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Carcinogenic Effects of Benzene: An Update

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National Center for Environmental Assessment–Washington Office
Office of Research and Development
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ABSTRACT

The major issue addressed in this document involves the nature and magnitude of the inhalation risk of cancer to humans exposed to low levels of benzene. Occupational studies continue to provide the bulk of evidence of benzene's carcinogenicity. Workers are exposed at much higher levels than is the general public. This document reconfirms that benzene is a "known" human carcinogen by all routes of exposure. This finding is supported by evidence from human epidemiologic studies, animal data, and an improvement in understanding of mechanism(s) of action. Human epidemiologic studies of highly exposed occupational cohorts have demonstrated that inhalation exposure to benzene can cause acute nonlymphocytic leukemia and other blood disorders, that is, preleukemia and aplastic anemia. Additionally, changes in blood and bone marrow consistent with hematotoxicity are recognized in humans and experimental animals. Currently, there is insufficient evidence to deviate from using an assumption of a linear dose-response curve for benzene, hence, the Agency's past approach of using a model with low-dose linearity is still recommended. Of the various approaches employing a linear assumption, utilizing the Pliofilm workers cohort, the inhalation risk at 1 ppm ranges from 7.1×10^{-3} to 2.5×10^{-2}. This reflects a modest change from the EPA's 1985 interim risk assessment which provided only a single estimate of risk (i.e., 2.6×10^{-2}).

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PREFACE

The National Center for Environmental Assessment-Washington Office, Office of Research and Development, has prepared this document on the *Carcinogenic Effects of Benzene: An Update* to serve as a source document for the Office of Air and Radiation, Office of Mobile Sources, to support decision making regarding regulation of benzene as a hazardous air pollutant.

In the development of this document, the scientific literature has been reviewed, key studies have been evaluated, and summary/conclusions have been prepared so that the carcinogenicity and related information are qualitatively and quantitatively characterized. This updated evaluation and review of benzene carcinogenicity was conducted under the standing guidance of the 1986 cancer risk assessment guidelines, but with a recognition of the proposed 1996 cancer risk assessment guidelines emphasizing mode of action and dose-response analysis. Relevant literature has been reviewed through July 1997.

The emphasis of this document is a detailed discussion of the relevancy of the 1985 cancer unit risk assessment of benzene in light of new information.

This final document reflects a consideration of all comments received on an External Review Draft dated June 1997 (EPA/600/P-97/001A) provided by an expert panel at a peer review workshop (July 16, 1997) and comments received during a public review and comment period (June - July 1997).

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GLOSSARY OF ACRONYMS AND ABBREVIATIONS

ALL	acute lymphocytic leukemia
AML	acute myeloid leukemia
AMML	acute myeloid and monocytic leukemia
ANLL	acute nonlymphocytic leukemia
ATSDR	Agency for Toxic Substances and Diseases Registry
C.I.	confidence interval
CFU-GM	colony-forming unit-granulocyte/macrophage
CLL	chronic lymphocytic leukemia
CML	chronic myeloid leukemia
DNA	desoxyribonucleic acid
FISH	fluorescent in situ hybridization
GM-CSF	granulocyte/macrophage-colony-stimulating factor
GPA	glycophorin A
GSH	glutathione
i.p.	intraperitoneal
IARC	International Agency for Cancer Research
IL-1	interleukin 1
IPCS	International Programme on Chemical Safety
MA	trans, trans-muconaldehyde
MDS	myelodysplasic syndromes
MOE	margin of exposure
MPO	myeloperoxidase
NCEA	National Center for Environmental Assessment (EPA)
NIOSH	National Institute for Occupational Safety and Health
NQO1	NAD(P)H:P quinone oxidoreductase
OAQPS	Office of Air Quality Planning and Standards (EPA)
OAR	Office of Air and Radiation (EPA)
OMS	Office of Mobile Sources (EPA)
OSHA	Occupational Safety and Health Administration
PHA	phytohemaglutin
POD	point of departure
ppb	parts per billion
ppm	parts per million
RBC	red blood cell
RH	rubber hydrochloride
RR	relative risk
RR	rate ratio
SMR	standard mortality ratio
TWA	time weighted average
WBC	white blood cell

EXECUTIVE SUMMARY

In 1992, the U.S. Environmental Protection Agency's (EPA's) Office of Air and Radiation, Office of Mobile Sources, requested that the National Center for Environmental Assessment (NCEA) provide an updated characterization of the cancer risk to humans from inhalation exposure to benzene. The previous characterization of the carcinogenic risk of exposure to benzene was done in 1985 by the Office of Health and Environmental Assessment (the predecessor organization to NCEA). Additional scientific data relevant to the carcinogenicity of exposure to benzene have been published in the literature since that time. This has brought into question the relevancy of the earlier quantitative cancer risk estimates. The 1985 cancer unit risk estimates were based on assumptions about the effects of low-level benzene exposure on humans derived from occupational health studies. The scope of this report is limited to issues related to the carcinogenicity of exposure to benzene. Specifically, this report evaluates and discusses studies published since 1985 to ascertain if there has been sufficient new scientific information that would significantly alter the 1985 interim benzene cancer unit risk estimate.

This updated evaluation and review of the benzene risk assessment is being conducted under the standing guidance of the 1986 *Guidelines for Carcinogen Risk Assessment* but with a recognition of additional areas of emphasis in the 1996 *Proposed Guidelines for Carcinogen Risk Assessment*. Thus, this updated review of the benzene risk assessment contains a discussion of how recent evidence on mode of action can be incorporated into hazard characterization and dose-response approaches.

The major issue addressed in this document involves the magnitude of the risk of cancer to humans exposed to low inhalation levels of benzene. Occupational exposure studies provide the bulk of evidence of carcinogenicity, since workers were exposed at higher levels than is the general public.

It has been clearly established and accepted that exposure to benzene causes acute nonlymphocytic leukemia and a variety of other blood-related disorders in humans. The existing EPA Group A classification of benzene based on the 1986 guidelines is replaced with a narrative incorporating the "known/likely" descriptor under the 1996 proposed guidelines. The narrative discusses the uncertainties about the following: the shape of the dose-response curve at low doses, mode of action, and exposure in human studies. The study of Pliofilm rubber workers at

three facilities in Ohio provides the best published set of data to date for evaluating human cancer risks from exposure to benzene. Compared to other published studies, this cohort has the fewest reported co-exposures in the workplace to other potentially carcinogenic substances that might confound risk analysis for benzene. Since the 1985 interim assessment, this cohort has been expanded to include workers who were employed for at least one day between 1940 and 1965. It included an additional 6.5 years of follow-up from the earlier study as well as individual estimates of personal exposure, which were not included in the previous study. One myeloblastic leukemia was subsequently noted after the additional follow-up. However, because of the compensating increase in expected deaths due to the additional person-years of follow-up, only a small change occurred in the overall relative risk.

Several investigators have employed various assumptions to estimate occupational exposure levels prior to 1950, when exposures were most intense. The estimates of exposure made by Rinsky were generally the lowest, but there is no consistent pattern among the estimates for particular years. Even with the differences in the exposure levels produced by utilizing these sets of estimates of exposure for the employees, the cumulative SMRs differed by no more than a factor of 3.

In the 1985 interim benzene document, a single overall unit risk estimate was calculated as the geometric mean of four maximum likelihood unit risk estimates generated from the three available exposed worker studies. The result was a probability of 2.6×10^{-2}. Neither of the chemical worker studies has sufficient power for independent calculations, and the net result of discarding data from them has only a small effect on the unit risk estimate.

Recently, the National Cancer Institute, in cooperation with the Chinese Academy of Preventive Medicine, published early results from a comprehensive study of 74,828 benzene-exposed workers employed from 1972 to 1987 in 672 factories in 12 cities of China. This study, one of the largest of its type ever undertaken, enabled its authors to claim detection of significantly elevated risks at extraordinarily low levels of exposure. Their findings suggested that workers exposed to benzene at average levels of less than 10 ppm are subject to a higher risk of hematologic neoplasms. Although most of the attributes considered important in a long-term retrospective study of this nature and magnitude were addressed by the authors, some uncertainties and potential weaknesses still remain. The derivation of the cohort from many different factories across China suggested the possibility that this cohort was exposed to mixtures

of many different chemicals. Exposure to other carcinogens in the workplace could have produced confounding effects, especially if exposures were to chemicals that increase the risk of leukemia. In addition, the derivation of early exposure estimates to benzene may be biased and life-style and socioeconomic factors may have had impacts. It is clear that this study contains thought-provoking new findings and conclusions. It is, however, premature to assume that the Chinese data should now replace Rinsky's Pliofilm workers cohort in the derivation of risk estimates.

At present, identification of the mechanisms by which exposure to benzene and its metabolites exerts their toxic and carcinogenic effects remains elusive. Animal studies showed that benzene itself was unlikely to be the actual toxicant, but rather that metabolism (i.e., metabolic transformation) was required for toxic effects to occur. The central issues in integrating the mechanistic data from the laboratory animal experiments with the occupational epidemiologic data to estimate risk of the anticipated ambient low-level human scenario are establishing whether the mechanisms that are operative in laboratory animals are similar to mechanisms operative in humans and accounting for the dose dependency of those mechanisms. Two pathways have been commonly postulated as being responsible for benzene toxicity. The first involves the major hepatic metabolites of benzene—phenol, catechol, and hydroquinone—while the second involves ring-opened forms of benzene. The majority of data suggest that the hepatic conversion of benzene to phenolic metabolites is an important primary event in benzene toxicity. Catechol and hydroquinone have been shown to persist in bone marrow after benzene exposure and the bone marrow is rich in peroxidase activity, and phenolic metabolites of benzene can be activated by peroxidases to reactive quinone derivatives. Although much less is known about the metabolism of benzene in humans than in laboratory animals, existing studies indicated that both metabolize benzene along similar pathways. The rates of some reactions, however, may differ. A recent series of investigations on Chinese workers highly exposed to benzene provides insights into particular enzymes underlying the hematotoxicity of benzene in humans. Overall, individuals with the rapid hydroxylator phenotype exhibited a 2.5-fold increased risk of benzene poisoning as compared to those with the slow hydroxylator phenotype.

