.995	0.984	2.2	1.10E-04	1.08E-04	0.000995	114.47	0.994	0.984	9.97	4.97E-04	4.89E-04	0.002341
6	25-29	117.9	0.994	0.979	2.3	1.15E-04	1.12E-04	0.001107	125.66	0.994	0.978	12.36	6.16E-04	6.03E-04	0.002943
7	30-34	156.4	0.992	0.973	2.8	1.39E-04	1.36E-04	0.001243	167.73	0.992	0.972	16.93	8.43E-04	8.20E-04	0.003763
8	35-39	206.8	0.990	0.965	3.2	1.59E-04	1.54E-04	0.001397	219.75	0.989	0.964	19.35	9.62E-04	9.28E-04	0.004690
9	40-44	270.8	0.987	0.955	4.7	2.33E-04	2.23E-04	0.001620	289.82	0.986	0.954	28.42	1.41E-03	1.35E-03	0.006036
10	45-49	362.1	0.982	0.943	6.3	3.12E-04	2.94E-04	0.001914	387.60	0.981	0.940	38.10	1.89E-03	1.77E-03	0.007809
11	50-54	530.9	0.974	0.926	9.9	4.88E-04	4.52E-04	0.002366	570.96	0.972	0.922	59.86	2.95E-03	2.72E-03	0.010529
12	55-59	810.5	0.960	0.901	14.9	7.30E-04	6.58E-04	0.003024	870.80	0.957	0.896	90.10	4.41E-03	3.95E-03	0.014478
13	60-64	1269.7	0.938	0.866	22	1.07E-03	9.23E-04	0.003947	1358.73	0.934	0.858	133.03	6.43E-03	5.52E-03	0.019993
14	65-69	1897.6	0.909	0.812	30.2	1.44E-03	1.17E-03	0.005117	2019.82	0.904	0.801	182.62	8.68E-03	6.96E-03	0.026952
15	70-74	2893.8	0.865	0.739	43.9	2.04E-03	1.51E-03	0.006627	3071.46	0.858	0.724	265.46	1.23E-02	8.91E-03	0.035865
16	75-79	4537.4	0.797	0.639	56.1	2.51E-03	1.60E-03	0.008231	4764.43	0.788	0.621	339.23	1.51E-02	9.38E-03	0.045241
17	80-84	7146.2	0.700	0.510	73.1	3.07E-03	1.57E-03	0.009797	7442.03	0.689	0.490	442.03	1.85E-02	9.03E-03	0.054276
18	85+	14746.4		0.356	83.8	2.03E-03	7.22E-04	0.010519	15085.53		0.337	506.73	1.13E-02	3.82E-03	0.058100

Difference, lifetime risk = 0.0476 1/ppm

(a) Probability of surviving to ith age interval, given survival to the beginning of that interval, p(i) = exp(-5*total death rate(i))
(b) Cumulative probability of surviving to the beginning of the ith interval, c(i) = c(i-1)*p(i-1)
(c) Probability of dying of leukemia in the ith interval, given survival to that interval, pleuk(i) = (leukemia death rate/total death rate)*p(i)
(d) Probability of dying of leukemia in the ith interval, not conditioned on surviving to the beginning of the ith interval = pleuk(i)* c(i).
(e) Cumulative probability of dying of leukemia through the end of the ith interval {I.e., sum of pleuk(i)*c(i) through the ith interval}
(f) Adjusted death rate, all causes, with exposure = total death rate + background leukemia death rate*(potency estimate * dose(i) - 1), where
from age interval 0-4 through 25-29 dose(i) is the cumulative dose to that age (e.g., 1 ppm * (midpoint of age interval in years - lag time));
from age interval 30-34 through 85+ dose(i) is a cumulative dose of 30 ppm-yrs.
(g) Predicted leukemia death rate with exposure = background leukemia death rate(1 + potency estimate * dose(i)).

Rates of Leukemia in California 1990-1994 from Perkins et al. (1997).
Death rates, all causes, California 1990-1994 from California Department of Heath Services, Center for Health Statistics
Population statistics in California 1990-1994 from California Department of Finance, Demographic Research Unit, http://www.dof.ca.gov/html/Demograp/data.htm.
Formulas for this life table from Health Effects of Cadmium, California Department of Health Services (DHS, 1986) and Crump and Allen (1985)

leukemia do not differ significantly. Average annual age-adjusted rates per 100,000 for 1990-1994 were 6.0 in California versus 6.3 in the U.S. for all races, both sexes. Also, rates for total leukemia have not changed appreciably over time (Perkins et al., 1997; Ries et al., 1997). Age-specific incidence rates for leukemia and leukemia subtypes in California (1991-1995) are reproduced in Appendix A.

Annual U.S. incidence and mortality rates from total leukemias and non-Hodgkin's lymphoma (1990-1994) were published by U.S. National Cancer Institute (NCI) (Ries et al., 1997). Death rates from all causes (1990-1994) were obtained from the CDC Vital Statistics Reports (U.S. DHHS, 1993a,b; 1995; 1996a,b). In all endpoints, rates were for all races and both sexes. Incidence and mortality rates for ANLL and MDS were not published by NCI (Ries et al., 1997) and were not available upon direct request.

Lifetime risk estimates from the Pliofilm Cohort data

Risk estimates (ppm^{-1}) for total leukemia stemming from a constant lifetime exposure of the general population to benzene are presented in Table 25. These estimates are based on individual survival data of the 1212 male, non-black 'wetside' workers using the linear relative risk model, fitted with Poisson regression/maximum likelihood techniques. The Rinsky exposure estimates are most consistent with the available information and are judged to provide the best estimate. However, risk estimates using the Crump exposure matrices provide a reasonable alternative considering the uncertainties involved. The Paustenbach exposure estimates are likely to be unreasonably high (Utterback and Rinsky, 1995) and were not utilized for the risk computations.

Table 25. Lifetime risk estimates for total leukemia from continuous exposure of the general population to benzene, based on potency estimates from the Pliofilm Cohort[a]

Exposure matrix	Model[b]	β_{life} (adjusted)	β_{life} (95 % upper bound)	Lifetime risk (ppm^{-1})	
All data points				Mean	95 %UCL
Rinsky	RR	0.136	0.168	0.039	0.048
Crump	RR	0.061	0.076	0.018	0.022
Rinsky	AR	1.20E-05	1.49E-05	0.021	0.026
Crump	AR	5.81E-06	7.19E-06	0.010	0.012
Exposures < 400 ppm-yr				Mean	95 %UCL
Rinsky	RR	0.118	0.153	0.034	0.044
Crump	RR	0.124	0.157	0.036	0.045
Rinsky	AR	1.08E-05	1.405E-05	0.019	0.024
Crump	AR	1.012E-05	1.291E-05	0.017	0.022

[a] Estimates based on individual worker exposure and mortality data provided by NIOSH (see text).
[b] Absolute and relative risk models. β has different units for the AR and RR model forms (see Table 24).

Selection of the various input parameters into the life table has a large effect on the magnitude of the final risk estimate. Estimates of additional benzene-induced leukemia risk (using all data points for non-black 'wetside' males) were compared for the following: incidence versus mortality rates, insignificant excess risk from exposures more than 30 years in the past, and white males versus total general population (Table 26). Also shown in Table 26 is a comparison of risk estimates using U.S. versus California total leukemia rates. Comparisons utilized the mean cancer potency estimate, adjusted for continuous exposure, from the relative risk model utilizing all data points for the Pliofilm Cohort (Table 20). The use of leukemia incidence rates resulted in risk estimates that were higher (about 30 percent) than those based on mortality rates. Risk estimates that were based on the concept that exposures more than 30 years in the past do not contribute significantly to leukemia risk were considerably lower (greater than two-fold) compared to estimates based on cumulative lifetime exposure.

Moreover, leukemia risk estimates from benzene were higher for white males (roughly 20 percent) than for the general population (all races, both sexes). Because of the higher background rate of leukemia in white males relative to other groups, white males might be thought of as a sensitive subpopulation to the effects of benzene. Also, as expected, estimates based on U.S. death and leukemia rates were equivalent to those based on California death and leukemia rates. Thus, all risk estimates generated in this assessment should also be appropriate for other state or federal regulatory programs.

Table 26. Impact of various input parameters on the estimates of lifetime leukemia risk from constant exposure to benzene

Life table inputs			**Lifetime additional risk** (ppm^{-1})	
Leukemia rates	Significance of past exposures	Population	Rinsky[a] (upper 95 % CI)	Crump[a] (upper 95 % CI)
CA Incidence[b]	30-year[b]	All races, both sexes[b]	0.039 (0.048)	0.018 (0.022)
CA Mortality	30-year	All races, both sexes	0.027 (0.033)	0.012 (0.015)
CA Incidence	Lifetime	All races, both sexes	0.083 (0.101)	0.039 (0.048)
U.S. Incidence	30-year	All races, both sexes	0.038 (0.046)	0.017 (0.021)
U.S. Incidence	30-year	White males	0.046 (0.057)	0.021 (0.026)

[a] Potency estimates based on the Rinsky or Crump exposure matrices, relative risk model, all data points (Table 20). Estimates derived from individual worker data as provided to OEHHA by NIOSH.
[b] Parameters selected as most appropriate.

Lifetime risk estimates from the Chinese Worker Cohort data

Cancer potency estimates obtained from Poisson regression analysis (Table 24) were applied to the life table to calculate the risk to the general population from lifetime exposure to benzene. Lifetime risk estimates are provided in Table 27.

Risk estimates from lifetime exposure to benzene were based on methodology adapted from the U.S. EPA Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996). The potency estimate, adjusted for general population exposure (e.g., 24 hr/d, 365 days/yr), would correspond to a lifetime risk of 0.087 ppm^{-1}. An equivalent lifetime risk estimate for ANLL/MDS in the same subgroup of workers is about 0.098 ppm^{-1}, based on ANLL incidence rates. As expected, these risk values are considerably higher than risk estimates that utilize additional dose groups in the estimation.

Table 27. Lifetime risk estimates for total leukemia from continuous exposure of the general population to benzene, based on potency estimates from the Chinese Worker Cohort (Hayes et al., 1997)

Neoplasm/Dose categories	Model/ Method[a]	Lifetime Risk from Exposure to One ppm Benzene (ppm^{-1})[b] Mean	95 %UCL
Total Leukemia			
All dose groups	RR	0.0084	0.011
Top dose removed	RR	0.045	0.056
All dose groups	AR	0.00063	0.00091
Top dose removed	AR	0.0054	0.0070
Lowest dose	U.S. EPA	0.087	
ANLL/MDS[c]			
All dose groups	RR	0.0088	0.011
Top dose removed	RR	0.032	0.041
All dose groups	AR	0.0014	0.0016
Top dose removed	AR	0.0048	0.0062
Lowest dose	U.S. EPA	0.098	
ANLL			
All dose groups	RR	0.0053	0.0068
Top dose removed	RR	0.021	0.029
All dose groups	AR	0.00082	0.0011
Top dose removed	AR	0.0034	0.0045
NHL[d]			
All dose groups	RR	0.010	0.013
All dose groups	AR	0.00022	0.00031

[a] RR, relative risk model; AR, absolute risk model; U.S. EPA, risk estimate using methods described in the U.S. EPA *Proposed Carcinogen Guidelines (U.S. EPA, 1996)* where the point-of-departure is the lowest dose group.
[b] Additional risk of lifetime exposure of the general population to benzene was calculated using life table methods (Figure 6). Life table calculations employed California leukemia incidence data, and exposures greater than 30-years in the past were not considered to contribute significantly to benzene-induced risk (see text).
[c] Additional risk estimates are based on ANLL incidence rates (Appendix A), thus are likely to underestimate the risk of ANLL/MDS.
[d] Based on results of all workers (i.e., cumulative exposure group) since no cases were observed for the mid-dose group of the 'constant' exposure group. A lag time of ten years was used in the life table calculations.

Selection of best estimates of cancer risk for general population exposure to benzene

Although a range of lifetime risk estimates is consistent with the available data, a best (most justifiable) estimate was selected for setting the PHG for benzene. Estimates based on the relative risk model were preferred over estimates from the absolute risk model. The relative risk model generally provided higher (more health protective) potency estimates and equivalent model fits compared to the absolute risk model. The relative risk model was preferred by the BEIR V committee for assessing radiation-induced leukemia (NRC, 1990), and has been more often used in setting regulatory standards than the absolute risk model.

The range of reasonable estimates and the uncertainties in the estimates are described here and in the Risk Characterization section below.