Benzene affects bone marrow cells in several different ways. Based on our current understanding, these effects are produced by the interactive effects of multiple metabolites. Genotoxic effects are a critical component of the leukemogenic properties of benzene. As more

information becomes available about the epigenetic effects of benzene and the role these effects play in the leukemogenic process in general, it is likely that these will be shown to have an important role. Evidence supports the hypothesis that more than one toxic effect contributes to the leukemogenic process, especially because benzene metabolic products may be able to cause general disruption of protein functions in bone marrow cells. Protein damage is likely to result in pleiotropic effects, including general toxicity, alteration of growth factor responses, and DNA damage. Therefore, the overall picture of benzene-induced leukemogenesis is an increased rate of genetic damage to hematopoietic cells that occurs in the context of disrupted bone marrow function. This situation could encourage not only the production of cells with key genetic changes, but also the selection and expansion of such cells due to the abnormal marrow. However, data are not sufficient at this time to state precisely which of the various documented effects, genotoxic or otherwise, are the critical ones for benzene-induced leukemogenicity.

In the 1985 benzene risk assessment, the lifetime leukemia risk due to benzene was developed using the geometric mean of risk estimates that were calculated on the basis of data from three studies of exposed workers. Subsequently, several risk assessments based on Rinsky's cohort have become available with individual risk estimates using varying assumptions and/or models, with outcomes ranging more than six orders of magnitude. Some recent evidence suggests the possibility that the low-dose curve could be supralinear since the formation of toxic metabolites plateaus above 25 ppm benzene in air. Thus, it is possible that the unit risk is underestimated if linearity is assumed at low doses. However, none of the approaches can be said to have greater scientific validity than any other; hence there is no clear basis for choosing a single best estimate. Rather, the set of risk estimates reflects both the inherent uncertainties in the risk assessment of benzene and the limitations of the epidemiologic response and exposure data. While the risk estimates would be significantly different if a nonlinear exposure response model was found to be more plausible, the shape (i.e., the nonlinearity) of the exposure-response curve cannot be determined without a better understanding of the biological mechanism of benzene-induced leukemia. The arguments made in favor of benzene-induced leukemia being nonlinear at low doses can be matched by arguments opposing this as a most likely occurrence. Thus, there is not sufficient evidence currently to reject a linear dose-response curve for benzene in the low-dose region, nor is there sufficient evidence to demonstrate that benzene is, in fact, nonlinear in its effects. Since this knowledge is not available at the present time, the EPA default

approach of using a model with low-dose linearity is still recommended. Of the various approaches employing a linear assumption, the risk at 1 ppm ranges from 7.1×10^{-3} to 2.5×10^{-2}, within which any calculated unit risk estimate would have equal scientific validity.

The effects from exposure to benzene could be potentially different among subpopulations, including children. However, there is insufficient data on differential susceptibility from environmental exposure to benzene, and it is not possible to make quantitative adjustments for these factors at this time.

Data insufficiencies in several areas are noted and research in these areas ultimately should provide a better understanding of how benzene causes cancer, particularly the mechanism of benzene-induced leukemia. Specific measures of early genetic damage in humans with known exposure to benzene will help define the biological events leading up to the disease by providing internal markers of its progression. Such information may be forthcoming in the near future from a large cohort of benzene-exposed workers under study in China. A need exists to further validate toxicokinetic models and to assess metabolic susceptibility factors in human subjects. Continued basic research in hematopoiesis and leukemia is critical for identifying the mechanisms of leukemogenesis. There remain important unanswered questions about the cell population that contains targets for leukemic transformation, such as cell number and rate of division, quiescence patterns, maturation, regulation, and apoptotic behavior. Particular emphasis should be placed on research on those sensitive subpopulations who are believed to be at increased risk (e.g., infants and children, the elderly).

1. INTRODUCTION

In 1992, the U.S. Environmental Protection Agency's (EPA's) Office of Air and Radiation (OAR), Office of Mobile Sources (OMS) requested that the National Center for Environmental Assessment (NCEA) provide an updated characterization of the cancer risk to humans of inhalation exposure to benzene. The previous interim characterization of the carcinogenic risk of exposure to benzene was done in 1985 by the Office of Health and Environmental Assessment (the predecessor to NCEA). Additional scientific data relevant to the carcinogenicity of benzene exposure have been published since that time. This has brought into question the relevancy of the earlier quantitative cancer risk estimates. The 1985 estimates were based on assumptions about the effects of low-level benzene exposure on humans derived from occupational health studies.

The regulatory authority (Clean Air Act Amendments, 1990) for controlling fuel emissions from vehicles resides in OAR. OMS has asked NCEA to provide a scientific support document based on health implications of continued exposure to benzene.

The scope of this report is limited to issues related to the carcinogenicity of exposure to benzene. Specifically, this report evaluates and discusses studies published since 1985 to ascertain if there has been sufficient new scientific information that would significantly alter the 1985 interim benzene cancer unit risk estimate.

1.1. HISTORY OF THE 1985 INTERIM DOCUMENT

In 1985, the Office of Research and Development prepared estimates of the inhalation unit risk for benzene (U.S. EPA, 1985) at the request of Office of Air Quality Planning and Standards (OAQPS). The previous cancer risk assessment of benzene by the Agency was completed in January 1979 (U.S. EPA, 1979). Subsequently, this assessment became out of date as new scientific information became available. In response to the need to update the 1979 assessment, the 1985 Interim Quantitative Cancer Unit Risk Estimate Due to Inhalation of Benzene was developed. It reviewed and incorporated information from three epidemiologic studies at the time (Rinsky et al., 1981; Ott et al., 1978; Wong et al., 1983). In addition, animal inhalation studies in male rats and mice (Goldstein et al., 1982) and in male and female rats (Maltoni et al., 1983) were added to the information base.

Data from the occupational cohorts of Rinsky et al. (1981), Ott et al. (1978), and Wong et al. (1983) were pooled and analyzed by Crump and Allen (1984) to provide exposure (cumulative dose) estimates for use in the development of a benzene cancer risk assessment for the Occupational Safety and Health Administration (OSHA, 1987) independently of EPA. These exposure estimates were available for use by the Agency. Crump and Allen (1984) made their

exposure estimates using three separate approaches (cumulative, weighted cumulative, and window) and two risk models (absolute and relative). The cumulative dose approach assumes that the risk depends on the air concentration times duration of exposure. The weighted cumulative dose approach assumes that the contribution of an exposure to risk varies depending on when exposure occurred. The window approach assumes that benzene exposure for longer than 15 years induces no additional risk, but that exposure between 5 and 10 years induces a risk proportional to the air concentration and exposure duration. All three approaches assume a latency period that begins at the beginning of exposure, during which there is assumed to be no increased risk. An absolute risk model assumes that the risk from exposure is independent of the background risk of disease, whereas a relative risk model assumes that the risk from exposure is proportional to the background incidence of the disease (see Section 3.1).

The Agency concluded that the cumulative and the weighted cumulative exposure estimates were both valid and preferable to the window approach. EPA also concluded that the absolute and relative risk models had equal validity. It was decided to calculate the geometric mean of the four resulting estimates derived from the different exposure estimates and risk models and then multiply this by a correction factor based on the epidemiologic data of Wong et al. (1983). This correction factor (1.23) was the ratio of risk estimates (under the relative risk model and cumulative exposure estimate) when all three studies (Rinsky et al., 1981; Ott et al., 1978; Wong et al., 1983) were used to the risk estimate generated when only the Rinsky et al. (1981) and Ott et al. (1978) studies were used. The Wong et al. (1983) study was not used under the absolute risk model because its information was considered insufficient by Crump and Allen (1984), whose study formed the basis for EPA risk numbers. The resulting quantitative cancer unit risk of 2.6×10^{-2} per ppm air concentration was about 10 times greater than the human risk estimate based on the three animal inhalation studies and 1.5 times higher than the pooled estimates from the three gavage studies. This estimate compared well with the original estimate from the 1979 benzene risk document (U.S. EPA, 1979) of 2.41×10^{-2}, which was based on the geometric mean of three unit risk estimates derived from the occupational cohort studies of Infante et al. (1977), Aksoy (1976, 1977), Aksoy et al. (1974), and Ott et al. (1977).

1.2. PROPOSED 1996 GUIDELINES FOR CARCINOGEN RISK ASSESSMENT

The Agency recently published its Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996). When final, these guidelines will supersede the existing Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986). The 1996 proposed cancer risk assessment guidelines include a number of changes that encompass a more detailed understanding of the carcinogenic processes and provide a framework for the use of mechanistic data. It should be noted, however, that the results of an assessment under the new guidelines will not differ greatly

from those under the 1986 guidelines, unless new kinds of information are forthcoming from research on mechanisms and toxicokinetics.

The proposed guidelines are intended to provide for greater flexibility in incorporating the rapidly increasing data in decisions to implement the Agency's regulatory authority. Risk characterizations are important components of the new guidelines and serve to explain the key lines of evidence and conclusions, discuss the strengths and weaknesses of the evidence, present alternative conclusions, and point out significant issues and uncertainties deserving serious consideration. A risk characterization summary would integrate technical characterizations of exposure, hazard, and dose response to form the overall synthesis and conclusions about human health risk. This document is limited to discussions of the hazard and dose-response characterizations.

The hazard assessment component emphasizes use of information about an agent's mode of action to reduce the uncertainty in describing the likelihood of harm and to provide insight into appropriate extrapolation procedures. Mode of action is defined as the agent's influence on molecular, cellular, and physiological functions. Because it is the sum of the biology of the organism and the chemical properties of an agent that leads to an adverse effect, evaluation of the entire range of data (i.e., physical, chemical, biological, and toxicological) permits a reasoned judgement of an agent's mode of action. Although cancer is a complex and diverse process, a risk assessment must analyze the presumed critical events, at least those that can be measured experimentally, to derive a reasonable approximation of risk. Understanding the mode of action helps interpret the relevancy of the laboratory animal data and guides the dose-response extrapolation procedure, i.e., it helps to answer the question of the shape of the dose-response function at low doses. The conditions (i.e., route, duration, pattern, and magnitude of exposure) under which the carcinogenic effects of the agent may be expressed should also be considered in the hazard characterization.

The weight-of-evidence narrative for the hazard characterization includes classification descriptors. Three standard descriptors ("known/likely," "cannot be determined," and "not likely") were proposed to replace the six letter categories used in the 1986 guidelines (i.e., A-E). Because of the wide variety of data sets encountered, these descriptors are not meant to stand alone; rather, the narrative context is intended to provide a transparent explanation of the biological evidence and how the conclusions were derived.

The dose-response assessment under the new guidelines is a two-step process. In the first step, the response data are modeled in the range of empirical observation. Modeling in the observed range is done with biologically based or appropriate curve-fitting models. The second step, extrapolation below the range of observation, is accomplished by modeling if there are sufficient data or by a default procedure.

This updated evaluation and review of the benzene risk assessment is being conducted under the standing guidance of the 1986 cancer risk assessment guidelines, but with a recognition of these areas of emphasis in the 1996 proposed cancer risk assessment guidelines. Thus, this updated review contains a discussion of how recent evidence on mode of action can be incorporated into hazard characterization and dose-response approaches. Earlier dose-response assumptions or alternative approaches will be discussed in this context.

The major issue addressed in this document involves the magnitude of the risk of cancer to humans exposed to low levels of benzene. Occupational exposure studies provide the bulk of evidence of carcinogenicity, since workers are exposed at much higher levels than is the general public. The 1996 proposed cancer risk assessment guidelines recommend a detailed discussion of the basis for developing the quantitative unit risk estimate, drawing on mode-of-action, metabolism, and pharmacokinetics information replete with uncertainty discussions as appropriate. The 1985 interim risk estimate calculation for benzene was based on science policy using a procedure incorporating the geometric mean of maximum likelihood estimates because little information was available regarding carcinogenicity at low exposure levels.

2. HAZARD ASSESSMENT AND CHARACTERIZATION

The "known/likely" category of the proposed 1996 cancer risk assessment guidelines includes agents for which adequate epidemiologic evidence (known) or a combination of epidemiologic and experimental evidence demonstrates an association between human exposure and cancer.

It has been clearly established and accepted that exposure to benzene causes acute nonlymphocytic (myelogenous) leukemia (ANLL) and a variety of other blood-related disorders in humans (ATSDR, 1997; IARC, 1982; U.S. EPA, 1979, 1985). The existing Group A classification of benzene based on the 1986 guidelines would be replaced with a narrative incorporating the "known/likely" descriptor under the 1996 proposed guidelines. The narrative discusses the uncertainties about the following: the shape of the dose-response curve at low doses, mode of action, and exposure in human studies; these topics are addressed in this section.

2.1. HUMAN DATA

Epidemiologic studies and case studies provide clear evidence of a causal association between exposure to benzene and leukemia, especially ANLL and, to a lesser extent, chronic nonlymphocytic leukemia as well as chronic lymphocytic leukemia (CLL) (Vigliani and Saita, 1964; Aksoy et al., 1974; Aksoy, 1976, 1977; Infante et al., 1977; Rinsky et al., 1981, 1987;

IARC, 1982; ATSDR, 1997). A number of studies, including the Pliofilm cohort, have indicated that benzene exposure is associated with various types of lymphohematopoietic neoplasia other than acute nonlymphocytic leukemia (ANLL) (Savitz and Andrews, 1996). However, the specific types associated with benzene exposure remain unidentified. Lymphocytic leukemia, commonly found in children, may have a genetic component as well as an environmental exposure component (Linet, 1985). Exposure to benzene and other environmental chemicals cannot be ruled out. A higher risk of multiple myeloma was once thought to be associated with exposure to benzene (DeCouflé et al., 1983; Rinsky et al., 1987). However, later studies have failed to confirm this (Hayes et al.,1996, 1997). One new site-specific cancer, Hodgkin's lymphoma, appears to be associated with exposure to benzene as well as with hematologic neoplasms in general, which includes AML and related myelodysplasic syndromes (Hayes et al., 1997).

The study of Pliofilm rubber workers at three facilities in Ohio (Rinsky et al., 1981) provides the best published set of data to date for evaluating human cancer risks from exposure to benzene. Compared to other published studies (Hayes et al., 1996; Bond et al., 1986a; Wong, 1987; Schnatter et al., 1996a; Rushton et al., 1997), Rinsky et al. (1981) has the fewest reported co-exposures in the workplace to other potentially carcinogenic substances that might confound risk analysis for benzene. Except for the cohort studied by Bond et al. (1986a), the Pliofilm workers, furthermore, experienced a greater range of estimated exposure to benzene than the cohorts of other studies in which efforts were made to estimate individual exposures. The value of Bond et al. (1986a) for analysis of the effects of exposure to benzene was diminished by reported coexposures to styrene, arsenic, and other potentially carcinogenic substances. Hence, the Pliofilm workers are the preferred population for estimating the effects of exposure to benzene. Since the 1985 interim assessment, this cohort has been expanded (Rinsky et al., 1987) to include workers who were employed at least 1 day between January 1, 1940, and December 31, 1965. (In the previous study, employment after December 31, 1950, was not considered.)

Three questions have been raised concerning the impact of these more recent data on the present updated assessment of benzene and its use in a quantitative risk assessment. First, does the update lead to any substantial changes in the estimated relative risk ratios that were derived in the 1981 study? Second, do the various approaches used to estimate exposure in the those early years lead to risk estimates that differ by a substantial amount? One of the major problems with exposure estimates used by Rinsky et al. (1981, 1987) and others in deriving relative risk estimates for use in developing quantitative unit risk estimates is that no ambient air measurements of exposure to benzene in the Pliofilm workplace were taken before 1946, and in that year there were only four samples measured. The absence of earlier definitive ambient air measurements has led to many quantitative risk estimates by numerous investigators over the

past several years that have differed from each other partially on the basis of differences in the assumptions made about what those earlier exposures to benzene were. Third, because the Rinsky et al. (1987) Pliofilm cohort is currently the best set of data available for estimating exposure and the risk of leukemia, would it be advisable to calculate the quantitative unit risk estimates utilizing that cohort only; and what would the effect be if the Ott et al. (1978) and Wong et al. (1983) epidemiologic studies were included in the calculation of a unit risk estimate?

To answer the first question, the first study (Rinsky et al., 1981) of Pliofilm workers in the rubber industry covered three facilities in Ohio and consisted of 1,165 male workers who had been employed between 1940 and 1965 and followed through 1981. The second study (Rinsky et al., 1987) included an additional 6.5 years of follow-up from the earlier study. It also included individual estimates of personal exposure, which were not included in the previous study. Duration of employment and personal exposure estimates during that employment were used to generate risk estimates based on grouped data. The updated follow-up made it possible to evaluate dose-response relationships and estimate risks at low exposure levels in terms of ppm-years of exposure. One myeloblastic leukemia was subsequently noted after the additional follow-up. However, because of the compensating increase in expected deaths due to the additional person-years of follow-up, only a small change occurred in the overall relative risk. Altogether, 9 leukemias were observed versus 2.66 expected in this cohort by December 31, 1981 (Rinsky et al., 1987). The relative risks were found to increase with cumulative exposure as shown in Table 1.

Cumulative exposure expressed in terms of ambient respirable benzene multiplied by length of exposure (parts per million times years exposed) is a variable that has been used by researchers to measure individual dose in most epidemiologic studies. However, some recent limited epidemiologic evidence supports certain alternative dose measures as perhaps better than the measurement of cumulative dose. These alternative measures may or may not more closely

Table 1. Relative risk as a function of cumulative exposure

Cumulative exposure (ppm-years)	Relative risk
0-40	1.1
40-200	3.2
200-400	11.9
More than 400	66.4

approximate the actual dose that induces leukemia. It is uncertain whether it is benzene or an active metabolite of benzene (or some combination of metabolites with or without benzene), either delivered to the hematopoietic system or formed within the stem cells, that is responsible for leukemogenesis.

Hayes et al. (1997) suggested that recent exposure to benzene may be more relevant to the risk of ANLL/MDS (myelodysplasic syndromes) than distant past exposures, although the earliest exposure to benzene may have been the most intense. However, measurements of these earliest exposures are almost nonexistent. Recent epidemiologic studies of petroleum workers suggest that there are few or no apparent effects to workers exposed to exceedingly low levels of benzene for long periods of time (Raabe and Wong, 1996). However, some possible effects can be detected when these data are subjected to alternative dose metrics. In a case control study, Rushton and Romaniuk (1997) examined 91 cases of leukemia and matched controls (4 to 1) on the basis of age and controls alive at the time of case occurrence. These authors applied a number of dose metrics to determine which was the most appropriate for determining the risk of specific leukemia types in workers exposed to benzene. At low levels of exposure (generally 80% had cumulative exposure of less than 5 ppm-yrs.), no elevated risks could be detected. The authors found no relationship to any of several alternative exposure scenarios for acute lymphocytic leukemia (ALL) and chronic myeloid leukemia (CML). However, for CLL, there was a nonsignificant increase in risk, which tended to increase with duration of employment for white-collar employees who had only background exposure to benzene. On the other hand, for acute myeloid and monocytic leukemia (AMML), a significantly increased risk tended to be associated with "peaked" exposures to benzene but not with cumulative exposure, maximum intensity of exposure (which was not all that intense; levels for the most part never exceeded 0.4 ppm, time-weighted average, 8-h day), or mean intensity of exposure if treated as a continuous variable. This could be important when using the dose metric, intensity times time (ppm-years). This analysis suggests that a person exposed to 40 ppm of benzene for 1 year might be at a greater risk of leukemia than a person exposed to 1 ppm for 40 years.