Best estimates for the rubber hydrochloride workers (Pliofilm Cohort) should be based on the following. (1) The most appropriate subset of workers is the 1212 non-black male workers that NIOSH felt was most suitable for continued follow-up and whose mortality history was updated through 1987. These workers were employed in jobs with direct processing of the rubber hydrochloride and exposure assignments were based on actual measurements. (It should be noted that inclusion of the workers not directly involved in the Pliofilm manufacturing, referred to as 'dryside' workers, did not affect the cancer potency estimates.) (2) The Rinsky exposure estimates appear to be the most consistent with the available information regarding benzene concentrations at the plant over time. Because of the uncertainties associated with exposure estimates, particularly in the early years of the cohort (Kipen et al., 1989; Cody et al., 1993; Ward et al., 1996), the Crump estimates provide a reasonable alternative. Cancer potency estimates using the Rinsky or Crump exposure estimates were essentially the same when the workers with the highest exposures were removed from the analyses. (3) Linear risk models are most appropriate for the data. Linear models provide adequate fits, and most non-linear models provide poor fits (Crump, 1994). Based on visual inspection (Figure 3), the data are consistent with a linear relationship with dose for the Rinsky and Crump exposure estimates. Also, the dose-response relationships observed in the much larger study of Chinese workers (Hayes et al., 1997), and possibly the Pliofilm workers using the Crump exposure estimate appear supralinear (i.e., the trend in relative risk falls below a linear trend with dose). Thus, exponential models were not considered. Additionally, epidemiological and mechanistic evidence also supports the use of a linear model and suggests against the notion of a threshold (see section on Low Dose Linearity, page 88). (4) The risk estimate from analyses where exposures > 400 ppm-yr were removed is selected as the most appropriate to ensure that risks at low exposures are not underestimated (see text). (5) The upper 95 percent CI of the risk estimate should be utilized to account for latent uncertainties in the data and for variability in susceptibility in the general population. As discussed in detail above, there are myriad sources of potential susceptibility to benzene hematotoxicity, including genetic susceptibilities and environmental and host factors. For example, a 7.6-fold difference in relative risk of benzene-induced hematotoxicity among different phenotypes of CYP2E1 and NQO1 was observed (Rothman et al., 1997).

Thus from the Pliofilm Cohort, the most justifiable estimate for estimation of leukemia risk to the general population is 0.044 ppm^{-1} (Table 25, exposures < 400 ppm, Rinsky exposure estimates, 95 percent CI, total leukemia). However, a reasonable range of mean and upper bound risk estimates within the scientific band of uncertainty would include those based on Crump exposure estimates, the absolute risk model, and workers with high exposures, 0.010 to 0.048 ppm^{-1}. The risk estimates for acute myeloid leukemia were usually slightly lower than the estimates of total leukemia (Crump, 1994).

Selection of risk estimates from the Chinese Worker Cohort is as follows. (1) The most appropriate subset of workers would be those in the 'constant' exposure group. For this subset of workers, which comprise roughly 75 percent of the cohort, the benzene concentrations were roughly the same throughout their entire work experience. Thus, confidence in their exposure assignment is greatest. (2) Although the data appear supralinear, a linear fit is not inconsistent with the data because of the large confidence bands surrounding the grouped estimates. An alternate approach would be to use the exposure level in the lowest dose group as a point of departure for assuming linearity from that point to the origin, consistent with proposed U.S. EPA guidelines (U.S. EPA, 1996). This approach leads to very high risk estimates. However, this assessment contends that a more reasonable approach is to estimate the potency from the data after dropping the high dose group from the analysis. Inclusion of the top dose group would likely underestimate the true risks based on observations of competitive metabolism among benzene and its metabolites in humans (Rothman et al., 1998). (3) For the reasons stated above, the 95 percent CI upper bound estimate is preferred to account for latent uncertainties and human variability.

Thus from the Chinese Worker Cohort, the best estimate for estimation of leukemia risk to the general population exposure to benzene is 0.056 ppm^{-1} (Table 27), which corresponds to the upper-bound estimate for total leukemia in the 'constant' exposure group with the top dose removed from the analysis. However, a range of mean and upper bound estimates within the scientific band of uncertainty would include those based on the use of the absolute risk model and those based on U.S. EPA methodology. Thus, risk estimates for total leukemia ranged from 0.0054 to 0.087 ppm^{-1}. Risk estimates for other endpoints, such as ANLL, generally fell within this range.

OEHHA believes that data from the Chinese Worker Cohort (Hayes et al., 1997) and the Pliofilm Cohort (Paxton et al., 1994) are suitable as the basis for risk assessment. The PHG for benzene-induced cancer is consistent with cancer potencies derived from the best upper 95 percent confidence bound lifetime risk estimate from the Pliofilm Cohort (0.044 ppm^{-1}), the Chinese Worker Cohort (0.056 ppm^{-1}), or the geometric mean of the estimates from the two studies (0.050 ppm^{-1})..

Although there is evidence to suggest that benzene may induce non-Hodgkin's lymphoma or multiple myeloma, the evidence for these endpoints has not been clearly established. Thus, risk estimates for these endpoints were not added to the risk estimates for leukemia.

Leukemia risk estimates from other cohort studies of benzene-exposed workers

Cancer potency estimates from other cohort studies were used to calculate lifetime risk estimates for the general population (Table 28). The estimates were compared to the results obtained from the Pliofilm Cohort and Chinese Worker Cohort. These studies included those reported by Ott (1978), Wong et al. (1987), Aksoy (1994), Jackobsson et al. (1993); Lynge et al. (1997), Ireland et al. (1997), and Fu et al. (1996). For those studies in which cases, expected deaths, and person-years were reported, potency estimates were calculated using Poisson regression, and additional lifetime risk was computed using life table methods as described above. For other studies, a simple risk calculation was made using the overall relative risk and exposure estimates (Equation 2). The lifetime risk estimates for total leukemia from the Dow Chemical Workers (Ott et al., 1978) and from the U.S. Chemical Workers (Wong, 1987) were consistent with those obtained from the Pliofilm Cohort and Chinese Worker Cohort. Ireland et al. (1997) studied workers employed at one of the facilities included in the Wong (1987) study, but broadened the inclusion criteria and extended the follow-up time.

Lifetime risk estimates calculated from the Ireland et al. (1997) study were much higher than from other cohorts. However, the results are not statistically significant, which may be due to the low-level exposures (< 15 ppm-yrs). Also, the authors noted that early exposures of the cohort may be underestimated which would result in an overestimation of the true risk. Lifetime risk estimates for AML from Scandinavian service station workers (Jakobsson et al., 1993) were higher than those for the Pliofilm Cohort and the Chinese Worker Cohort, although a similar and larger study by Lynge et al. (1997) yielded lower, non-significant risk estimates. Crude risk estimates of benzene-exposed shoe workers (Fu et al., 1996) ranged from zero in English shoe workers where exposures were not quantified to 0.0015 (0.0041, upper 95 percent CI) for shoe workers in Florence. Crude risk estimates for Turkish shoe workers (Aksoy, 1994) were about ten-fold lower than for the European shoe workers (Table 28). In summary, the risk estimates obtained from other occupational studies of benzene are generally consistent with the range of risk estimates obtained from the Pliofilm Cohort and the Chinese Worker Cohort. The differences in the cancer potency and associated lifetime risk estimates among these studies may be explained in part if the true dose-response relationship is supralinear (i.e., the trend in relative risk falls below a linear trend with dose). Generally, the studies with highest exposures (Aksoy, 1994; Fu et al., 1996) provided the lowest potency which would be expected from linear extrapolation from the high end of a supralinear curve. Likewise, the studies providing the lowest exposure levels (Ott et al., 1978; Wong, 1987; Jakobsson et al., 1993; Ireland et al, 1997; Lynge et al., 1997) provided the highest potency estimates, which is consistent with linear extrapolation from the low end of a supralinear curve.

Table 28. Estimates of leukemia risk from several cohort studies

Study/cohort	Potency estimate ($ppm\text{-}yr^{-1}$)	SE	Lifetime risk to the general population continuously exposed to one ppm benzene	
			Mean	Upper 95 % CI
Ott et al. (1978)	0.041	0.0065	0.036	0.039
Wong (1987)	0.047[a]	0.032	0.041 NS[b]	0.058
Ireland et al. (1997)	0.225	0.182	0.173 NS	0.245
Aksoy (1980, 1985, 1994)			0.00018 to 0.00025[c1]	--
Jakobsson et al. (1993)			0.070[c2] (AML)	0.147
Lynge et al. (1997)			0.0082[c3] (AML) NS	0.032
Fu et al. (1996)			0 (English cohort) to 0.0015[c4] (Florence)	0.0041

[a] From Crump and Allen (1984) using equivalent unpublished data from Wong, assuming an SMR of 50 in the unexposed controls

[b] NS, not statistically significant.

[c] Crude risks of total leukemia or AML were estimated by Equation 2: $(RR-1)I_{referent}/ppm$. These estimates do not account for the wave-like pattern of leukemia risk following exposure to benzene, adjustment for this factor would reduce the estimates roughly two-fold (Table 26). Risk estimates indicated in Table 28 were derived as follows:

1. (2.26-1)(0.0105)/150 to 210 ppm/0.33 (occupational/continuous) = 0.00018 to 0.00025 ppm^{-1}.
2. (3.6-1)(0.0027)/0.31 ppm/0.33 = 0.070 ppm^{-1}.
3. (1.3-1)(0.0027)/0.3 ppm/0.33 = 0.0082 ppm^{-1}.
4. (2.14-1)(0.0105)/25 to 600 ppm/0.33 = 0.000060 to 0.0015 ppm^{-1}.

Comparison of benzene-induced leukemia risk to smoking-induced leukemia risk

Benzene is a constituent of tobacco smoke, and for smokers in the general population cigarette smoking is the major source of benzene exposure (Wallace, 1996). Based on U.S. EPA TEAM studies, an average individual receives a dose of approximately 55 µg benzene from each cigarette smoked (Wallace, 1996). Thus a typical heavy smoker who smokes 32 cigarettes per day receives a dose of about 1800 µg benzene/day. Cigarette smoking is associated with increased risk of leukemia, based on several large cohort studies (Kinlen and Rogot, 1988; McLaughlin et al., 1989; Garfinkel and Boffetta, 1990; Siegel, 1993). In this section, we used the smoking data in humans to calculate potency and lifetime risk estimates for leukemia from benzene in cigarettes and compared those results to the estimates developed above for benzene-induced leukemia in occupationally exposed workers. If the benzene-induced lifetime risk estimates for leukemia from the worker cohorts exceed the estimates from smokers, then this would indicate that we have potentially overestimated cancer potency and lifetime risk.

Several epidemiological studies of smoking and leukemia are available (reviewed in Siegel, 1993); however, the two largest studies (McLaughlin et al., 1989; Garfinkel and Boffetta, 1990) have the greatest statistical power to detect an association (Siegel, 1993). McLaughlin et al. (1989) studied 293,916 male U.S. veterans (1588 leukemia deaths) whose mortality experience was followed for 26 years. Relative risks for total leukemias for nonsmokers, < 10, 10-20 and > 20 cigarettes per day were 1.00, 1.07, 1.33 and 1.34, respectively. Relative risks for acute leukemias for nonsmokers, < 10, 10-20 and > 20 cigarettes per day were 1.00, 1.10, 1.47 and 1.16, respectively.

Garfinkel and Boffetta (1990) reported results from two extremely large prospective cohort studies on U.S. citizens enrolled by the American Cancer Society, referred to as Cancer Prevention Study (CPS) I and II. CPS I followed the mortality experience of more than one million individuals, age 30 years or

greater, from 25 U.S. states who were enrolled in the study in 1959 and followed through 1972. CPS II studied approximately 1.2 million U.S. citizens, 30 years and older, drawn from every state in the U.S., who were enrolled in 1982 and followed through 1986. In males in CPS I, relative risks for total leukemia for former smokers, current smokers (1-19 cigarettes/day) and current smokers (>20 cigarettes/day) were 1.36, 1.32 and 1.61 respectively compared to nonsmokers. Relative risks for myeloid leukemia in males were 2.23, 2.25, and 2.87 respectively. In males in CPS II, relative risks for total leukemia for former smokers, current smokers (1-19 cigarettes/day) and current smokers (>20 cigarettes/day) were 1.35, 1.55 and 1.41, respectively, compared to nonsmokers. Relative risks for myeloid leukemia in males were 1.12, 1.65, and 1.75, respectively. All associations of leukemia and smoking in males were significant ($p<0.05$). Relative risks of leukemia among smoking females were not significantly different from non-smokers in either CPS I or CPS II.

It should be noted that the shape of the dose-response curve for smoking-induced leukemia in males is supralinear (i.e., the trend in relative risk falls below a linear trend with dose), similar to that observed for benzene-induced leukemia in the Chinese Worker Cohort. The reason for the supralinear relationship may be that more highly exposed smokers are dying from other causes, thus effectively reducing the observed risks from leukemia. Alternately, the true relationship may be supralinear for reasons not apparently clear. Cancer potency estimates for total leukemia were estimated from the Garfinkel and Boffetta (1990) data with the high dose group removed in a manner equivalent to this assessment's treatment of the Chinese Worker Cohort and Pliofilm Cohort data for benzene. Since the number of deaths and person-years for each category were not reported, a mean potency was calculated using linear regression of the excess relative risk (RR-1) versus exposure (using the mid-point of the low-dose group, ten cigarettes/day). Ten cigarettes per day are equivalent to an average intake of 550 μg benzene/day or 7.86 μg/kg-day for a standard 70 kg male. This is equivalent to inhaling an average daily concentration of 0.0172 ppm benzene. If one assumes that all cohort members smoked for 50 years, then the average cumulative exposure for the smokers in the low-dose group is 0.86 ppm-yr. With these estimates, cancer potency values for total leukemia from benzene from cigarette smoking of 0.372 and 0.640 ppm-yr^{-1} were calculated for the CPS I and CPS II, respectively, for a geometric mean of 0.488 ppm-yr^{-1}. These potency estimates assume that all the leukemia risk associated with smoking is related to benzene which is unlikely, since cigarette smoke contains small quantities of other leukemogens such as 1,3-butadiene, ^{210}Po (α-emitter) and ^{210}Pb (β-emitter) (Austin and Cole, 1986). Also, cigarette smoke contains quantities of hydroquinone and catechol (Hoffmann and Wynder, 1986), metabolites of benzene.