Utilizing a nested case-control design, Schnatter et al. (1996a) conducted a similarly designed study of Canadian petroleum distribution workers exposed to benzene. Fourteen leukemia cases were matched 4 to 1 on birth date and time at risk with members from the same cohort. Average benzene concentrations ranged from 0.01 to 6.2 ppm during the history of the plant. The authors reported that at those exposure levels no significant increase in the risk of leukemia was detected utilizing either cumulative exposure or other dose metrics. The authors used several dose metrics, i.e., intensity, duration of exposure, family history of cancer, cigarette smoking, and years of exposure at certain levels of intensity (+0.5 ppm or +1.0 ppm). Only family history and whether the victim smoked cigarettes produced an elevated but still

nonsignificant risk of leukemia. The study, however, had little power to detect an elevated risk of leukemia because it was based on only 14 cases.

As was pointed out by Raabe and Wong (1996), few employees in the petroleum industry reported large amounts of exposure to benzene measured in terms of ppm-years, although many employees worked in the industry for long periods of time. In their meta-analysis of 208,000 workers combined from 19 different cohorts, only a small fraction of the workers accumulated as much as 200 ppm-yrs., the level the authors suggested was the "threshold" for carcinogenesis. In fact, Raabe and Wong (1996) reported that the average benzene level was only 0.22 ppm based on 14,824 samples from industrial hygiene surveys.

It has been suggested also that metabolic saturation may have taken place in the early years of the Pliofilm cohort, if the Paustenbach et al. (1993) exposure estimates are considered. But based upon a discussion of their estimates by Utterback and Rinsky (1995), it is unlikely that such high levels of exposure could have occurred to these Pliofilm workers. Thus, adjusting for metabolic saturation may be an irrelevant issue. Furthermore, since the exact mechanism(s) of leukemogenesis in benzene-exposed workers is not known, an adjustment to the exposure metric is not possible at this time.

Also, almost all of the 9 leukemia victims in the Pliofilm cohort received long intervals of exposure and 7 experienced latent periods of 15 years or longer. One subject succumbed to AML in 1954 after only 3-1/2 years of exposure. The shortest latent period is that of a Pliofilm worker with CML who died in 1950 after 2 years' exposure. The next earliest death from leukemia was in 1957 after a latent period of 15 years. There was little evidence of "recent" exposure being responsible for the leukemia deaths seen in the Rinsky cohort. Suppression of the hematopoietic system from exposure to benzene, which was suggested by the rising blood count data in the early years (1940-1946) of the Pliofilm cohort, appears not to have facilitated the diagnosis of leukemia at an early stage in such workers, based upon latent factors. Furthermore, after 1946 some measurements were available that made it possible to calculate rough estimates of personal cumulative exposure for each member of the cohort. These estimates tended to be similar among different investigators. However, Rinsky et al. (1981, 1987), Crump and Allen (1984), and Paustenbach et al. (1992, 1993) employed various assumptions to estimate personal exposure levels before 1950, when exposures were most intense. The estimates of exposure made by Rinsky et al. (1981, 1987) were generally the lowest of the three sets, thus giving rise to the highest cancer unit risk estimates.

Paustenbach et al. (1992, 1993) used a variety of assumptions to derive the highest estimates of personal exposure of any of the investigators. They cited seven factors that influenced their estimates: (1) inaccuracy of devices used for monitoring airborne concentrations of benzene; (2) length of the work week; (3) rubber shortages during World War II; (4) installa-

tion of local exhaust systems to reduce airborne concentrations of benzene; (5) additional exposure to benzene by skin contact; (6) ineffectiveness of respiratory devices; and (7) medical evidence of overexposure of workers to benzene. The authors concluded that these pliofilm workers were exposed to the highest levels during the early years of exposure.

These estimates, however, were severely criticized by Utterback and Rinsky (1995), who observed that the Paustenbach et al. (1992) exposure estimates were based upon worst-case assumptions concerning actual exposure levels that may have existed during the earlier years of the rubber hydrochloride (RH) cohort. Utterback and Rinsky (1995) claimed that Paustenbach et al. used selected information, improperly cited, to inflate estimates of exposure and produce risk estimates that were off by an order of magnitude. Perhaps one of the more important examples of this was the conclusion of Paustenbach et al. (1992) that detector tube readings underestimated benzene concentrations in RH plants. They cited the "validity studies" of Hay (1961) to support this position. To compensate for this supposed inaccuracy in exposure estimates made with the instrument, Paustenbach et al. apparently inflated the readings by about 50% and used these inflated estimates as the basis for benzene exposure received by members of the RH cohort. However, Utterback and Rinsky (1995) pointed out that Hay (1961) indicated in his paper that there were sufficient problems in determining the accuracy of these detector tube kits that the conclusions presented in that paper (Hay, 1961) might not be correct. Hay (1961) additionally stated that "the results of this study show all of these (detector tube) kits to be unsatisfactory in terms of the quantitative results obtained. Their value seems to be limited to semiquantitative indications of benzene concentrations in environments to which the tubes have been calibrated." Utterback and Rinsky (1995) further noted that prolonged exposure to the elevated levels of benzene postulated by Paustenbach et al., 100-200 ppm, for as long as a decade would have produced an "epidemic of serious benzene poisoning" that would have resulted in much sickness and many more deaths than actually seen in the RH cohort.

Rinsky et al. (1981, 1987), on the other hand, after analyzing data from various sources (Industrial Commission of Ohio in 1946 and 1955, Ohio Department of Health in 1956, the University of North Carolina in 1974, NIOSH in 1976, and company surveys from 1946 to 1950 and 1963 to 1976), assumed that the levels of benzene, as measured by the 8-h time-weighted average (TWA) exposure of the workers, were close to the recommended standards for specific years as follows: 100 ppm (1941), 50 ppm 8-h TWA (1947), 35 ppm 8-h TWA (1948), 25 ppm 8-h TWA (1957 and 1963), and 10 ppm TWA (1969). This analysis produced the lowest set of estimates.

Crump and Allen (1984) developed a third set of exposure estimates based on the concept that benzene levels declined progressively as more restrictive standards were implemented in the workplace. Their estimates lie somewhere between those of Rinsky et al. (1981, 1987) and of

Paustenbach et al. (1993). These estimates were used in deriving the quantitative unit risk estimates in EPA's Interim Quantitative Cancer Unit Risk Estimates Due to Inhalation of Benzene (U.S. EPA, 1985). Even with the differences in the these three sets of estimates of exposure for employees, the cumulative standardized mortality ratios (SMRs) differed from the Crump and Allen estimates by no more than a factor of 2 (Table 2).

During the period 1940 through 1975, Kipen et al. (1988) studied over 17,000 peripheral blood counts that were collected from 459 benzene-exposed Pliofilm workers from the same company studied by Rinsky et al. (1987). The most important observation from this study (as evidenced by WBCs and RBCs) was that the mean blood counts were somewhat lower in the early 1940s and gradually rose until around 1948, when mean levels attained the levels of the most recent measurements. The authors attributed this phenomenon to a reduction in the ambient airborne exposure levels of benzene received in the workplace. These authors assumed that the mean estimated exposure to workers in the plants from 1940 to 1948 was 75 ppm, based upon the Crump and Allen estimates of exposure (Crump and Allen, 1984). During the period from 1940 to 1948, Crump and Allen assumed the estimated exposure was declining from a mean of 137 ppm in 1940 to a mean of 32 ppm in 1948. From 1948 to 1975, the average estimated mean exposure was 15 to 20 ppm.

This assumed reduction in estimated mean levels of benzene in the air, from a high in 1940 to a low in 1948, based upon the educated but entirely subjective guesswork of Crump and Allen, was paired with the mean blood count data from 1940 to 1948 by year. Significant inverse correlations of the estimated exposure levels of benzene with WBC and RBC measurements were produced over that period. This calculation tends to support the theory that elevated levels of exposure to benzene may indeed suppress the body's ability to produce red and white blood cells if the increase is not temporal or if it is not due to changes or improvements in diagnostic procedures.

Table 2. Standardized mortality ratios for deaths from leukemia among Pliofilm workers based on the estimated cumulative exposure of the selected investigators

Investigators	0-5 ppm-yrs	5-50 ppm-yrs	50-500 ppm-yrs	>500 ppm-yrs
Rinsky et al., 1981, 1987	2.0	2.3	6.9	20
Crump and Allen, 1984	0.9	3.2	4.9	10.3
Paustenbach et al., 1993	1.3	1.8	2.8	11.9

Source: Adapted from Paxton, 1996.

However, a significantly negative correlation does not necessarily support any assumptions about the actual levels in those early days. This point is made clearly by Kipen et al. (1989). Their purely mathematical calculation provides no information on what the early levels actually were. A similar association could also be achieved if one assumes lower levels of benzene in 1940, as was done by Rinsky et al. (1981, 1987), that fall to even lower levels by 1948 (although still above the levels seen after 1948). These calculations suggest only that, at some elevated level of exposure to benzene, the body's ability to produce red and white blood cells is compromised.

In a later study of the same blood count data, Ward et al. (1996) came to a similar conclusion through the use of the Rinsky et al. (1987) exposure estimates, which they also assumed to be elevated during the early years of exposure, although not by as much as the Crump and Allen (1984) exposure estimates. Both groups of researchers agree that elevations in exposure to benzene tend to suppress blood counts. They do not, however, agree on the exact level at which this happens. Both find support for their choice of estimates of exposure during the period 1940 to 1948. Differences in these estimates result mainly from the choice of methodology utilized. As a result, the blood count data cannot be used to determine which set of exposure estimates is more appropriate in determining the unit risk.

With the proportional hazards dose-response model, as used by Paxton et al. (1992) and Paxton (1996), the estimated relative risks differed by no more than a factor of 4 from the Crump and Allen (1984) estimates within each cumulative dose-response category (Table 3). Hence, the use of Rinsky et al. (1981, 1987) or Paustenbach et al. (1993) exposure estimates would have little effect on the quantitative risk estimate.

More recently, Schnatter et al. (1996b) provided a new exposure analysis of the Pliofilm cohort that used the median of the sets of exposure estimates described above to develop a new set of indices of exposure per person. This technique differed from the standard method of

Table 3. Estimated relative risks of leukemia derived by the proportional hazards dose-response model according to the estimated cumulative exposure (ppm-years) of the selected investigators

Investigators	4.5 ppm-yrs	45 ppm-yrs	90 ppm-yrs	450 ppm-yrs
Rinsky et al., 1981, 1987	1.02	1.19	1.41	5.5
Crump and Allen, 1984	1.00	1.04	1.07	1.43
Paustenbach et al., 1993	1.01	1.07	1.14	1.96

Source: Adapted from Paxton et al., 1992.

measuring total exposure to benzene (i.e., cumulative exposure = length of exposure × concentration) in that an "average" total concentration per person was determined from the job category with the greatest exposure (maximally) and of longest duration. This method made it possible to isolate subgroups with less exposure to specified concentrations of benzene and then to calculate the risk of leukemia in those subgroups. In theory, these subgroups were unlikely to be exposed to concentrations greater than a specified concentration.

The results of the Schnatter et al. (1996b) analysis indicated that for the lowest exposure estimates (Rinsky et al., 1981, 1987), the "critical" concentration is between 20 and 25 ppm for the risk of acute myelogenous leukemia (AML) "to be expressed," and for the median, the risk is between 50 and 60 ppm, although there appeared to be little power to detect a significant effect in these risk estimates. Interestingly, for total leukemia, the "critical" concentrations for the median were lower and appeared to fall in the range of 35 to 40 ppm, and the risk estimates appeared somewhat less erratic.

These risk estimates are not inconsistent with estimates of Wong (1995), who utilized cumulative exposure to estimate the risk of AML in Pliofilm workers. The Schnatter et al. (1996) analysis suffers from the same problems as the Wong (1995) and the Rinsky et al. (1987) studies of Pliofilm workers: the data lack sensitivity to detect the effects of benzene exposure at low levels. To assume from this data set that a critical concentration exists at the levels indicated and from which a "threshold" could be inferred is unwarranted. In fact, the lower estimates of the critical concentration based on the sum total of leukemia deaths versus just those deaths from AML seem to suggest that there might be a lower critical region for AML, if a larger data set were available.

The National Cancer Institute, in cooperation with the Chinese Academy of Preventive Medicine, has been conducting a comprehensive study of 74,828 benzene-exposed workers employed from 1972 to 1987 in 672 factories in 12 cities of China (Dosemeci et al., 1994; Hayes et al., 1996, 1997; Yin et al., 1987, 1989, 1994, 1996). A comparison group of workers consisting of 35,805 employees was assembled from non-benzene-exposed units of 69 of the above factories and 40 factories elsewhere. A variety of job categories were studied in the painting, printing, footwear, rubber, and chemical industries. Workers in both groups were followed for an average of slightly less than 12 years. Less than 0.3% were lost to a follow-up in both the exposed and the unexposed group. Work histories were utilized to link benzene-exposure data to individual time-specific estimates for each worker (Dosemeci et al., 1994).

This study, one of the largest of its type ever undertaken, enabled its authors to claim detection of significantly elevated risks at extraordinarily low levels of exposure. Their findings suggested that workers exposed to benzene at average levels of less than 10 ppm are subject to a higher risk of hematologic neoplasms (RR = 2.2, 95% C.I .= 1.1-4.2). A combination of ANLL

and MDS produced a relative risk of 3.2 (95% C. I. = 1.0-10.1). For exposure to a sustained concentration of 25 ppm benzene, the risk of ANLL and MDS increased to 7.1 (95% C.I. = 2.1-23.7). These risks were associated with more recent exposure to benzene (less than 10 years). The risk of other leukemias (other than ANLL), including chronic myeloid and monocytic leukemia, was also elevated (RR = 2.0), although not significantly so. Additionally, the risk of non-Hodgkin's lymphoma was significantly elevated (RR = 4.2 with 95% C.I. = 1.1-15.9) for those with a sustained exposure to benzene that occurred 10 years prior to diagnosis. The authors concluded that benzene exposure "is associated with a spectrum of hematologic neoplasms and related disorders in humans and that risks for these conditions are elevated at average benzene-exposure levels of less than 10 ppm."

Although most of the attributes considered important in a long-term retrospective study of this nature and magnitude were addressed by the authors, some uncertainties and potential weaknesses still remain. The derivation of the cohort from many different factories across China suggested the possibility that this cohort was exposed to mixtures of many different chemicals. Exposure to other carcinogens in the workplace could have produced confounding effects, especially if exposures were to chemicals that increase the risk of leukemia. Although not specifically stated, concurrent exposures to many other chemicals, some hazardous, must have occurred because benzene was used as a solvent for paints, varnishes, glues, coatings, and other products (Dosemeci et al., 1994) that were part of the occupational environment for this cohort. These products contain a myriad of chemicals, of which some undoubtedly were carcinogens.

The authors claim that since there was little movement of the workforce from one job to another (i.e., 1.4 jobs held during an average work history), little confounding could have taken place. In the absence of information on exposure to other chemicals, the conclusions of the authors are questionable. It would seem nonmobility among the workforce would ensure that longer exposure to a few confounders could occur, rather than shorter exposures to many confounders. To reduce the uncertainties of confounding under these circumstances, it would be necessary to eliminate from the cohort all jobs where exposure to other hazardous substances occurred. This would be helpful in determining more precisely the impact on the risk from any one confounder, since multiple confounders would not then be present.

The second potential problem was the development of exposure estimates for the 74,828 benzene-exposed workers and the 35,805 unexposed workers. According to the authors (Dosemeci et al., 1994), only 38% of the 18,435 exposure estimates were based upon actual measurements of benzene concentrations; the remainder were numbers generated by factory industrial hygienists based upon their estimates of benzene concentrations. During the earliest period, only 3% of the exposure estimates were based on actual measurements. Accuracy and precision of these subjective estimates was unknown. As a consequence, derivations of dose per

individual may have been subject to random error and to bias. Such bias, if present, might have contributed to the supralinearity at higher benzene concentrations evident in the results of this study.

It is clear that this study contains thought-provoking new findings and conclusions. These include derivation of stable estimates of risk at lower exposure levels owing to the massive size of the cohort, the suggestion that non-Hodgkin's lymphoma may be a consequence of exposure to benzene, the possibility that elevated risk of non-AML (which presumably includes CML) also occurs as a consequence of exposure to benzene, and the finding of some evidence for supralinearity in the mode of action, since the risk ratios based on these data are significantly elevated at 10 ppm and tend to plateau as the dose increases to somewhat higher levels. It is, however, premature to assume that the Chinese data (Hayes et al., 1997) should now replace Rinsky's Pliofilm workers cohort in the derivation of risk estimates. Further work must be accomplished to identify the other potentially hazardous chemicals in addition to benzene to which the Chinese workers were exposed. In addition, it is important to analyze lifestyle and socioeconomic factors impacting this cohort and to determine how they differ from those of workers in similar occupational settings in Western countries.

To answer the question posed earlier (p. 6), the net result of discarding data from the Ott et al. (1978) and the Wong et al. (1983) studies would have little effect on the unit risk estimate. The Ott et al. (1978) cohort and its later update (Bond et al., 1986a) rely on a smaller data set. Both the Ott study and its update by Bond have insufficient power to detect a risk of leukemia at low doses. Furthermore, Bond et al. (1986a) also stated that their data for risk assessment purposes should not be used for determining unit risk estimates, for several reasons (i.e., small number of events, competing exposures to other potentially hazardous materials, and the uncertain contribution of unquantified brief exposures).

Even though the unpublished Wong et al. (1983) cohort has ample power to detect a risk of leukemia, and the published version (Wong, 1987) includes estimates of personal exposure to benzene, the estimates apparently were not reliable. Wong (1987) stated that the estimated historical industrial hygiene data were not precise enough for absolute quantitative risk assessment. The Rinsky et al. (1981, 1987) cohort has ample power, latency, and better estimates of later exposure to airborne benzene. However, during certain time frames (i.e., levels of ambient air benzene before 1950), the actual airborne measurements of benzene in the workplace were either meager or nonexistent.

In the 1985 interim benzene document (U.S. EPA, 1985), a single overall unit risk estimate was calculated as the geometric mean of four maximum likelihood unit risk estimates generated from the Ott et al. (1978) and Rinsky et al. (1981, 1987) studies using both absolute and relative risk models, and then "correcting" this mean by multiplying it by the ratio of the

largest unit risk estimate from the four separate unit risk numbers above to the unit risk estimate calculated from the Wong (1987) cohort. The result was a probability of 2.6×10^{-2}, which is close to that calculated by Crump (1992) assuming a similar approach, that is, a linear model and Crump and Allen (1984) exposure estimates but excluding Ott at al. (1978), Bond et al. (1986a), and Wong (1987). These risk estimates range from 1.1×10^{-2} to 2.5×10^{-2} and can be found in Section 3.1 (Table 4). By inspection, the inclusion of data from Ott et al. (1978), Bond et al. (1986a), or Wong (1987) changes these unit risk estimates little.

It is apparent that the calculation of a new unit risk estimate based on a reordering of the assumptions about what the earlier distribution of ambient air measurements of benzene might have been, or from the elimination of data sets that add little to the knowledge of risk at low doses, has questionable validity. Such a recalculation likely would result in little change from EPA's 1985 interim quantitative unit risk estimate, based on the current epidemiologic data and associated uncertainties.