The mean potency estimate for total leukemia and benzene in cigarette smoke, 0.488 ppm-yr^{-1}, was used in a life table calculation of lifetime additional risk. This calculation was equivalent to that used to estimate additional risk for leukemia and benzene exposure among workers (Figure 6, page 108), except that exposure (smoking) was assumed to begin at age 15 [7]. The estimate of lifetime additional risk to the general population for leukemia and benzene from smoking was calculated to be 0.122 ppm^{-1}.

The geometric mean of the central estimate for population lifetime risk of leukemia and benzene derived from occupationally exposed workers (in this assessment) is 0.039 ppm^{-1}. This estimate is 32 percent of the lifetime risk estimate (central estimate) derived from benzene in cigarette smoke. Thus, we conclude that the leukemia lifetime risk estimates developed from the benzene-exposed workers are consistent with the smoking data and do not appear to overpredict benzene-induced risk.

Risk Estimates from Animal Carcinogenicity Studies

A comparative analysis of the cancer potency estimates derived from the human worker (inhalation) studies and those derived from animal cancer studies, especially those employing oral exposures, is also

[7] If one assumes smoking begins at age 30, then the additional risk estimate would be 0.112 ppm^{-1}.

relevant to assess the validity of the PHG. Analyses and reviews of the cancer potency estimates from animal studies have been reported elsewhere (ARB, 1984; Crump and Allen, 1984; U.S. EPA, 1985). ARB (1984) estimated risks from the NTP[8] gavage studies and from the Maltoni et al. (1983) gavage and inhalation studies. Mathematical models using maximum likelihood estimation including the multistage and the Mantel-Bryan models were applied to incidence data on mouse preputial gland carcinomas, mouse mammary gland carcinomas, mouse leukemia and lymphoma (combined), mouse Zymbal gland carcinomas, and rat Zymbal gland carcinomas.

Table 29. Comparison of human equivalent 95 percent upper-bound oral cancer potency estimates, $(mg/kg\text{-}d)^{-1}$, from sensitive tumor sites in animal and human cancer studies of benzene[a]

Study	Leukemia	Leukemia/ Lymphoma	Preputial Gland	Zymbal Gland	Mammary Gland
Animal studies					
NTP (gavage, male mouse)		0.1	0.2	0.04	
NTP (gavage, female mouse)					0.07
Maltoni et al. (1983) (inhalation, male/female rat)				>0.03[b]	
Human studies					
Chinese Worker Cohort	0.1				
Pliofilm Cohort	0.1				

[a] Sources of data are ARB (1984) and this assessment. Animal cancer potency estimates are based on the multistage model.

[b] Retention of inhaled benzene by rats at the doses used by Maltoni et al. (1983) would likely be less than 50 percent (Sabourin et al., 1987). Thus, the human equivalent cancer potency of 0.014 $(mg/kg\text{-}d)^{-1}$ as reported by ARB (1984) would be expected to be greater than 0.03 $(mg/kg\text{-}d)^{-1}$.

As shown in Table 29, the cancer potency estimates from animal studies are very similar to the best estimates obtained from the human studies. The cancer potency estimate of 0.1 $(mg/kg\text{-}d)^{-1}$ for leukemia among benzene-exposed workers was the same as the estimate for lymphoma and leukemia (combined) in the NTP mouse gavage study. The most sensitive tumor site and species, the preputial gland of male mice, yielded an upper-bound cancer potency estimate of 0.2 $(mg/kg\text{-}d)^{-1}$, which is two-fold higher than the best estimate of 0.1 $(mg/kg\text{-}d)^{-1}$ from the Chinese Worker Cohort or the Pliofilm Cohort (this assessment).

In a similar analysis, U.S. EPA (1985) adapted analyses of animal experiments conducted by Crump and Allen (1984). Crump and Allen (1984) utilized three animal bioassays, reported by Goldstein et al., 1980; Maltoni et al., 1983; and NTP[8]. Doses corresponding to increased risk of one in a million were estimated using the multistage model and, where time-to-tumor data existed, the multistage-Weibull model was used. Various tumor endpoints were modeled, including leukemia in rats, Zymbal gland carcinoma in rats, all squamous cell carcinomas in rats and mice, lung tumors in mice, mammary tumors in mice, and

[8] Assessments used 1983 pre-published data from NTP which were published in final form in 1986.

all tumors combined in rats. The range of 95 percent upper bound human equivalent cancer potency estimates derived from these analyses were very similar to those reported in Table 29 above. The range of estimates obtained from animal studies (U.S. EPA, 1985) overlapped the range of estimates derived from the human worker studies (this assessment).

Thus, the cancer potency estimates from the animal studies employing oral exposures, 0.04 to 0.2 (mg/kg-d)$^{-1}$, are consistent with the estimates obtained from the human studies, 0.1 (mg/kg-d)$^{-1}$ (Table 29). This comparison provides support for the validity of the PHG for benzene.

CALCULATION OF PHG

Calculations of concentrations of chemical contaminants in drinking water associated with negligible risks for carcinogens or non-carcinogens must take into account the toxicity of the chemical itself, as well as the potential exposure of individuals using the water. Tap water is used directly as drinking water and for preparing foods and beverages. It is also used for bathing, showering, washing, flushing toilets, and other household uses that may result in dermal and inhalation exposures.

Occupational exposures to benzene occur primarily by inhaling benzene vapors. Thus in order to calculate the PHG for benzene in drinking water, cancer risk estimates were derived for the inhalation exposures and then extrapolated to risks expected from tap water use. This approach is reasonable for three reasons. First, roughly one-half of an individual's total benzene exposure from use of contaminated tap water is expected from inhalation due to transfer of benzene to indoor air (e.g., showering, washing) (see Table 3). Second, no human studies of benzene-induced leukemia via the oral route are available in the scientific literature. Third, the metabolism and distribution of benzene does not appear to be appreciably different by different routes of exposure.

Noncarcinogenic Effects

Calculation of a public health-protective concentration (C, in mg/L) for benzene in drinking water for noncarcinogenic endpoints follows the general equation:

$$C = \frac{\text{NOAEL/LOAEL} \times \text{BW} \times \text{RSC}}{\text{UF} \times \text{L/day}}$$

where,

NOAEL/LOAEL = No-observed-adverse-effect-level or lowest-observed-adverse-effect-level: a NOAEL of 0.53 ppm was observed for hematological effects in U.S. male refinery workers exposed to benzene for up to 21 years (Tsai et al., 1983). (See Appendix B for study selection and details). Adjusting this value from an occupational exposure to a continuous population exposure results in a concentration of 0.19 ppm. Converting to units of mg/kg-d yields: 0.19 ppm*(3190 μg/m^3 air / ppm)*(1/70 kg)*(20 m^3/d)*(0.5 absorbed)*(mg/1000 μg) = 0.087 mg/kg-d, where 70 kg is the standard adult male body weight, and 20 m^3/d is the default estimate for the volume of air inhaled per day. Benzene absorption efficiencies of 50 percent for inhalation and 100 percent for oral ingestion were estimated from the available literature (Appendix C).

BW = Adult body weight (default of 70 kg).

RSC = For the relative source contribution (RSC), a default value of 0.2 was selected. As discussed in the section on Environmental Occurrence, benzene is not

routinely found in tap or surface water. In cases where benzene was detected, concentrations rarely exceeded the federal MCL. Given benzene-contaminated water at the current California MCL (1 μg/L) or federal MCL (5 μg/L), the total benzene intake would be 4.6 or 23 μg/day, respectively (based on dose estimates from Lindstrom et al., 1994 and adjusted as described in Table 3). Wallace (1996) estimated that the average daily intake of benzene from all sources was about 2000 μg for smokers and 200 μg for non-smokers. Thus, the RSC from benzene-contaminated water at the California MCL would be 0.002 for smokers and 0.02 for non-smokers. The RSC from benzene-contaminated water at the federal MCL would be 0.01 for smokers and 0.1 for non-smokers. Thus, the low-end default value of 0.2 appears reasonable.

UF = Uncertainty factors: an UF of ten is utilized to account for inter-individual variability.

L/day = Adult daily water consumption rate (the default is two L/day): estimates from studies of households with benzene-contaminated tap water indicate that additional exposures via inhalation and dermal exposure (e.g., showering) are expected and result in daily water consumption equivalents of 4.7 (best estimate, Lindstrom et al., 1994, adjusted value, see Table 3, page 10). This estimate includes adjustments for differences in absorption by route of exposure.

Thus, the health protective concentration based on non-cancer hematotoxic effects is

$$C = \frac{0.087 \text{ mg/kg-d} \times 70 \text{ kg} \times 0.2}{10 \times 4.7 \text{ L/day}} = 0.026 \text{ mg/L or } 26 \text{ ppb}$$

Carcinogenic Effects

For carcinogens, the following general equation can be used to calculate the public health-protective concentration (C) for benzene in drinking water (in mg/L):

$$C = \frac{BW \times R}{CSF \times L/day} = \text{mg/L}$$

where,

BW = Adult body weight (a default of 70 kg)

R = *De minimis* level for lifetime excess individual cancer risk (a default of 10^{-6})

CSF = Cancer slope factor was estimated from the upper 95 percent confidence limit on the lifetime risk of total leukemia estimated for general population exposure to benzene, 24 hr/d, 365 d/yr. The same potency estimate is derived from the Pliofilm Cohort (Paxton et al., 1994) or the Chinese Worker Cohort (Hayes et al., 1997), after rounding. For example, the mean lifetime risk estimate (0.044 ppm^{-1}) from the Pliofilm cohort has been converted to a population-based cancer potency in units of $(\text{mg/kg-day})^{-1}$: risk/(mg/kg-d) = (0.044/ppm)*(ppm/3190 $\mu g/m^3$ air)*(70 kg)*(1/20 m^3/d)*(1/0.5

absorbed)*(1000 μg/mg) = 0.1, where 70 kg is the standard adult male body weight, and 20 m^3/d is the default estimate for the volume of air inhaled per day. This computation is based on absorption efficiencies of 50 percent for inhalation and 100 percent for oral ingestion of benzene, which were derived from the available literature on benzene uptake and metabolism in humans and animals (Appendix C).

L/day = Daily volume of water consumed by an adult: although the standard default is 2.0 L/day, studies of household use of benzene-contaminated drinking water (Lindstrom et al., 1994; Beavers et al., 1996) indicate that additional exposures via inhalation (e.g., from stripping of benzene to air via showering, dish washing) and via dermal absorption (e.g., bathing, showering) are expected. A best estimate of the daily water consumption equivalents is 4.7 L (Lindstrom et al., 1994, adjusted values, Table 3). This value is supported by an estimate obtained from CalTOX (DTSC, 1999) of 4.6 L-equivalents. This estimate includes adjustments for differences in absorption by route of exposure.

Thus, the health protective concentration for benzene in drinking water based on carcinogenicity is:

$$C = \frac{70\ kg \times 10^{-6}}{0.1\ (mg/kg\text{-}d)^{-1} \times 4.7\ Leq/day} = 1.5 \times 10^{-4}\ mg/L, \text{ or } 0.15\ ppb$$

The PHG is calculated as 0.15 ppb based on carcinogenicity (total leukemia) which is also protective of non-cancer hematological effects from chronic exposure.

RISK CHARACTERIZATION

Risk conclusions and summary of supporting evidence

Exposure to benzene is associated with increases in numerous adverse effects including bone marrow damage, changes in circulating blood cells, developmental and reproductive effects, alterations of the immune system, and cancer. Benzene is absorbed through all routes of exposure, and the metabolism and distribution do not appear to depend significantly on route of exposure. In humans, the most sensitive responses to benzene are those related to the blood-forming organs. There is a wealth of evidence demonstrating benzene's ability to cause bone marrow damage giving rise to clinical outcomes such as various anemias, myelodysplastic syndromes, and leukemia. More than 25 studies are available which examined the carcinogenic effects in workers exposed to benzene, primarily via inhalation. No cancer studies of humans exposed to benzene by ingestion are available. A PHG for benzene in drinking water of 1.5×10^{-4} mg/L (0.15 ppb) was calculated for carcinogenic risks (total leukemia) of lifetime exposure of the general population. The PHG is based upon a population-based cancer potency estimate of 0.1 $(mg/kg\text{-}d)^{-1}$ for oral exposures to benzene. The same cancer potency estimate is derived from either of two well-conducted epidemiological studies of benzene-induced leukemia in workers. A large amount of information is available to suggest that there may be wide inter-individual variability in susceptibility to benzene-induced hematotoxicity among adults. The differences in susceptibility stem from genetic variability in the key enzymes that metabolize benzene and from environmental and host factors. Also, white males may be more sensitive (about 20 percent) to the leukemogenic effects of benzene than other

subpopulations because they have increased background rates of leukemia. Additional evidence suggests that benzene may also be associated with increased incidence of childhood leukemia and other adult cancers. A health protective concentration of benzene in drinking water for non-cancer effects of 0.026 mg/L (26 ppb) was also determined based on a study of hematotoxicity among U.S. male refinery workers chronically exposed to benzene.

Benzene is ubiquitous in the environment, present in ambient and indoor air. Thus, exposure of the entire population to benzene is expected. Major sources of benzene exposure in the environment are volatilization and combustion of gasoline and other fuels, and wood smoke. Personal exposures to benzene are expected from cigarette smoke, driving-related activities, and indoor and outdoor air. Exposures via drinking water may arise from leaking underground tanks or other discharges to drinking water sources. Occupational exposures are also expected since benzene is produced during petroleum refining and is used as a major feedstock in the production of solvents, resins and other chemical products.

It is difficult to calculate the risks to Californians associated with benzene exposure via drinking water since exposures are not widespread and usually arise out of a spill or other localized contamination of water sources. However, we can readily calculate individual risks given a concentration of benzene in tap water. For example, risk from lifetime exposure to benzene at the current California drinking water standard (MCL) of 1×10^{-3} mg/L is:

$$\text{Lifetime upper-bound risk} = \frac{(1 \times 10^{-3}\ \text{mg/L}) * (0.1\ (\text{mg/kg-d})^{-1}) * (4.7\ \text{L equivalents/d})}{70\ \text{kg body weight}} = 7 \times 10^{-6},$$

where 0.1 $(\text{mg/kg-d})^{-1}$ is the upper 95 percent confidence limit of the cancer slope factor, and 4.7 L equivalents/d is the adjustment for ingestion, dermal and inhalation exposures associated with household use of tap water containing benzene. (The 4.7 L value accounts for differences in absorption by route of exposure.) In other words, it is unlikely that lifetime use of tap water contaminated with benzene at the MCL would result in more than seven additional cancer cases per one million people exposed.

Based upon the estimated cancer potency and the widespread use and exposure via air, benzene will continue to be a major occupational and public health concern.

Summary of toxicity information

The toxic effects observed in both animals and humans following exposure to benzene were reviewed. These effects include acute toxicity, subchronic and chronic hematotoxic effects, genetic toxicity, developmental and reproductive toxicity, immunotoxicity, and cancer. Studies in animals indicate that benzene acts as a male reproductive toxicant and also adversely affects development. Human evidence regarding reproductive effects is weak and does not support or refute the animal evidence. In humans, the blood-forming organs appear to be the most sensitive to benzene toxicity. There is a wealth of evidence demonstrating benzene's ability to alter the bone marrow and lymph system -- giving rise to clinical outcomes such as various anemias, lymphocyte depression, myelodysplastic syndromes, and leukemia. Benzene is clearly genotoxic, causing chromosomal damage and mutations in test systems *in vitro*, in animals *in vivo*, and in occupationally exposed humans.

Benzene caused cancers at many sites in rats and mice, administered by multiple routes of exposure. However, there is considerable debate whether benzene is associated with all subtypes of leukemia and other forms of cancer in humans. This assessment reviewed the evidence and arguments for and against causal associations between benzene and different cancers. Benzene exposure in humans is most commonly associated with acute non-lymphocytic leukemia (ANLL), but is also associated with other types of leukemia. Total leukemia (all leukemia subtypes as a group of related diseases) was selected as

an appropriate endpoint for the basis of the benzene PHG. The impact of this decision on the magnitude of the risk estimates is minimal, since cancer risk estimates based on ANLL/MDS (Table 27, Chinese Worker Cohort) or AMML (Table 18, Pliofilm Cohort) (Crump, 1994) differed by about 20 to 25 percent from those based on total leukemia. Epidemiological and mechanistic evidence, as well as practical issues related to diagnosis and reporting, support the use of total leukemia. There is evidence to suggest that benzene also causes non-Hodgkin's lymphoma and multiple myeloma. Since the evidence has not been as well established as it has been for leukemia, lifetime risk estimates for lymphoma and multiple myeloma were not added to the risk estimates for leukemia. The risk manager may want to consider the possible associations of benzene with lymphoma and multiple myeloma as significant sources of uncertainty such that the true cancer risks from exposure to benzene may be underestimated.

Summary of exposure assessment

Benzene is ubiquitous in the environment, present in indoor and outdoor air. Benzene is an important chemical to many industries and is produced and used in high quantities. Benzene is a component of gasoline and other fuels and is produced during combustion of fuel, wood and other materials. Widespread worker exposure is expected, although air concentrations are expected to be low in the U.S. since benzene is tightly regulated. For individuals not exposed occupationally, primary sources of benzene exposure include cigarette smoke, environmental tobacco smoke, indoor and outdoor air, and driving-related activities. It is estimated that the average daily intake of benzene is roughly 2.0 mg for a moderate to heavy smoker and about 0.2 mg for non-smokers (Wallace, 1996).

Drinking water is not usually a major source of exposure to benzene, but may arise when drinking water sources are contaminated, such as from leaking underground gasoline storage tanks. Exposure estimates from benzene-contaminated tap water were developed as part of this assessment. In addition to direct ingestion of tap water, exposure estimates of benzene in tap water must take into account potential exposure from dermal contact during showering and bathing and from inhalation when benzene is volatilized from water into household air. Estimates of total benzene exposure were developed from two studies of homes with benzene-contaminated water and compared with estimates obtained from CalTOX, a multimedia total exposure computer program. Estimates from the three sources were consistent and indicated that roughly an equal amount of benzene would be inhaled and ingested from contaminated water use. A smaller proportion of the total exposure would come from dermal exposure. Assuming ingestion of 2.0 L of water per day, an estimated 4.7 L-equivalents of total benzene intake would be expected from household use of benzene-containing tap water. Uncertainties related to the extrapolation from inhalation to oral exposures are expected to be small relative to other sources of uncertainty such as genetic susceptibility and estimates of worker exposures.

An extensive exposure assessment, which developed ranges of expected exposures, identified high exposure groups, and characterized exposure to susceptible populations, was not undertaken for the purposes of this assessment.

It has been reported that high exposures to gasoline (300 to 2000 ppm) which contains benzene may be less hazardous than an equivalent exposure to benzene alone, based on studies in mice and from modeling predictions (Bond et al., 1997). Volatile aromatics in gasoline including toluene, ethylbenzene and xylenes compete with benzene for CYP2E1, thus these co-exposures significantly reduce the overall metabolism of benzene relative to equivalent exposures of pure benzene. However, these experiments employed relatively high concentrations of test material (1) 300 ppm gasoline plus six ppm benzene, and (2) 2000 ppm gasoline plus 40 ppm benzene), levels at which metabolic saturation is expected. At low levels of exposure, competition for the P450 binding sites would be minimal; thus, reduction in benzene toxicity due to co-exposures would also be minimal.

Summary of dose-response assessment

<u>Non-cancer endpoints</u>

Values for health protective concentrations of benzene in drinking water were developed for both non-cancer and cancer endpoints. The non-cancer health protective concentration (0.026 mg/L) was based on a NOAEL of 0.53 ppm benzene observed for hematological changes among U.S. refinery workers chronically exposed (Tsai et al., 1983) (Appendix B). A RSC of 0.2 (low-end default) was used in the computation, although the RSC could be significantly lower (especially for individuals who smoke), based on predictions of total intake from benzene contaminated-tap water at the current MCL compared to published intake estimates from other sources (Wallace, 1996). An uncertainty factor of ten was applied for inter-individual variability.

Other studies of hematotoxic effects in benzene-exposed workers and animals were available and were considered. The NOAEL or LOAEL from these alternate studies would yield results that are consistent with the value derived from the Tsai et al. (1983) study, after application of standard uncertainty factors. Thus, confidence in the selected toxicity estimate is high.

The non-cancer health protective concentration for drinking water was based upon a study of workers exposed via inhalation. Absorption and pharmacokinetic information in humans was used to extrapolate across the two routes of exposure (Appendix C). Absorption of benzene through the oral route is complete, whereas by inhalation an estimated 50 percent is retained. Metabolism and distribution do not appear to differ substantially based on route of exposure.

The database of dose-response information on benzene-induced toxicity is large. One deficiency is the lack of a two-generation reproduction study. However, unlike U.S. EPA, it is not current Cal/EPA policy to add an UF for database deficiency.

Cancer endpoints

A major focus of this assessment was benzene-induced cancer, since cancer is the endpoint that is associated with the greatest potential risk at low exposures. The PHG (1.4×10^{-4} mg/L) is based upon an upper-bound population-adjusted cancer potency estimate of 0.1 $(mg/kg\text{-}d)^{-1}$ for oral exposures, and would correspond to a cancer potency of 0.05 $(mg/kg\text{-}d)^{-1}$ for inhalation exposures. The same estimates are derived from observations of increased rates of total leukemia from two epidemiological studies of benzene-exposed workers. The inhalation estimate was converted to that expected for oral exposures using information on route-to-route extrapolation (Appendix C). The uncertainties associated with extrapolation from inhalation to oral exposures, especially at lower exposure levels, are expected to be small.

Of the over 20 epidemiological studies of benzene, two studies were judged to be superior and were selected for analysis. The first was a study of 74,828 exposed and 35,805 unexposed workers in China, conducted jointly by NCI and the Chinese Academy of Preventive Medicine (Hayes et al., 1997; Yin et al., 1996; Dosemeci et al., 1994). The second was a study of 1868 U.S. rubber hydrochloride (Pliofilm) workers (Paxton et al., 1994a). Each study was evaluated separately.

Potency estimates were obtained for the cohorts and used to calculate lifetime additional risk to the general (target) population using standard life table methods. Lifetime risk estimates were consistent for the two studies. The central and 95 percent upper confidence bound best estimates for total leukemia in the Pliofilm Cohort were 0.034 and 0.044 ppm^{-1}, respectively. Mean and upper-bound risk estimates for total leukemia based on alternate exposure estimates, alternate models and differing assumptions ranged from 0.010 to 0.048 ppm^{-1}. The risk estimates for acute myeloid or monocytic leukemia were about 20 percent lower than the values estimated for total leukemia using relative risk models (Table 18) (Crump, 1994). The central and 95 percent upper-confidence bound estimates, chosen as most appropriate for that dataset, for total leukemia in the Chinese Worker Cohort were 0.045 and 0.056 ppm^{-1}, respectively. However, lifetime risk estimates for total leukemia using alternate risk models and differing assumptions (see above) ranged from 0.00063 to 0.087 ppm^{-1}. Risk estimates for other endpoints, such as ANLL, generally fell within this range (Table 27).

Poisson regression of linear risk models was utilized to analyze the data, a procedure that uses maximum likelihood estimation. Individual exposure and worker history data were used in the analysis of the Pliofilm Cohort. Grouped summary data as published by Hayes et al. (1997) was used in the analysis of the Chinese Worker Cohort. This assessment evaluated the evidence regarding the shape of the carcinogenic dose-response curve of benzene (see Low Dose Linearity, page 88) and concluded that significant evidence exists to support the use of linear risk models. Two linear risk models were employed, a relative risk model and an absolute risk model. The relative risk model assumes that background rates of disease (leukemia) are a multiplicative function of risk. The absolute risk model assumes that background rates of disease are an additive function of risk. Our understanding of benzene-induced leukemogenesis is insufficient to know which model is the most biologically viable. This assessment preferred the relative risk model to the absolute risk model for the following reasons. The relative risk model provided the most health protective potency estimates, provided equivalent model fits, it was preferred by the BEIR V committee for assessing radiation-induced leukemia (NRC, 1990), and has been more often used in setting regulatory standards than the absolute risk model.

Several approaches taken in this assessment differ significantly from prior assessments of benzene; the impact of these differences should be clearly understood by the reader. Previous assessments defined the target population as white males, whereas this assessment chose all races, both sexes as the target population. Although white males have a higher background rate of leukemia than other subpopulations which translates to higher risk estimates (about 20 percent) when using relative risk models, they were not treated as a sensitive subpopulation. The increase in 'susceptibility' is model dependent, and the magnitude of the increase is likely to be small compared to inter-individual variability. Another difference is this assessment's use of incidence rates to calculate lifetime additional risk in the general population, a decision that increases the risk estimates by about 30 percent. This effectively means that the estimates of risk derived from this assessment will protect against contracting leukemia as opposed to dying from leukemia, a distinction that will become more and more important as medical advances are made to increase survival. Contracting leukemia is a catastrophic event that entails a huge emotional and financial cost; California and federal guidelines for carcinogen assessment support use of incidence rates in this manner. Another significant difference is the incorporation of the concept of a changing pattern of risk following exposure to benzene. Leukemia and bone cancers, unlike most other cancers, appear to follow a wave-like pattern of risk following exposure to a leukemogen. Risks increase rapidly within ten years and decline to or near background levels by 30 years after cessation of exposure. Like radiation and chemotherapeutic agents, analyses of the Chinese Worker Cohort data (Hayes et al., 1997) and the Pliofilm Cohort data (see Table 21, and Finkelstein, 2000) suggest that this wave-pattern of leukemia risk following exposure also functions for benzene. Incorporation of this concept reduces the overall risk estimates by roughly two-fold compared to previous assessments which assumed that risk was a function of lifetime cumulative exposure to benzene.