2.2. LABORATORY ANIMAL DATA

Studies on the carcinogenicity of benzene in rodents include inhalation exposures to Sprague-Dawley rats, C57BL/6 mice, AKR mice, CD-1 mice, and CBA mice, and gavage treatment of Sprague-Dawley rats, Wistar rats, F344 rats, RF/J mice, Swiss mice, and $B6C3F_1$ mice (Cronkite et al., 1989; Goldstein et al., 1982; Huff et al., 1989; Maltoni et al., 1983, 1988; NTP, 1986; Snyder et al., 1980, 1982, 1984; Farris et al., 1993). Inhalation concentrations ranged from 0 to 1,000 ppm and gavage doses ranged from 0 to 200 mg/kg. Upon exposure via inhalation, benzene was found to be carcinogenic in rats and mice in multiple target organs, including oral and nasal cavities, liver, forestomach, preputial gland, lung, ovary, and mammary gland. It is noted that in humans the cancer induced by benzene exposure is predominantly acute nonlymphocytic leukemia, while in rodents lymphocytic leukemia was observed in two series of experiments in C57BL/6 mice (Snyder et al., 1980) and CBA/Ca mice (Cronkite et al., 1989).

Although the reason for the difference in lineage of hematopoietic cancers induced in mice and humans is not fully understood, it may be related to differences in hematopoiesis. Lymphocytes make up a larger portion of the nucleated cells in mouse bone marrow than in human bone marrow (Parmley, 1988) and could simply represent a larger target cell population for benzene metabolites. The target organs for benzene carcinogenicity in rodents are rich in enzymes that may confer tissue sensitivity to benzene, as is human bone marrow (Low et al., 1989, 1995). The bone marrow, Zymbal gland, and Harderian gland all contain peroxidases, which can activate phenols to toxic quinones and free radicals. Sulfatases, which remove conjugated sulfate and thus reform free phenols, are also present at high levels in these target organs. The selective distribution of these two types of enzymes in the body may explain the

accumulation of free phenol, hydroquinone, and catechol in the bone marrow and the target organ toxicity of benzene in humans and animals. Therefore, the animal bioassay results may have some relevance to human leukemia, but it should be emphasized that there is no demonstrated and reproducible animal model for leukemia resulting from benzene exposure. Thus, the mechanism of leukemia development following benzene exposure is not well understood.

2.3. MODE-OF-ACTION INFORMATION

The mechanisms by which exposure to benzene and its metabolites exerts its toxic and carcinogenic effects remain elusive. Animal studies showed that benzene itself was unlikely to be the actual toxicant but rather required metabolism to exert its toxic effects (Andrew et al., 1997; Gad-El Karim et al., 1986; Sammett et al., 1979; Sawahata et al., 1985). The central issues in integrating the mechanistic data from the laboratory animal experiments with the occupational epidemiologic data to estimate risk are establishing whether the mechanisms in laboratory animals are similar to mechanisms in humans and accounting for the dose dependency of those mechanisms. That is, understanding the mode of action permits rational extrapolation across species and from high to low doses. Characterization of dosimetry, i.e., description of the uptake, internal disposition, and translation of an exposure concentration to the effective dose at the target site, is necessary. Processes such as altered gene regulation, cytotoxicity, and cell proliferation are thought to be important for benzene leukemogenesis.

2.3.1. Metabolism

The primary pathways involved in benzene metabolism are shown in Figure 1; the majority of these metabolites were isolated from in vivo studies. Phase II conjugation pathways (not shown in Figure 1) are also extremely important in benzene metabolism and toxicity: extensive glucuronidation and sulfation of phenols were reported in the original studies of Parke and Williams (1953) and have been demonstrated in human systems (Seaton et al., 1995). Because many reactive metabolites may be formed during benzene metabolism, it is difficult to elucidate the pathway responsible for benzene toxicity. Indeed, it is certainly a possibility that more than one metabolic pathway is responsible and that the mechanisms underlying benzene toxicity may be multifactorial.

Two pathways have been commonly postulated as being responsible for benzene toxicity. The first involves the major hepatic metabolites of benzene (phenol, catechol, and hydroquinone), whereas the second involves ring-opened forms of benzene (Figure 1). Cytochrome P450 2E1 has been shown to metabolize benzene to phenol (Johansson and Ingleman-Sundberg, 1988). The majority of data suggest that the hepatic conversion of benzene to phenolic metabolites is an important primary event in benzene toxicity (Smith et al., 1989).

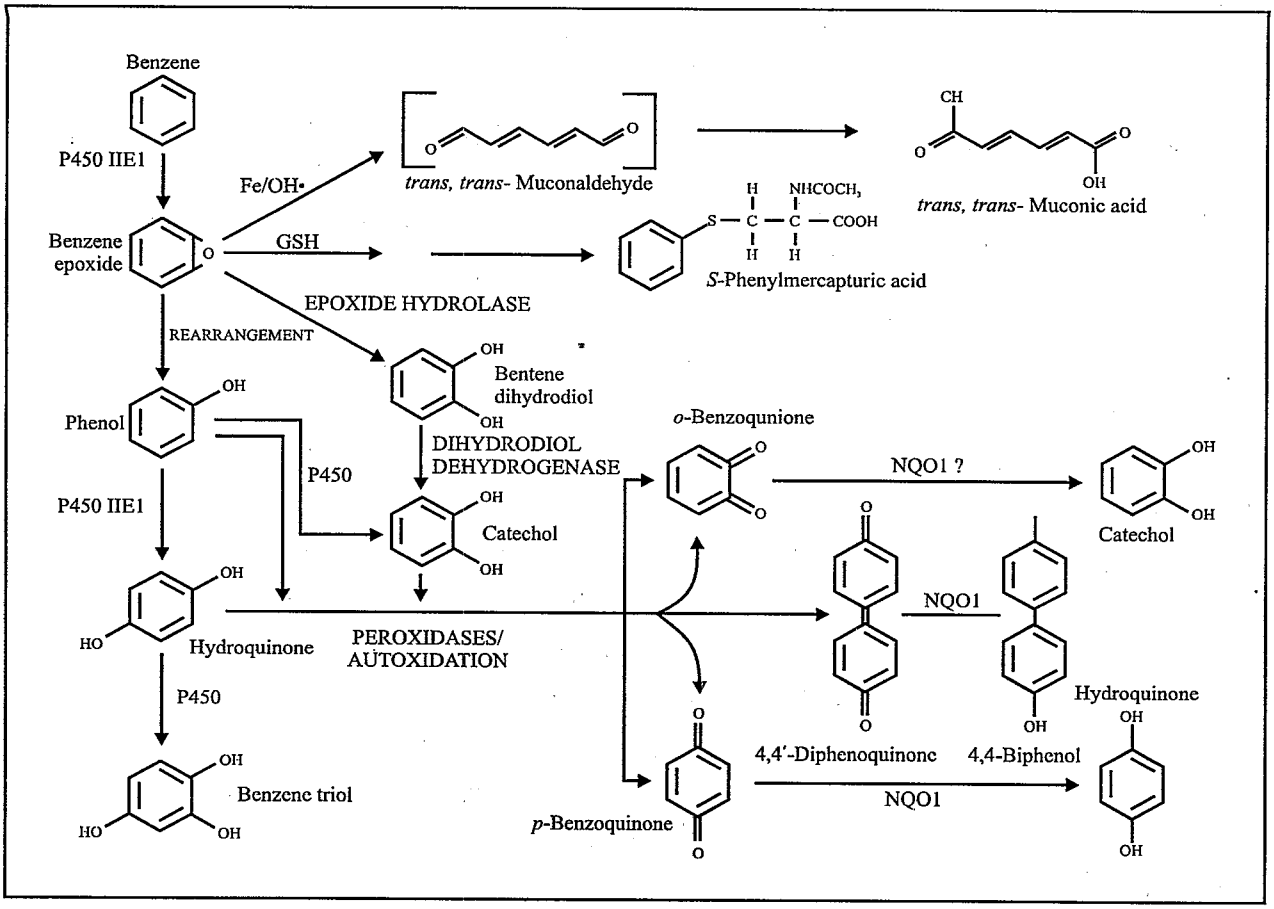

Figure 1. Major metabolic pathways in benzene toxicity.

Source: Ross, 1996.

Catechol and hydroquinone have been shown to persist in bone marrow after benzene exposure (Rickert et al., 1979). The bone marrow is rich in peroxidase activity (Bainton et al., 1971), and phenolic metabolites of benzene can be activated by peroxidases to reactive quinone derivatives (Subrahmanyam et al., 1991). Peroxidase activation of hydroquinone is known to result in covalent binding to protein (Subrahmanyam et al., 1989; Ganousis et al., 1992) and the formation of DNA adducts, as detected by ^{32}P-post-labeling (Lévay et al., 1993), in both murine and human bone marrow in vitro. Peroxidases reported to be present in bone marrow include myeloperoxidase (MPO) (Bainton et al., 1971), prostaglandin synthase (Gaido and Weirda, 1987), and eosinophil peroxidase; of these, MPO is known to be present in bone marrow in high concentrations, and such a peroxidase can readily bioactivate benzene-derived phenolics to reactive quinones (Figure 1) in situ in bone marrow (Bhat et al., 1988).

The major problem with the phenolic hypothesis, however, is that phenol does not reproduce the myelotoxicity associated with benzene (Tunek et al., 1981). Kenyon and Medinsky (1995), have suggested the inability of phenol to induce myelotoxicity may be related to its preferential conjugation in the periportal region of the liver, whereas benzene is metabolized to phenol and further to hydroquinone in the pericentral region. Preferential removal of phenol by conjugation in the periportal region of the liver could explain why more hydroquinone is produced from benzene than from phenol. An interaction of phenol and hydroquinone has been reported to reproduce the myelotoxicity of benzene (Eastmond et al., 1987), and increased covalent binding of ^{14}C-hydroquinone has been observed in bone marrow when a combination of both compounds was administered to mice (Subrahmanyam et al., 1990). Catechol also has been reported to markedly stimulate peroxidatic bioactivation of hydroquinone in murine stroma (Ganousis et al., 1992), and a synergistic genotoxic effect of catechol and hydroquinone has been observed in human lymphocytes (Robertson et al., 1991). These data support the hypothesis that an interaction of phenolic metabolites may induce or at least contribute to benzene toxicity.

The second metabolic mechanism commonly proposed to explain benzene toxicity involves ring opening of benzene to reactive muconate derivatives. When benzene is given in vivo, trans, trans-muconic acid can be detected as a urinary metabolite. A precursor of trans, trans-muconic acid (trans, trans-muconaldehyde [MA]), has been suggested to be the ultimate toxic species obtained during benzene metabolism (Latriano et al., 1986). The major problem with this hypothesis is that MA has never been demonstrated as an in vivo metabolite of benzene, although formation of MA has been reported from benzene in mouse liver microsomes. The reactivity and rapid further metabolism of MA may preclude its isolation in vivo. The proposed mechanism of formation of MA has been suggested to involve an iron-catalyzed ring opening of

the benzene epoxide (Figure 1) and appears not to be derived from the dihydrodiol (Zhang et al., 1993b).

The metabolism of benzene was more rapid and extensive in the mouse than in the rat, and this observation appears to correlate with the greater sensitivity of the mouse to benzene's myelotoxic and genotoxic effects (Henderson et al., 1992; Sabourin et al., 1988). Irons and co-investigators showed that very little metabolism of benzene occurs in the bone marrow (Irons et al., 1980; Sawhata et al., 1985). The combination of metabolism in the liver and toxicity in the bone marrow suggests that one or more relatively stable metabolites formed in the liver is transported to the bone marrow, where it exerts its toxic effects.

Although much less is known about the metabolism of benzene in humans than in laboratory animals, existing studies indicate that both metabolize benzene along similar pathways (Cooper and Snyder, 1988; Inoue et al., 1988a,b; IPCS, 1993; Sabourin et al., 1989). The rates of some reactions, however, may differ (Henderson et al., 1989; IPCS, 1993; Sabourin et al., 1989). A recent series of investigations on Chinese workers highly exposed to benzene provides insights into particular enzymes underlying the hematotoxicity of benzene in humans. Overall, individuals with the rapid hydroxylator phenotype exhibited a 2.5-fold increased risk of benzene poisoning as compared to those with the slow hydroxylator phenotype (Rothman et al., 1995). In the Chinese worker study, DNA was collected to investigate the potential role of genetic polymorphisms affecting NAD(P)H:P quinone oxidoreductase (NQO1) in the susceptibility of workers to benzene (Rothman et al., 1996a). NQO1 (also known as DT-diaphorase) is an enzyme that catalyzes a two-electron reduction of quinones to hydroquinones and, on the basis of several in vitro and animal experiments, appears to provide protection against quinones formed during benzene metabolism (Ross et al., 1990; Smart and Zannoni, 1984; Zhu et al., 1995). A point mutation in the NQO1 gene results in a loss of enzyme activity in homozygous individuals. Analyses performed on 38 workers and 35 controls showed that individuals homozygous for this mutation had a 3.2-fold increased risk of myelotoxicity. These results, which were consistent with the results of previous animal and in vitro studies, identified groups of individuals with increased susceptibility to benzene's hematopoietic effects (Seaton et al., 1994; Smart and Zannoni, 1984). Recent biochemical studies also suggest a role for NQO1 in the greater sensitivity of mice than rats to the myelotoxic effects of benzene (Zhu et al., 1995).

Recent biochemical studies also suggested a role for NQO1 in the greater sensitivity of mice than rats to the myelotoxic effects of benzene (Zhu et al., 1995). Metabolic activation by P450 2E1 and detoxification of benzene-derived quinones appeared to be important steps in benzene hematotoxicity. Levels of P450 2E1, which are known to vary substantially between individuals and between ethnic groups and may partially explain the differences in response seen in different studies (Stephens et al., 1994). Recent studies showed that allelic differences at the

CYP2E1 and NQO1 loci were associated with susceptibility to benzene hematotoxicity (Rothman et al., 1997). The role of hematotoxicity in benzene-induced leukemia, however, still remains unclear. Furthermore, P450 2E1 is involved in the metabolism of ethanol and is readily inducible, indicating that alcohol consumption may affect a person's susceptibility to benzene poisoning (Koop et al., 1989; Stephens et al., 1994).

There has been a considerable amount of progress in understanding and quantifying the factors that contribute to the distribution and metabolism of benzene and its metabolites in experimental animal species (Schlosser et al., 1993, 1995; Medinsky et al., 1994; Low et al., 1995). The quantity of benzene metabolites produced in different species result from the subtle interplay of oxidation and conjugation pathways and the distribution of enzyme systems in the liver and other organs as well as relative rates of perfusion in different organs and different species. These differences have been explored using a physiologically based pharmacokinetic model (Schlosser et al., 1995; Medinsky et al., 1996), but their application in predicting metabolism and dosimetry in humans remains a subject of considerable debate. Recent studies using genetically engineered animals (transgenic CYP2E1 knockout mice) indicate that CYP2E1 is the primary isozyme responsible for benzene metabolism in vivo and that metabolic activation of benzene is required for the development of both cytotoxicity and genotoxicity following benzene exposure. These observations are important because humans vary in their expression of CYP2E1 activity, as well as in their ability to metabolically activate benzene (Lee et al., 1996; Valentine et al., 1996). Although, there is a scientific consensus that metabolism of benzene is required for resultant toxicity and carcinogenic response, the role of a metabolite or metabolites of benzene responsible for these adverse effects is controversial and more research data is needed to better define sequelae of pathogenesis following exposure to benzene and its metabolites.

In summary, it is generally agreed that benzene toxicity in both experimental animals and humans results from the biotransformation of the parent compound to reactive species. Furthermore, current evidence indicates that benzene-induced myelotoxicity and genotoxicity results from a synergistic combination of phenol with hydroquinone, muconaldehyde, or catechol. The pathways for benzene oxidative metabolism are generally understood and involve the cytochrome P-450 family of enzymes (CYP2E1). Phenol, hydroquinone, catechol, and trans, trans-muconic acid are the major metabolites produced in experimental animals and in humans (in vitro and in vivo). The present studies further demonstrate that benzene oxidative metabolism correlates with observed genotoxicity and cytotoxocity in bone marrow, blood, and lymphoid tissues after benzene exposure. Recent studies also demonstrate the importance and magnitude of CYP2E1 in benzene metabolism and toxicity and that CYP2E1 expression may play a significant role in human variability, genetic polymorphism, and resultant differential risk from benzene exposure.

2.3.2. Mutagenicity and Genotoxicity

The literature on the genotoxic effects of benzene is extensive, with more than 220 publications with original data. Reviews of the earlier literature (Dean, 1978, 1985) present clear evidence that benzene exposure results in chromosome aberrations in a variety of in vitro and in vivo assays and in persons occupationally exposed to benzene over long periods of time. Benzene generally has yielded negative results in gene mutation assays in bacteria or in vitro mammalian cell systems (Ashby et al., 1985; Oberly et al., 1984, 1990). However, Ward et al. (1992) reported dose-related increases in mutations at the *hprt* locus in lymphocytes of CD-1 mice exposed to benzene (40, 100, and 1,000 ppb) by inhalation for 6 weeks (22 h/day, 7 days/week). Also, Mullin et al. (1995) detected increased mutant frequencies in the *lacI* transgene from lung and spleen but not liver from C57BL/6 mice exposed to 300 ppm benzene for 6 h/day, 5 days/week for 12 weeks.

Benzene exposure results in a variety of both structural and numerical chromosome damage (see cited reviews). Experiments in rodents have provided consistent evidence from a number of studies that benzene exposure causes increased frequency of micronucleated cells (summarized in ATSDR, 1997). Micronuclei also are seen in human cells exposed in vitro to various metabolites and combinations of metabolites (Zhang et al., 1993a; Eastmond, 1993; Yager et al., 1990; Hogstedt et al., 1991; Robertson et al., 1991). Synergistic increases in micronuclei were induced by catechol and hydroquinone, but not catechol and phenol or phenol and hydroquinone (Robertson et al., 1991). However, in mice treated intraperitoneally with binary or tertiary mixtures of these three metabolites, synergistic effects resulted only from mixtures of phenol and hydroquinone (Marrazzini et al., 1994); adding catechol to the mixture was no more effective than hydroquinone alone in inducing micronuclei. Chen and Eastmond (1995a) corroborated the phenol and hydroquinone synergy. Using an antikinetichore-specific antibody and fluorescent in situ hybridization (FISH), they demonstrated that both chromosome breakage and loss were induced and that the relative frequency of these events was indistinguishable whether mice were treated with benzene (440 mg/kg) or the binary mixture of hydroquinone and phenol (60/160 mg/kg).

The evidence that human exposure to benzene produces the types of chromosomal rearrangements associated with AML and MDS, such as interstitial deletions, inversions, or translocations, continues to accumulate. Earlier studies of patients with benzene-induced hematopoietic disorders demonstrated increased chromosome aberrations in lymphocytes and bone marrow cells (Dean, 1985). The rearrangements observed included stable and unstable aberrations (Aksoy, 1989; Forni, 1971, 1994; Sarto et al., 1984; Sasiadek, 1992; Van den Berghe et al., 1979). Tompa et al. (1994) performed cytogenetic analyses on groups of workers occupationally exposed to benzene. Improved working conditions over a 3-year period resulted

in reducing average peak concentrations from 68.7 mg/m^3 in 1990 to 27.1 mg/m^3 in 1991 and 18.4 mg/m^3 in 1992. Striking decreases in chromosome aberrations were seen by 1992 in those workers with less than 10 years' exposure. Workers exposed for more than 10 years showed lesser reductions. Recently, Rothman et al. (1995) used the glycophorin A (GPA) gene mutation assay to examine the type of mutations produced by benzene exposure in human bone marrow among 24 workers heavily exposed to benzene and 23 matched controls. The assay detects a spectrum of mutational mechanisms, and the results indicated significant increases in NN-type variants but not in NO-type variants. The NN-type cells are presumed to be consequences of mitotic recombination or gene conversion processes, while the NO-type are derived from point mutations or deletion events.