There are several additional sources of uncertainty with respect to this analysis and benzene-induced leukemia of which the risk manager should be aware. The first is the available evidence describing the inter-individual variability to the toxic effects of benzene (see Inter-individual Variability and Benzene-induced Hematotoxicity, page 71). The range of susceptibility to benzene-induced toxicity within the general population cannot be accurately quantified at this time but is potentially very large. Benzene's toxicity is intimately tied to its complex metabolism and distribution. Key enzymes involved in the metabolism of benzene, including CYP2E1, the quinone reductase NQO1, and myeloperoxidase are polymorphic. For example, a 7.6-fold difference in benzene-induced hematotoxicity in workers was observed among gene variants of CYP2E1 and NQO1 (Rothman et al., 1997). In addition to genetic susceptibilities, dietary and environmental factors may also add to the inter-individual variability. Dietary and endogenous sources of phenol, hydroquinone, catechol and 1,2,4-benzenetriol, the primary phenolic metabolites of benzene, are found in high concentrations in the urine of unexposed individuals (McDonald et al., 2001). The roles that these background 'metabolites' play in benzene toxicity are

unknown. Other environmental factors that may confer additional variability include ethanol consumption, infection, and co-exposures to radiation and other leukemogens.

Another source of uncertainty relates to the shape of dose-response curve for benzene-induced leukemia. The dose-response relationship for leukemia in the Chinese Worker Cohort is supralinear (e.g., trend in relative risk falls below a linear trend with increasing dose). The reasons for the supralinearity are not clearly known, although it may relate to competitive or saturable metabolism at higher exposures (Rothman et al., 1998) or may be related to the use of a cumulative dose measure and differing susceptibilities among workers. Linear Poisson regression was performed on the Chinese data with the top dose group removed and with all dose groups, since the error bars are large and are not inconsistent with a linear relationship. The removal of the top dose group increased the cancer potency by five-fold, using the linear relative risk model. A similar approach was taken for the analysis of the Pliofilm Cohort data for two reasons. Three sets of exposure estimates are available for the Pliofilm Cohort (Crump and Allen, 1984; Rinsky et al., 1981, 1987; Paustenbach et al., 1992). The Paustenbach estimates are likely to be high (Utterback and Rinsky, 1995) and were not used to compute lifetime risk. The Crump and Rinsky estimates differed mainly on how they handled the early exposure estimates when few actual measurements were made. Thus, potencies were calculated for all dose points and for workers whose exposures were less than 400 ppm-yr. Interestingly, the potency (and lifetime risk) estimates were essentially the same for the Crump and Rinsky exposure estimates after removal of the highly exposed workers (Table 20). Thus, this assessment selected as its best estimates those values derived from workers with lower exposures since inclusion of individuals with high exposures may underestimate the risks if the true relationship is supralinear. However, estimates that used all of the exposed workers were included in the range of reasonable risk estimates.

Beyond the uncertainties described above, some researchers believe that the true dose-response relationship for benzene-induced leukemias is non-linear at low doses or exhibits a threshold of response (Raabe and Wong, 1996; Schnatter et al., 1996a,b). OEHHA considered this information in its overall assessment of benzene, but concluded that a linear-to-low dose model was most consistent with the available evidence and the guidelines for carcinogen assessment.

Lifetime risk estimates for leukemia derived from the Chinese Worker Cohort and the Pliofilm Cohort were compared to estimates obtained from studies of other benzene-exposed workers and from animal bioassays. In general, some risk estimates from other occupational studies of benzene were higher and some were lower than the range obtained for the Chinese Worker Cohort and Pliofilm Cohort. Two studies, one of U.S. chemical workers (Ireland et al., 1997) and of Scandinavian service station workers (Jakobsson et al., 1997), yielded cancer risk estimates that were approximately five-fold and three-fold higher, respectively, than the best estimates from the Chinese Worker Cohort and the Pliofilm Cohort. These two studies estimated relatively low exposures to benzene, whereas relatively low risk estimates were calculated from Turkish shoe workers (Aksoy, 1980, 1985, 1994) where very high exposures to benzene were reported. These observations are also consistent with a supralinear dose-response relationship. Potency estimates from animals, including those based on oral exposure, were very similar to the estimates from the human data (Table 29). Compared to cancer potency estimates from human inhalation studies, estimates from animal ingestion studies were equivalent for lymphoma and leukemia (combined) and two-fold higher for the most sensitive species and site (mouse preputial gland).

The assessment attempted to indirectly validate the magnitude of the cancer potency and lifetime risk estimates derived from the Chinese Worker Cohort and the Pliofilm Cohort by comparing the results to those obtained for leukemia and benzene exposure from cigarette smoking (see analysis, page 114). Some of the largest epidemiological datasets available relate smoking to various ailments including leukemia. Benzene is a component of cigarette smoke and constitutes the highest non-occupational exposure of the general population to benzene (Wallace, 1996). Potency estimates for leukemia and benzene from cigarettes were calculated from two epidemiological studies that followed the mortality experience of over one million individuals from the U.S. (Garfinkel and Boffetta, 1990). These estimates

were compared to potency estimates obtained from the benzene-exposed workers. The mean lifetime risk estimate for total leukemia among the benzene-exposed workers was 32 percent of the mean estimate from smokers. Since cigarette smoke contains small quantities of other leukemogens such as 1,3-butadiene and two radioactive elements, the worker-based estimates are reasonably consistent with the smoking data. This comparison adds to the confidence in the estimates of cancer potency and the PHG, and suggests that the lifetime risk estimates derived from benzene-exposed workers are less likely to be overestimates of the true risk.

Confidence in the PHG and the cancer potency on which it is based is high. As discussed above, some researchers have suggested that a threshold may exist for benzene-induced cancers. However, this assessment reviewed the evidence regarding the shape of the dose-response curve for benzene-induced leukemia and concluded that a significant amount of epidemiological and mechanistic evidence suggests that benzene induces leukemia linearly to low doses. Thus, a non-linear or threshold approach is not warranted. Lifetime risk estimates for leukemia derived from other epidemiological studies of benzene-exposed workers, from epidemiological studies of benzene and cigarette smoking, and from animal cancer studies all provide support for the selected PHG value.

OTHER REGULATORY STANDARDS

The PHG of 0.00015 mg/L is seven-fold lower than the current California MCL (0.001 mg/L) and 33-fold lower than the federal MCL for benzene (0.005 mg/L) in drinking water. DHS set the current California MCL for benzene in 1989 based on economic and technical feasibility considerations, but also considered health-based estimates. The previous health based estimates, developed by DHS (1987), were 0.00063 mg/L for 2 L daily water consumption and 0.00018 mg/L for 7 L daily water consumption (DHS, 1987). The federal MCL for benzene of 0.005 mg/L was also determined in part by technical feasibility criteria. As a matter of policy, the U.S. EPA MCLG, the federal equivalent of the PHG, is zero mg/L for all Group A and B carcinogens.

Benzene is classified as a Group A (human carcinogen) by U.S. EPA (U.S. EPA. 1999); as a Group 1 carcinogen (carcinogenic to humans) by IARC (IARC, 1982); as “Known to be a Human Carcinogen” by NTP (NTP, 1998); and as “known to the State ” of California to cause cancer in 1987, and developmental and reproductive toxicity in 1997 under Proposition 65 (OEHHA, 1999b).

Benzene drinking water standards set for other U.S. states include Alabama (0.005 mg/L), Arizona (0.005 mg/L), Connecticut (0.001 mg/L), Florida (0.003 mg/L), Maine (0.005 mg/L), Minnesota (0.01 mg/L), and New Jersey (0.001 mg/L) (ATSDR, 1997). The World Health Organization’s international drinking water standard is 0.01 mg/L (ATSDR, 1997).

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APPENDIX A. AGE-SPECIFIC LEUKEMIA INCIDENCE RATES FOR CALIFORNIA

The following are unpublished incidence rates for leukemia subtypes provided to OEHHA by the California Cancer Registry.

These rates were used in the computation of additional risk to the general population from exposure to benzene.

Average Annual Incidence Rates per 100,000 by Age of Leukemia and Specific Leukemia Subtypes in California 1991-1995.
All races, both sexes

Age interval (years)	Total leukemias	Total lymphatic leukemia	Total myeloid leukemia	Acute myeloid leukemia (a)	Chronic myeloid leukemia	Other myeloid leukemia	Total monocytic leukemia	Acute monocytic leukemia (b)	Other leukemia	Other acute leukemia (c)	Acute non-lymphocytic leukemia (a+b+c)
0-4	7.6	6.2	0.8	0.7	0.1	0*	0.2	0.2	0.4	0.2	1.1
5-9	4.2	3.6	0.5	0.3	0.1	0.1	0*	0*	0.1	0.1	0.4
10-14	3.1	2.2	0.8	0.6	0.1	0.1	0*	0*	0.1	0.1	0.7
15-19	3.1	1.8	1.2	0.8	0.2	0.2	0*	0*	0.1	0.1	0.9
20-24	2.3	0.9	1.2	0.8	0.3	0.1	0*	0*	0.2	0.1	0.9
25-29	2.3	0.6	1.5	0.8	0.5	0.2	0.1	0.1	0.1	0.1	1.0
30-34	2.9	0.5	2.1	1.1	0.8	0.2	0.1	0.1	0.2	0.1	1.3
35-39	3.1	0.7	2.1	1.1	0.8	0.2	0.1	0.1	0.3	0.1	1.3
40-44	4.7	1.1	3.0	1.6	1.2	0.2	0.1	0.1	0.5	0.1	1.8
45-49	6.4	1.9	3.4	1.9	1.3	0.2	0.1	0.1	0.9	0.2	2.2
50-54	10	3.6	5.1	3.2	1.5	0.3	0.2	0.2	1.1	0.4	3.8
55-59	14.5	6.2	6.7	4.5	2.0	0.3	0.2	0.2	1.4	0.3	5.0
60-64	22.1	9.3	9.8	6.2	3.2	0.5	0.6	0.6	2.5	0.8	7.6
65-69	30.2	12.6	14.0	9.3	4.1	0.5	0.5	0.4	3.1	1.3	11.0
70-74	43.4	17.6	19.4	13.3	5.4	0.7	0.9	0.8	5.5	2.3	16.4
75-79	56	22.5	25.5	16.1	8.4	1.0	1.1	0.8	6.8	3.5	20.4
80-84	72.7	28.3	31.2	20.2	9.8	1.2	2.0	1.7	11.2	5.8	27.7
85+	82.4	34.4	32.8	19.1	12.1	1.5	1.6	1.3	13.6	5.8	26.2

* based on 5 or fewer cases

APPENDIX B. SUMMARY DOCUMENT: CHRONIC NON-CANCER REFERENCE EXPOSURE LEVEL

The following is a reproduction of the final summary document describing the derivation of the chronic REL, a regulatory standard for the Air Toxics "Hot Spots" Program mandated by the Air Toxics Hot Spots Information and Assessment Act of 1987, as amended (OEHHA, 2000). Data used to derive the chronic REL was also used to estimate a health protective concentration of benzene in drinking water for non-cancer endpoints.

CHRONIC TOXICITY SUMMARY

BENZENE

(Benzol; Benzole; Cyclohexatriene)

CAS Registry Number: 71-43-2

I. Chronic Toxicity Summary

Inhalation reference exposure level	**60 μg/m^3 (20 ppb)**
Critical effect(s)	Lowered red and white blood cell counts in occupationally exposed humans
Hazard index target(s)	Hematopoietic system; development; nervous system

II. Physical and Chemical Properties (HSDB, 1994; 1999)

Description	Colorless liquid
Molecular formula	C_6H_6
Molecular weight	78.1 g/mol
Density	0.879 g/cm^3 @ 25° C
Boiling point	80.1°C
Vapor pressure	100 torr @ 26.1°C
Solubility	Soluble in ethanol, chloroform, ether, carbon disulfide, acetone, oils, and glacial acetic acid; slightly soluble in water
Conversion factor	1 ppm = 3.2 mg/m^3 @ 25° C

III. Major Uses or Sources

Benzene has been widely used as a multipurpose organic solvent. This use is now discouraged due to its high toxicity, including carcinogenicity. Present uses include use as a raw material in the synthesis of styrene, phenol, cyclohexane, aniline, and alkyl benzenes in the manufacture of various plastics, resins, and detergents. Syntheses of many pesticides and pharmaceuticals also involve benzene as a chemical intermediate (HSDB, 1994). The tire industry and shoe factories use benzene extensively in their manufacturing processes. Annual demand in the U.S. was

estimated to be 6 million tons in 1990 (HSDB, 1994). Benzene exposure also occurs as a result of gasoline and diesel fuel use and combustion (Holmberg and Lundberg, 1985). In 1996, the latest year tabulated, the statewide mean outdoor monitored concentration of benzene was approximately 0.7 ppb (CARB, 1999a). Annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 750,364 pounds of benzene (CARB, 1999b). (This does not include the large amount of benzene emitted by mobile sources.)

IV. Effects of Human Exposure

The primary toxicological effects of chronic benzene exposure are on the hematopoeitic system. Neurological and reproductive/developmental toxic effects are also of concern at slightly higher concentrations. Impairment of immune function and/or various anemias may result from the hematotoxicity. The hematologic lesions in the bone marrow can lead to peripheral lymphocytopenia and/or pancytopenia following chronic exposure. Severe benzene exposures can also lead to life-threatening aplastic anemia. These lesions may lead to the development of leukemia years after apparent recovery from the hematologic damage (DeGowin, 1963).

Kipen et al. (1988) performed a retrospective longitudinal study on a cohort of 459 rubber workers, examining the correlation of average benzene exposure with total white blood cell counts taken from the workers. These researchers found a significant ($p < 0.016$) negative correlation between average benzene concentrations in the workplace and white blood cell counts in workers from the years 1940-1948. A reanalysis of these data by Cody et al. (1993) showed significant decreases in RBC and WBC counts among a group of 161 workers during the 1946-1949 period compared with their pre-exposure blood cell counts. The decline in blood counts was measured over the course of 12 months following start of exposure. During the course of employment, workers who had low monthly blood cell counts were transferred to other areas with lower benzene exposures, thus potentially creating a bias towards non-significance or removing sensitive subjects from the study population. Since there was a reported 75 percent rate of job change within the first year of employment, this bias could be highly significant. In addition, there was some indication of blood transfusions used to treat some "anemic" workers, which would cause serious problems in interpreting the RBC data, since RBCs have a long lifespan in the bloodstream. The exposure analysis in this study was performed by Crump and Allen (1984). The range of monthly median exposures was 30-54 ppm throughout the 12-month segment examined. Despite the above-mentioned potential biases, workers exposed above the median concentrations displayed significantly decreased WBC and RBC counts compared with workers exposed to the lower concentrations using a repeated measures analysis of variance.

Tsai et al. (1983) examined the mortality from all cancers and leukemia, in addition to hematologic parameters in male workers exposed to benzene for 1-21 years in a refinery from 1952-1978. The cohort of 454 included maintenance workers and utility men and laborers assigned to benzene units on a "regular basis". Exposures to benzene were determined using personal monitors; the median air concentration was 0.53 ppm in the work areas of greatest exposure to benzene. The average length of employment in the cohort was 7.4 years. The analysis of overall mortality in this population revealed no significant excesses. 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 the presence of a healthy worker effect. An internal comparison group of 823 people, including 10 percent of the workers who were employed in the same plant in operations not related to benzene, showed relative risks for 0.90 and 1.31 for all causes and cancer at all sites, respectively ($p < 0.28$ and 0.23). A subset of 303 workers was followed for medical surveillance. Up to four hematological tests per year were conducted on these workers. Total and differential white blood cell counts, hemoglobin,

hematocrit, red blood cells, platelets and clotting times were found to be within normal (between 5 percent and 95 percent percentile) limits in this group.

Collins *et al*. (1997) used routine data from Monsanto's medical/industrial hygiene system to study 387 workers with daily 8-hour time-weighted exposures (TWA) averaging 0.55 ppm benzene (range = 0.01 – 87.69 ppm; based on 4213 personal monitoring samples, less than 5 percent of which exceeded 2 ppm). Controls were 553 unexposed workers. There was no increase in the prevalence of lymphopenia, an early, sensitive indicator of benzene toxicity, among exposed workers (odds ratio = 0.6; 95 percent confidence interval = 0.2 to 1.8), taking into account smoking, age, and sex. There also was no increase in risk among workers exposed five or more years (odds ratio = 0.6; 95 percent confidence interval = 0.2 to 1.9). There were no differences between exposed and unexposed workers for other measures of hematotoxicity, including mean corpuscular volume and counts of total white blood cells, red blood cells, hemoglobin, and platelets.

Rothman et al. (1996) compared hematologic outcomes in a cross-sectional study of 44 male and female workers heavily exposed to benzene (median = 31 ppm as an 8-hr TWA) and 44 age and gender-matched unexposed controls from China. Hematologic parameters (total WBC, absolute lymphocyte count, platelets, red blood cells, and hematocrit) were decreased among exposed workers compared to controls; an exception was the red blood cell mean corpuscular volume (MCV), which was higher among exposed subjects. In a subgroup of 11 workers with a median 8 hr TWA of 7.6 ppm (range = 1-20 ppm) and not exposed to more than 31 ppm on any of 5 sampling days, only the absolute lymphocyte count was significantly different between exposed workers and controls ($p = 0.03$). Among exposed subjects, a dose response relationship with various measures of current benzene exposure (i.e., personal air monitoring, benzene metabolites in urine) was present only for the total WBC count, the absolute lymphocyte count, and the MCV. Their results support the use of the absolute lymphocyte count as the most sensitive indicator of benzene-induced hematotoxicity.

An examination of 32 patients, who were chronically exposed to benzene vapors ranging from 150 to 650 ppm for 4 months to 15 years, showed that pancytopenia occurred in 28 cases. Bone marrow punctures revealed variable hematopoeitic lesions, ranging from acellularity to hypercellularity (Aksoy et al., 1972).

Central nervous system disorders have been reported in individuals with pancytopenia following chronic occupational benzene exposure to unknown concentrations for an average length of time of 6 years (Baslo and Aksoy, 1982).

Runion and Scott (1985) estimated a composite geometric mean benzene concentration in various workplaces containing benzene to be 0.1 ppm (0.32 mg/m^3) (geometric standard deviation = 7.2 ppm, 23.3 mg/m^3). This estimate was based on samples collected by industrial hygienists between the years 1978 and 1983.

V. Effects of Animal Exposure

A number of animal studies have demonstrated that benzene exposure can induce bone marrow damage, changes in circulating blood cells, developmental and reproductive effects, alterations of the immune response, and cancer. With respect to chronic toxicity, hematological changes appear to be the most sensitive indicator.

Wolf et al. (1956) studied the effects of repeated exposure to benzene in rabbits (80 ppm, 175 total exposures), rats (88 ppm, 136 total exposures) and guinea pigs (88 ppm, 193 total exposures). The observed effects included leukopenia, increased spleen weight, and histological

changes to the bone marrow. Hematologic effects, including leukopenia, were observed in rats exposed to mean concentrations of 44 ppm (143 mg/m^3) or greater for 5-8 weeks (Deichmann et al., 1963). Exposure to 31 ppm (100 mg/m^3) benzene or less did not result in leukopenia after 3-4 months of exposure. Snyder et al. (1978) exposed Sprague-Dawley rats and AKR/J mice to 300 ppm benzene, 6 hours/day, 5 days/week for life. Lymphocytopenia, anemia and decreased survival time were observed in both species. Cronkite et al. (1982) exposed male mice to 400 ppm benzene, 6 hours/day, 5 days/week for 9.5 weeks and observed depressed bone marrow cellularity, decreased stem cell count, and altered morphology in spleen colony-forming cells.

Mice have been shown to be more sensitive than rats or rabbits to the hematologic and leukemic effects of benzene (Sabourin et al., 1989; IARC, 1982). Sabourin et al. (1988) showed that metabolism of benzene to the toxic hydroquinone, muconic acid, and hydroquinone glucuronide was much more prevalent in the mouse than in rats, whereas the detoxification pathways were approximately equivalent between the two species.

A study on the chronic hematological effects of benzene exposure in C57 Bl/6 male mice (5-6 per group) showed that peripheral lymphocytes, red blood cells and colony-forming units (CFUs) in the bone marrow and spleen were significantly decreased in number after treatment with 10 ppm (32.4 mg/m^3) benzene for 6 hours/day, 5 days/week for 178 days (Baarson et al., 1984).

Inhalation of 0, 10, 31, 100, or 301 ppm (0, 32.4, 100.4, 324, or 975 mg/m^3) benzene for 6 hours/day for 6 days resulted in a dose-dependent reduction in peripheral lymphocytes, and a reduced proliferative response of B- and T-lymphocytes to mitogenic agents in mice (Rozen et al., 1984). In this study, total peripheral lymphocyte numbers and B-lymphocyte proliferation to lipopolysaccharide were significantly reduced at a concentration of 10 ppm (32.4 mg/m^3). The proliferation of T-lymphocytes was significantly reduced at a concentration of 31 ppm (100.4 mg/m^3).

Male and female mice (9-10 per group) exposed to 100 ppm (324 mg/m^3) benzene or greater for 6 hours/day, 5 days/week for 2 weeks showed decreased bone marrow cellularity and a reduction of pluripotent stem cells in the bone marrow (Cronkite et al., 1985). The decrease in marrow cellularity continued for up to 25 weeks following a 16-week exposure to 300 ppm (972 mg/m^3) benzene. Peripheral blood lymphocytes were dose-dependently decreased with benzene exposures of greater than 25 ppm (81 mg/m^3) for 16 weeks, but recovered to normal levels following a 16-week recovery period.

Ward et al. (1985) exposed 50 Sprague-Dawley rats and 150 CD-1 mice of both sexes to 0, 1, 10, 30, or 300 ppm benzene, 6 hours/day, 5 days/week for 13 weeks. Serial sacrifices were conducted at 7, 14, 28, 56, and 91 days. No hematological changes were found for mice and rats at 1, 10, or 30 ppm in this study. Significant increases in mean cell volume and mean cell hemoglobin values and decreases in hematocrit, hemoglobin, lymphocyte percentages, and decreases in red cell, leukocyte and platelet counts were observed in male and female mice at 300 ppm. The changes were first observed after 14 days of exposure. Histological changes in mice included myeloid hypoplasia of the bone marrow, lymphoid depletion in the mesenteric lymph node, increased extramedullary hematopoiesis in the spleen, and periarteriolar lymphoid sheath depletion. Effects were less severe in the rats.

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.

The NTP (1986) conducted a bioassay in F344 rats and B6C3F1 mice of benzene by corn oil gavage. Doses were 0, 25, 50, and 100 mg/kg-day for females and 0, 50, 100, and 200 mg/kg-day for males. Dose-related lymphocytopenia and leukocytopenia were observed in both species

in all dosed groups but not controls. Mice exhibited lymphoid depletion of the thymus and spleen and hyperplasia of the bone marrow.

Cronkite et al. (1989) exposed CBA/Ca mice to 10, 25, 100, 300, 400 and 3000 ppm benzene 6 hours/day, 5 days/week for up to 16 weeks. No effects were observed at the 10 ppm level. Lymphopenia was observed in the 25 ppm exposure group. Higher concentrations of benzene produced dose-dependent decreases in blood lymphocytes, bone marrow cellularity, spleen colony-forming units, and an increased percentage of CFU-S in S-phase synthesis.

Farris et al. (1997) exposed $B6C3F_1$ mice to 1, 5, 10, 100, and 200 ppm benzene for 6 hr/day, 5 days/week, for 1, 2, 4, or 8 weeks. In addition some animals were allowed to recover from the exposure. There were no significant effects on hematopoietic parameters from exposure to 10 ppm benzene or less. Exposure to higher levels reduced the number of total bone marrow cells, progenitor cells, differentiating hematopoietic cells, and most blood parameters. The replication of primitive progenitor cells was increased. The authors suggested that this last effect, in concert with the genotoxicity of benzene, could account for the carcinogenicity of benzene at high concentrations.

Reproductive and developmental effects have been reported following benzene exposure. Coate et al. (1984) exposed groups of 40 female rats to 0, 1, 10, 40, and 100 ppm (0, 3.24, 32.4, 129.6, or 324 mg/m^3) benzene for 6 hours/day during days 6-15 of gestation. In this study, teratologic evaluations and fetotoxic measurements were done on the fetuses. A significant decrease was noted in the body weights of fetuses from dams exposed to 100 ppm (324 mg/m^3). No effects were observed at a concentration of 40 ppm (129.6 mg/m^3).

Keller and Snyder (1986) reported that exposure of pregnant mice to concentrations as low as 5 ppm (16 mg/m^3) benzene on days 6-15 of gestation (6 hr/day) resulted in bone-marrow hematopoietic changes in the offspring that persisted into adulthood. However, the hematopoietic effects (e.g. bimodal changes in erythroid colony-forming cells) in the above study were of uncertain biological significance. In a similar later study, Keller and Snyder (1988) found that exposure of mice *in utero* to 20 ppm (64 mg/m^3) benzene on days 6-15 of gestation resulted in neonatal suppression of erythropoietic precursor cells and persistent, enhanced granulopoiesis. This effect was considered significant bone-marrow toxicity by the authors. No hematotoxicity was seen in this study at 10 ppm (32 mg/m^3).

An exposure of 500 ppm (1,600 mg/m^3) benzene through days 6-15 gestation was teratogenic in rats while 50 ppm (160 mg/m^3) resulted in reduced fetal weights on day 20 of gestation. No fetal effects were noted at an exposure of 10 ppm (Kuna and Kapp, 1981). An earlier study by Murray et al. (1979) showed that inhalation of 500 ppm benzene for 7 hours/day on days 6-15 and days 6-18 of gestation in mice and rabbits, respectively, induced minor skeletal variations in the absence of maternal toxicity. Red and white blood cell counts in the adults of either species were measured by Murray et al. (1979) but were not significantly different from control animals. However, fetal mouse hematological effects were not measured.

Tatrai et al. (1980) demonstrated decreased fetal body weights and elevated liver weights in rats exposed throughout gestation to 150 mg/m^3 (47 ppm).

Staff identified Tsai et al. (1983) as the most appropriate study for a chronic REL derivation. The authors examined hematologic parameters in 303 male workers exposed to benzene for 1-21 years in a refinery from 1952-1978. Follow-up success was 99.3 percent in the entire cohort of 359. A total of approximately 1400 samples for hematological tests and 900 for blood chemistry tests were taken between 1959 and 1979. Exposures to benzene were determined using personal monitors. Data consisting of 1394 personal samples indicated that 84 percent of all benzene samples were less than 1 ppm; the median air concentration of benzene was 0.53 ppm in

the work areas of greatest exposure to benzene ("benzene related areas", for example, production of benzene and cyclohexane and also of cumene). The average length of employment in the cohort was
7.4 years. 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 the presence of a healthy worker effect. An analysis using an internal comparison group of 823 people, including 10 percent of the workers who were employed in the same plant in operations not related to benzene, showed relative risks for 0.90 and 1.31 for all causes and cancer at all sites, respectively ($p < 0.28$ and 0.23). Total and differential white blood cell counts, hemoglobin, hematocrit, red blood cells, platelets and clotting times were found to be within normal (between 5 percent and 95 percent percentile) limits in this group. Although the exposure duration averaged only 7.4 years, the study was considered to be chronic since
32 percent of the workers had been exposed for more than 10 years.

VI. Derivation of Chronic Reference Exposure Level (REL)

Study	Tsai *et al.* (1983)
Study population	303 Male refinery workers
Exposure method	Occupational exposures for 1-21 years
Critical effects	Hematological effects
LOAEL	Not observed
NOAEL	0.53 ppm
Exposure continuity	8 hr/day (10 m^3 per 20 m^3 day), 5 days/week
Exposure duration	7.4 years average (for the full cohort of 454); 32% of the workers were exposed for more than 10 years
Average occupational exposure	0.19 ppm
Human equivalent concentration	0.19 ppm
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	1
Intraspecies uncertainty factor	10
Cumulative uncertainty factor	10
Inhalation reference exposure level	0.02 ppm (20 ppb; 0.06 mg/m^3; 60 $\mu g/m^3$)

VII. Data Strengths and Limitations for Development of the REL

Both the animal and human databases for benzene are excellent. Although the study by Tsai et al. (1983) is a free-standing NOAEL, the endpoint examined is a known sensitive measure of benzene toxicity in humans. In addition, the LOAEL for the same endpoint in workers reported by Cody et al. (1993) help form a dose-response relationship and also yield an REL which is consistent with that derived from Tsai et al. (1983). The study by Cody et al. (1993), since it failed to identify a NOAEL and was only for a period of 1 year, contained a greater degree of uncertainty in extrapolation to a chronic community Reference Exposure Level. The recent results of Collins et al. (1997) that included a NOAEL of 0.55 ppm and of Rothman et al. (1996) that included a LOAEL of 7.6 ppm are consistent with those of Tsai et al. Therefore the study by Tsai et al. (1983) was used as the basis for the chronic REL for benzene.

In the Cody et al. (1993) study, significant hematological effects, including reduced RBC and WBC counts, were observed 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 hematological effects. In this rubber plant, workers who had blood dyscrasias were excluded from working in the high benzene units. Furthermore, individual workers having more than a 25 percent 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 used in the Kipen et al. (1988) and Cody et al. (1993) studies. An equivalent continuous exposure of 10.7 ppm can be calculated by assuming that workers inhaled 10 m^3 of their total 20 m^3 of air per day during their work-shift, and by adjusting for a normal 5 day work week. Application of uncertainty factors for subchronic exposures, estimation of a NOAEL, and for protection of sensitive subpopulations (10 for each) results in an REL of 0.01 ppm (10 ppb; 30 $\mu g/m^3$). This is comparable to the REL based on Tsai *et al*. (1983).

Ward et al. (1996) determined a relationship between occupational exposures to benzene and decreased red and white cell counts. A modeled dose-response relationship indicated a possibility for hematologic effects at concentrations below 5 ppm. However, no specific measures of the actual effects at concentrations below 2 ppm were taken, and the Tsai et al. (1983) data were not considered in their analysis. The purpose of this study was to characterize the trend for effects at low concentrations of benzene. A NOAEL or LOAEL was not identified in the study. The selection of a NOAEL of 0.53 ppm is therefore not inconsistent with the results of the Ward et al. (1996) study.

The human data presented by Tsai and associates were selected over animal studies because the collective human data were considered adequate in terms of sample size, exposure duration, and health effects evaluation.

For comparison with the REL of 20 ppb based on human data, we estimated a REL based on the chronic inhalation study in mice by Baarson et al. (1984), which showed that bone-marrow progenitor cells were markedly suppressed after intermittent exposures (6 hr/day, 5 days/week) to 10 ppm benzene for 6 months. An extrapolation of this value to an equivalent continuous exposure resulted in a concentration of 1.8 ppm. Application of an RGDR of 1 for a systemic effect and uncertainty factors of 3 and 10 for inter- and intraspecies variability, and 10 for estimation of a NOAEL from the LOAEL would result in an REL of 6 ppb (20 $\mu g/m^3$). The Farris et al. (1997) 8 week study indicated a LOAEL of 100 ppm and a NOAEL of 10 ppm for hematological effects. Application of an RGDR of 1 and UFs of 10 for subchronic, 3 for interspecies and 10 for intraspecies extrapolation (total UF = 300) also resulted in an estimated

REL of 6 ppb, in reasonable agreement with the proposed REL of 20 ppb. One could also crudely approximate an inhalation REL from the oral NTP bioassay where a dose of 25 mg/kg-day was associated with hematological effects. The concentration approximately equivalent to a 25 mg/kg dose for a 70 kg human breathing 20 cubic meters per day is 27 ppm. Assuming this is a LOAEL and applying an RGDR of 1 for systemic effects, a 3 fold UF for extrapolation to humans, a 10-fold UF for LOAEL to NOAEL extrapolation and a 10-fold UF for intraspecies extrapolation yields a REL of 90 ppb. There are a number of uncertainties to this approach, yet it comes within a factor of 5 of the proposed REL based on human studies.

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APPENDIX C. ABSORPTION OF BENZENE AFTER INHALATION OR INGESTION EXPOSURE

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The best available human epidemiological data for evaluation of cancer risk for benzene derives from studies of occupational inhalation exposure. To apply the results of risk estimates derived from these occupational studies to the estimation of cancer risk arising from oral exposure to benzene, extrapolation between inhalation and oral doses of benzene is required.

A workshop organized by U.S. EPA and the ILSI Risk Science Institute concluded that route-to-route extrapolation for risk assessment is appropriate when similar toxic endpoints are observed with both routes of exposure and when toxicokinetic data are available (Gerrity et al., 1990). Although there are no cancer studies available on humans exposed to benzene via oral exposures, there is abundant evidence, discussed in the PHG document above, that indicates that similar cancers and hematotoxic endpoints occurred in several animal studies of both oral and inhalation exposure. Animal data also demonstrate that benzene metabolism is similar after ingestion and inhalation. Therefore, it is reasonable to extrapolate cancer risks from inhalation to oral exposures.

Some previous approaches to extrapolation from an inhalation to an oral slope factor for benzene assumed absorption after inhalation, by default, to be equivalent to absorption from oral exposure. In contrast, the most scientifically rigorous method for route-to-route extrapolation would involve development of a pharmacokinetic model able to predict concentrations of the ultimate carcinogen in bone marrow (the target tissue for benzene's carcinogenic effects) under a variety of different human exposure scenarios. There are currently several inadequacies of the scientific database required for this approach, addressed in detail in the PHG documentation. A major difficulty is that the particular chemical species responsible for the induction of leukemia in benzene-exposed people and animals is not known with certainty; therefore it is not clear what model quantities are most relevant. Further, pharmacokinetic models that include metabolism and distribution to the bone marrow have not been adequately validated for humans.

While the identity of the specific compounds responsible for leukemogenicity continues to be debated, most experts agree that benzene metabolites, or by-products of their formation are responsible for benzene leukemogenesis. This suggests that extrapolation between routes of exposure could be based on a dose metric defined as the total quantity of benzene metabolized in the body after uptake of equivalent amounts, a somewhat simpler metric than delivered dose of the unknown ultimate carcinogenic compound(s). However, the comparative kinetics of metabolite formation and clearance after inhalation or ingestion of benzene are not known for humans and the many uncertainties involved in using animal-based models to predict dosimetry for humans may preclude a risk assessment application for PBPK models dependent on animal-derived metabolism data at present.

Therefore, a simple approach to route-to-route extrapolation based on the ratio of the fraction absorbed by each route is reasonable at this time, from both scientific and practical standpoints. This appendix summarizes published literature addressing the absorption of benzene after oral exposure to laboratory animals, and after inhalation exposure in humans and animals. No relevant data were located for absorption of benzene after ingestion in humans.

Gastrointestinal Absorption

Benzene is efficiently absorbed in rabbits, hamsters, mice, and rats following administration by oral gavage. In an early study in rabbits, 90 percent of the radioactivity from a single bolus dose was recovered in urine and exhaled air (Parke and Williams, 1953). Sabourin and colleagues administered radiolabeled benzene orally, by corn oil gavage, and intraperitoneally (i.p.) to rats and mice (Sabourin et al., 1987). Doses of 0.5, 5, 14, 50 and 150 mg/kg were given to F344 rats and $B6C3F_1$ mice by the oral route; i.p. doses were 0.5 and 150 mg/kg. Exhaled air, urine, feces, pelt and body tissues were collected for analysis of radioactivity over the 48 hours following dosing. Table 1 summarizes the mean percent of the radiolabel for the highest and lowest dose groups in exhaled air (benzene plus metabolites), urine and feces. In some cases the standard deviations around the mean indicate considerable variability among the animals. However, despite the variability, the data suggest that benzene, over a wide range of doses, is almost completely absorbed through the gastrointestinal tract of rats and mice.

Table 1. Excretion of Orally Administered [^{14}C]Benzene in Rats and Mice (Sabourin et al., 1987)

Dose	**0.5 mg/kg-d**		**150 mg/kg-d**	
Test Animal	Oral (percent)[a]	I.p. (percent)[a]	Oral (percent)[a]	I.p. (percent)[a]
F344 Rats				
Exhaled Air	12.4	18.2	48.7	56.5
Urine	92	85	43	38
Feces	2.0	2.0	1.0	0.7
Sprague-Dawley Rats				
Exhaled Air	6.2	23.9	52.4	68.9
Urine	88	82	37	37
Feces	1.3	1.3	0.5	0.3
$B6C3F_1$ Mice				
Exhaled Air	2.5	10.2	70.2	60
Urine	77	67	23	11
Feces	12	17	8	3.8

[a] Percent of radiolabel (mean values from 2 to 4 animals).

In a recent study (Mathews et al., 1998), rats, mice and hamsters were treated by oral gavage with a range of benzene doses that overlapped and extended lower than the dose range used in the Sabourin et al. (1987) study. Nearly complete absorption from the gastrointestinal tract was confirmed in all three species. Both studies report a greater proportion of metabolites excreted in urine at low doses, with a shift to greater amounts of unmetabolized benzene excreted in exhaled air at high doses. The two papers identify the onset of metabolic saturation at oral doses around 10 to 15 mg/kg. However, at the oral doses at which humans are likely to be exposed, the animal results suggest a linear increase in total metabolite production with exposure level.

In humans, oral exposure occurs by ingestion of contaminated food or water. No animal studies are available that allow a comparison of absorption between gavage and drinking water administration. Theoretically,

benzene ingested in drinking water could be subject to volatilization loss from the stomach, which would be suppressed by the oil vehicle used in the animal gavage experiments. On the other hand, it might be expected that a greater proportion of large bolus doses would escape absorption, and pass through in the feces, while smaller doses would be better absorbed. The fact that essentially complete absorption was observed even at high gavage doses in the Sabourin et al. and Mathews et al. studies suggests that, in the absence of data to the contrary, it is reasonable to assume complete absorption of benzene ingested by humans.

Pulmonary Absorption

Pulmonary absorption of volatile organic compounds is not expected to be complete; some portion of the inhaled concentration is exhaled from the lung without entering the systemic circulation. Experimental evidence confirms incomplete absorption of benzene in both animals and humans.

Rats and Mice

In the Sabourin study cited above, rats and mice were also exposed to benzene by inhalation (Sabourin et al., 1987). The results are summarized in Table 2. Mice and rats were exposed to 13, 29, and 130 ppm benzene by inhalation. Rats were also given 260 and 870 ppm, while mice were exposed to one high dose of 990 ppm. The total inhaled dose of benzene was computed from the exposure concentration and measured breathing rate. The amount of benzene retained was then computed as a fraction of this quantity, based on the radioactivity remaining in the carcass or excreted in urine and feces. Benzene taken up, but subsequently excreted in exhaled air is not counted in the absorbed fraction; the resulting retention values thus somewhat underestimate total absorption.

Table 2. Percent of inhaled benzene retained in rats and mice. Data from Sabourin et al., 1987

Exposure concentration (ppm)		Average percentage retained after 6 hours (n=3)	
Rats	*Mice*	*Rats*	*Mice*
13	11	33	50
29	29	44	52
130	130	23	38
260	--	22	--
870	990	15	9.7

Humans

There is a significant database on benzene measured in the exhaled breath of humans exposed in occupational, environmental or experimental situations. Occupational and environmental exposures are highly variable from individual to individual and over time. This variability renders estimation of the actual exposure received quite complicated in many situations. Therefore, we focus here on studies of controlled human exposures to known concentrations of benzene for known duration, but use estimates from other studies for comparison.

Chamber studies are often designed to study the excretion of benzene and/or its metabolites. While useful information concerning half-time of benzene in the body and elimination kinetics can be obtained from the post-exposure period, computation of total benzene retained requires long follow-up times and measurement of all urinary and exhalation products. There are no human studies that combine total metabolite analysis and sufficient follow-up. Alternatively, instantaneous absorption can be computed from concurrent measurements of exposure concentration (Cinh) and benzene in exhaled air (Cexh). Measurements of this sort are available for a number of studies.

For this report, the percent of benzene absorbed is defined simply as: 100 * (Cinh - Cexh)/Cinh, where both concentrations are measured during the exposure period. Concentration data have been converted to this metric to facilitate comparison across studies. The results are summarized in Table 3.

Hunter and colleagues (Hunter, 1966, 1968; Hunter and Blair, 1972)

> In the first paper of this series, absorption of 47 percent was reported for one male subject exposed for 24 minutes to a concentration "a little above the threshold value of 25 ppm" (Hunter, 1966). In the next paper, one male subject exposed for two and four hours to approximately 30 ppm absorbed 55-60 percent of the inhaled concentration (Hunter, 1968). Hunter and Blair (1972) exposed five male subjects for 2-3 hr to concentrations ranging from about 30 to 100 ppm. However, inhaled and exhaled air concentrations are not reported for the time during exposure except for one subject (Table 3). The time of sampling was not given, neither was it clear whether the data represent a single sample or an average of multiple samples. For this single subject, exposed over a period of five days to concentrations ranging from 21 to 32 ppm, the percent absorbed (computed as above) ranged from 53 to 63 percent. It is not clear whether this is a different subject from the previous report.

Nomiyama and Nomiyama, 1974

> The authors determined both "retention" and "uptake" of benzene. Their calculation of retention is equivalent to the definition of absorption used in this report. Six subjects, three male and three female, were exposed to benzene concentrations ranging from 52 to 62 ppm for four hour periods. Exhaled air was sampled every hour. The authors report that absorption averaged over the six individuals was 30.2 percent. This figure is somewhat lower than the other studies discussed here. However, the data in Figure 2 of the publication indicate that a potential explanation is that absorption was averaged over the 3.0, 3.5 and 4.0 hour time points only. The percent absorption was time-dependent in these experiments: absorption was high early in exposure, and approached a steady state only after three hours. According to the data plotted in the figure, the average absorption at the one-hour time point was approximately 60 percent for women and 45 percent for men. A decrease to approximately 43 percent and 35 percent, respectively, occurred at the two-hour time point.

Pekari et al., 1992

> Three male subjects were exposed to 1.7 and 10 ppm for four hours. Six samples of exhaled air and blood were taken during exposure from each subject at each exposure level. Post-exposure follow-up consisted of exhaled air, blood and urinary phenol measurements. The average percent absorption was 48 percent +/- 4.3 at 10 ppm, and 52 percent +/- 7.3 at 1.7 ppm.

Table 3. Absorption of inhaled benzene in humans

Study	Percent absorbed, average (range)	Exposure concentration	Exposure duration	Number of subjects	Number of samples per exposure period
Fiserova-Bergerova et al., 1974	48 %	??	5 hr	14	1?
Hunter, 1966	47%	25-30 ppm	24 min.	1	na
Hunter, 1968	(55-60%)	Approx. 30 ppm	2 hr, 4 hr	1 (2 exposures)	na
Hunter and Blair, 1972	(53-63%)	21-32 ppm	3-4 hr	1 (10 exposures)	1
Nomiyama and Nomiyama, 1974	30 % +/- 6.7 (SD)	52-62 ppm	4 hr	6	30 % based on 3 samples
Pekari et al., 1992	52 % (SD 7.3)	1.7 ppm	4 hr	3	6
	48 % (SD 4.3)	10 ppm	4 hr	3	6
Srbova et al., 1950	50-62 % (one subject)	100 ppm	90 min	1	7
	20-50 % (group range after 2 hours)	47-110 ppm	2-3 hr	23	every 15 min
Yu and Weisel, 1998	64 % (range: 48-73%)	32-69 ppm (in tobacco smoke)	30 120 min	3 3	4 7

Sherwood, 1988

A single male subject was studied, and the author states that the methods used allow uptake to be "roughly estimated", but the uptake fraction is not reported. The method for collecting exhaled air during the exposure period did not involve an actual breath sample, but was based on the concentration of benzene in the outlet of a self-pressurized blouse in which the exposure occurred. Because of these problems, this study is not listed in Table 3.

Srbova et al., 1950

This was the largest study located, reporting on 27 exposures to 23 subjects. Exposure concentrations ranged from 47 to 100 ppm; exposure durations were two to three hours. Exhaled air samples were taken every 15 minutes. Unfortunately, specific absorption data are given for only one experiment. The authors report that, in general, absorption was greatest in the first five minutes but decreased to 20 to 60 percent after one hour and to 20 to 50 percent after a second hour. For the one subject on whom data were reported, absorption ranged from 50-62 percent over one exposure period in which samples were taken at 5, 15, 30, 45, 60, 75, and 90 minutes (computed from data in the first two columns of the table entitled "Experiment 27", using the formula specified above). Higher figures for absorption resulted from samples early in the exposure period; a steady decrease was observed as exposure progressed.

Teisinger et al., 1952 (data reported in Fiserova-Bergerova et al., 1974)

This study was published in Czech (Teisinger et al., 1952, as reported in Fiserova-Bergerova et al., 1974) and subsequently translated into French (Teisinger et al., 1955, as reported in Fiserova-Bergerova et al., 1974). Neither of these publications was reviewed for this report. Figure 2 from Fiserova-Bergerova et al. (1974) reports the data from the Teisinger study in graphic form. A mean absorption of about 47 percent, with standard error encompassing approximately 43 to 53 percent can be estimated from the figure. These data represent the average of measurements from 14 subjects exposed for five hours and sampled toward the end of the exposure period. The exposure level is not clear from the 1974 report, however, it has been cited as being 100 ppm (Travis et al., 1990).

Yu and Weisel, 1998

> In this recent study, benzene concentration in inhaled and exhaled air was reported for three female subjects, each sampled at four time points during one to three exposure episodes. However, the exposures were to sidestream tobacco smoke, rather than pure benzene. Smoke was generated from burning cigarettes in room air, resulting in variable benzene concentrations during exposure and incomplete mixing. Exposure sessions were of duration 30 or 120 minutes. Benzene concentrations ranged from 32 to 69 ppm. The mean percent absorbed in eight experiments was 64 percent, with a range of experiment averages from 48 to 73 percent. While several studies have reported that absorption is higher at the outset of inhalation exposure, there was no significant difference between the shorter and longer duration experiments in this study.

Discussion of Inhalation Absorption

The data summarized above clearly indicate that absorption of benzene from the inhalation route is incomplete. We consider the Pekari et al. study to be the most technically sound, due to the use of modern experimental methods and collection of a large number of samples per subject. Based on averaging the values for the two exposure levels in this study, we recommend the use of a 50 percent absorption factor for inhalation exposure to benzene. There is very good overall agreement among the studies, with most supporting an absorption factor close to 50 percent.

Corroboration of the 50 percent absorption factor can be found in the literature on exhaled air measurements arising from occupational and environmental exposure. Subjects in these studies generally have longer term exposure, and may represent equilibrium conditions. Analysis of exhaled breath measurements from control subjects in an occupational study, who had low background exposure to benzene, indicated average absorption of 55 percent (Perbellini et al., 1988). In most studies of this sort exhaled air samples were collected in the post-exposure period. The concentration of benzene in exhaled air falls very rapidly upon removal from exposure, and so post-exposure samples would be expected to predict a lower absorption fraction. Wallace et al. (1993) reported an absorption fraction of 70 percent for benzene, based on measurements of exhaled air for nonsmokers in the TEAM studies (Table 1 of the publication). Thus, occupational and environmental exposure studies suggest that an absorption fraction of 0.50 is a good estimate.

A recent PBPK modeling study applied data on benzene in blood and exhaled air supplied by Pekari and colleagues to a model describing benzene disposition in the body (Bois et al., 1996). After fitting model parameters to the dataset, the model predicted that 57 percent of benzene in inhaled air is metabolized in the body (90 percent CI: 47 to 67 percent). Since at low exposure levels, a majority of absorbed benzene is metabolized rather than excreted unchanged, the 57 percent figure can be roughly compared to the 50 percent absorption factor that Pekari and colleagues estimated from their measurements. Until the model is further validated by application to other human data, we recommend use of the measured data rather than the model predictions.

The general agreement of the animal and human data provides additional support for the Pekari study results. The two low exposure concentrations in Table 2 overlap with the range of concentrations tested in human studies. At these lower concentrations inhalation absorption efficiency is similar in animals and humans.

A figure of 50 percent absorption by inhalation is also consistent with other estimates in the literature. ACGIH (1998) cites Rusch's conclusion (Rusch, 1977) that approximately 46 percent of inhaled benzene is absorbed in humans. Another estimate, based on the studies of Hunter, Nomiyama and Srbova cited above, was 47 percent (Owen, 1990). The latter estimate was adopted by MacIntosh and colleagues for use in a recent population-based exposure model for benzene (MacIntosh et al., 1995). An analysis of short-term exposure limits for benzene assumed 50 percent absorption by inhalation (Paxman and Rappaport, 1990). Thus, there is a general consensus in the literature that supports replacing the default assumption of equivalence of absorption by oral and inhalation routes by an inhalation absorption estimate of 50 percent.

Potential Limitations

Dose dependence of absorption

A decrease in absorption was observed in both mice and rats as inhaled concentration increased from 30 to 130 ppm (Table 2). In a recent inhalation study in Sprague-Dawley rats, a shift in clearance of benzene from the chamber air (due to uptake by the animals) was noted between concentrations in a much lower range, suggesting the possibility of saturation of metabolism as low as ten ppm (Yoshida et al., 1998). Saturated metabolism would be expected to result in reduced absorption of benzene due to slower clearance of blood benzene concentrations. While air benzene concentrations used in controlled human exposure studies collectively covered nearly two orders of magnitude, no dose dependency can be observed when the studies are taken together. There is some indication that the high exposure levels (up to 110 ppm) used in the Srbova et al. study may have resulted in lower absorption (the lower end of the range was 20 percent, however, the analytical methods used in this early work may not be accurate. The results of the TEAM studies may indicate higher absorption at very low doses. Overall, it is not clear whether the lack of evidence of saturation in the human studies is because exposure levels did not reach those used in animal studies or because substantial inter-study and inter-individual variability obscures any possible relationship in these studies with their generally very small sample sizes.

Time dependence of absorption

Data from several of the chamber studies indicates that there is a lag time between the onset of exposure and the time at which steady state blood concentration is reached. Most studies averaged the absorption percentages from early and late exposure phases together. In an excretion study (not considered above, because only post-exposure exhaled air was sampled) it was found that benzene accumulated over a five day period in which a subject was exposed each day (Berlin et al., 1980). Higher blood levels would limit further uptake. This suggests that pulmonary absorption efficiency in chronically exposed people, or workers who are exposed for longer intervals than were the subjects of chamber studies, could be lower than is suggested by the relatively short term exposure studies discussed above.

Inter-individual variability in absorption

The drawbacks of experimental exposure studies for use in risk assessment include small numbers of people tested, and short exposure durations. While the human data demonstrate reasonably good agreement, indicating that approximately one half of inhaled benzene is absorbed into the bloodstream at exposure concentrations between 1 and 100 ppm, considerable inter-individual variability was observed in all studies that reported on multiple subjects. Many factors, including activity level, pulmonary health, and different rates of metabolic clearance are likely to influence the amount of benzene actually taken up in a diverse population exposed by the inhalation route. To date, characterization of the extent of variability is limited.

Gender dependence of absorption

Another potential source of inter-individual variability is gender-dependence. Nomiyama et al. (1974) reported that women had higher initial absorption of benzene, although at equilibrium, the percent absorbed was similar to men. The Yu and Weisel study was performed on female subjects only, and reported some of the highest estimates of absorption. Sato et al. (1975) exposed five men and five women to 25 ppm benzene for two hours. Exhaled air concentrations were measured for the post-exposure period only. Clearance of benzene appeared to be slower in women, a finding the authors attributed to differences in body fat. It is possible that the observations of Yu and Weisel (1998) and Nomiyama and Nomiyama (1974) can be explained by a slower approach to steady-state conditions in women due to more extensive partitioning into

fat. Because of the paucity of detailed data on female subjects, however, whether there are significant gender differences in absorption kinetics remains unclear.

Conclusions

The data summarized here provide support for use in risk assessment of adjustment factors for intake of benzene equal to 0.5 for the inhalation route and 1.0 for ingestion.

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