Aneuploidy, the loss and gain of whole chromosomes, is also found in some cases of myeloid malignancy. Patients with benzene-induced leukemia, rodents, and human cells treated in vitro display increased aneuploidy. Numerical changes in the C-group chromosomes 6-12 and X have been detected in the blood and bone marrow of patients with benzene-induced myelogenous leukemia, myelodysplasic syndrome, and pancytopenia (Vigliani and Forni, 1976). A recent report by Zhang et al. (1996) showed that the induction of aneuploidy of chromosome 9 as measured by FISH in interphase lymphocytes from benzene-exposed workers is significantly elevated only at high levels of exposure (>31 ppm in air). The human evidence for aneuploidy induction also is supported by in vitro experiments. Hydroquinone and 1,2,4-benzenetriol induce aneuploidy of chromosomes 7 and 9 in human cells (Zhang et al., 1994; Eastmond et al., 1994). Eastmond and co-workers also have reported that micronuclei containing centromeres are formed in bone marrow and spleen cells following oral benzene exposure in mice (Chen et al., 1994; Chen and Eastmond, 1995a). Centromere-containing micronuclei are thought to be formed when a whole chromosome is lost during mitosis. Thus, considerable evidence supports the assertion that exposure to benzene produces aneuploidy in a variety of systems.

DNA adducts of phenol, hydroquinone, or benzoquinone have been reported in a number of in vitro systems (Reddy et al., 1990; Lévay et al., 1993; Bodell et al., 1993). Reddy et al. (1990) did not detect DNA adducts in rat bone marrow, Zymbal gland, liver, or spleen after four daily gavage treatments of phenol or a 1:1 mixture of phenol and hydroquinone. Subsequently, the same group (Reddy et al., 1994) did not detect DNA adducts in liver, bone marrow, or mammary glands of mice sacrificed after receiving four daily intraperitoneal (i.p.) injections of 500 mg/kg benzene. Using the same P1-enhanced P^{32}-postlabeling procedure, Pathak et al. (1995) performed a series of in vivo experiments using concentrations ranging from 25 to 880 mg/kg and a variety of injection schedules for periods up to 14 days, as well as in vitro experiments with hydroquinone or 1,2,4-benzenetriol. One major and two minor DNA adducts were detected in the bone marrow of mice receiving i.p. injections of 440 mg/kg of benzene

twice a day for 3 days. No adducts were seen with any treatment regimen involving only a single injection per day, even at 880 mg/kg for 3 days. Co-chromatography indicated that the adducts were identical to those seen after in vitro treatment of bone marrow with hydroquinone. Using the same treatment regimen, the same adducts were detected in white blood cells of mice (Lévay et al., 1996).

The single-cell gel electrophoresis assay (comet assay) is a rapid and simple method for detecting single-strand breaks in DNA induced by oxidative damage as well as topoisomerase II inhibitors (McKelvey-Martin et al., 1993). Plappert et al. (1994) exposed mice to 100, 300, and 900 ppm of benzene 6 h/day, 5 days/week for 4 weeks with samples taken at 3 days and weekly thereafter. DNA damage measured as increased tail moment was seen in liver and bone marrow after 5 days at 100 ppm. Increased damage in peripheral blood was not recorded until 4 weeks at 100 ppm. Maximal damage was seen after 5 days at 300 ppm, with little increased or decreased damage at longer exposures. Damage returned to or approached control levels if animals were allowed 24 or 48 h recovery time after the cessation of benzene exposure. Tuo et al. (1996) treated mice by gavage with benzene at 40, 200, and 450 mg/kg and detected dose-related increases in DNA tail length in both peripheral lymphocytes and bone marrow. Pretreatment with the CYP2E1 inhibitor propylene glycol reduced the damage by almost half at all concentrations. The comet assay was used to detect damage induced by benzene and several metabolites in cultured human lymphocytes (Anderson et al., 1995). Increased tail length was seen after 0.5 h treatment with benzenetriol and catechol, after 1 h with benzene, and after 2 h with muconic acid, hydroquinone, and benzoquinone. Recently, Andreoli et al. (1997) reported increased DNA damage in gasoline station attendants monitored for 1 year with breathing zone air samplings. Using tail moment as the measure of DNA damage, the mean for exposed workers was twice that of controls matched for age and smoking habits. They also exposed peripheral lymphocytes from unexposed donors to hydroquinone, benzenetriol, and benzoquinone, and positive responses were recorded with all three. Hydroquinone-exposed cells stimulated to divide with PHA were more than 10-fold less sensitive than resting cells. Further, the DNA repair inhibitor ara-C dramatically increased the damage in hydroquinone-treated cells.

2.3.3. Epigenetic Effects

While the evidence is unambiguous that chromosome rearrangements are detected in leukemia and are observed following benzene exposure, it is also evident that such effects are only part of the complex process of leukemogenesis. Normal hematopoesis is a complex process in which differentiation and proliferation are coordinately linked, and several investigations have demonstrated that exposure to benzene and several metabolites adversely affects this process. Benzene has long been recognized as a hematotoxicant, causing bone marrow suppression and

aplastic anemia (Goldstein, 1988; Aksoy, 1988; Kipen et al., 1988) In humans, lymphocytes appear to be the peripheral blood cell population most sensitive to toxicity (Aksoy et al., 1971; Moszczynski and Lisiewicz, 1982). A recent investigation of Chinese workers reported a highly significant difference in absolute lymphocyte count between exposed and unexposed workers (Rothman et al., 1996b). Decreased lymphocyte counts correlated with increased levels of urinary benzene metabolites. Lymphocytes are the source of many regulatory cytokines, such as IL-3; depression of marrow lymphocyte populations might disrupt normal production of these cytokines. An abnormal regulatory environment could then contribute to bone marrow suppression or leukemogenesis. In addition to lymphocytopenia, erythrocytic populations are suppressed in cases of chronic benzene exposure. Peripheral granulocytic cells may be suppressed or increased in number. These effects on peripheral populations are, in general, borne out in experiments on rodents, although exposures and exposure rates are difficult to compare to those experienced by humans.

There has been debate about whether hematotoxicity, evidenced as depressed white blood cell counts, is a required precursor to benzene-induced leukemia. There have been reports of benzene-induced leukemia without proof of prior bone marrow suppression (Yin et al., 1989), and myeloid malignancy does not always arise in a setting of clinically detectable bone marrow suppression. However, hematotoxicity is probably involved in a large percentage of cases and probably increases the risk of malignancy. Extensive cell death in progenitor populations, combined with abnormal regulatory signals, could assist an abnormal clone to gain bone marrow dominance. Clonal hematopoiesis is thought to be a step toward MDS and/or AML. In addition, cell death and cytopenia are likely to promote expansion of remaining stem cells, which increases the chance that a cell carrying a genetic abnormality will be recruited into cycle. Irons and co-workers have shown that in clonogenic assays hydroquinone pretreatment increased the number of colonies formed by recombinant GM-CSF-induced CFU-GM at concentrations as low as 10^{-9} molar (Irons et al., 1992). This suggests that hydroquinone can increase recruitment of hematopoietic progenitor cells into the granulocyte-macrophage pathway, or increase the number of resting cells entering the cell cycle. Since myeloid progenitors are thought to be sensitive to genotoxicity through expression of myeloperoxidase, both scenarios could result in increased genetic damage occurring in the bone marrow by increased numbers of MPO-positive cells. Alternatively, increased recruitment could simply increase the probability of recruiting a previously genetically altered cell into cycle. Enhanced response to GM-CSF could occur by a number of different biochemical mechanisms, including alteration of signal transduction pathways. The phenomenon of increased clonogenicity in the presence of GM-CSF is also observed with other chemicals that cause myeloid leukemias, suggesting that it may play an important role in leukemogenesis (Irons and Stillman, 1993).

Kalf and co-workers have reported that benzene toxicity to the bone marrow can be prevented by IL-1 administration (Renz and Kalf, 1991). They postulate that hydroquinone is oxidized to benzoquinone, which then inhibits the conversion of pre-IL-1 to the active form in stromal macrophages (Niculescu et al., 1995). The role of IL-1 in early hematopoiesis is not entirely clear, but this cytokine is able to stimulate fibroblasts and endothelial cells to secrete other factors that, in turn, act on hematopoietic progenitors. Stromal macrophages also produce growth factors that directly regulate myelopoiesis. In long-term culture, developing granulocytes are found clustered near these cells, presumably because they are receiving either instructive or permissive signals necessary for their proper development. Therefore, disruption of IL-1 production and/or other stromal effectors of hematopoiesis could act as a selective pressure for the development of abnormal clones that have acquired the ability to grow under adverse conditions.

2.3.4. Pathogenesis

Lymphohematopoietic neoplasia can be defined as uncontrolled proliferation or expansion of lymphohematopoietic cells that no longer have the capacity to differentiate normally to form mature blood cells. Clones derived from the myeloid lineage are designated as chronic or acute leukemias. Within these general classes, leukemias represent a heterogeneous group of diseases. Heterogeneity is apparent even within the group classified as AML. MDS consist of a group of blood disorders with defects in hematopoietic maturation. They are considered as preleukemic because a significant portion of these progress to frank leukemia (Wright, 1995). Consistent with present models for the origin and progression of neoplasia, development of leukemia is thought to be a multistep process that involves several independent genetic and epigenetic events. Cell survival, differentiation, and proliferation are regulated processes under coordinated control by multiple factors in normal hematopoiesis. Particularly challenging to understanding the pathogenesis is having to discern the role of altered regulation of cell growth as it is superimposed on more normal hematopoiesis and cell population dynamics involving survival, proliferation, and differentiation.

Irons and Stillman (1996) have summarized much of the extensive literature relating to secondary leukemia involving either therapy or occupational exposures. Clonal chromosome aberrations involving more than 30 different abnormalities have been identified in the majority of patients diagnosed with AML (Caligiuri et al., 1997). In secondary leukemias associated with alkylating agent antineoplastic therapy, loss of genetic material from chromosomes 5 and 7 is found in the great majority, whereas leukemias following topoisomerase II inhibitory drugs more frequently involve aberrations involving chromosome band 11q23 (Pedersen-Bjergaard et al., 1995). Several interleukin genes (IL-3, IL-4, IL-5), granulocyte/macrophage-colony-stimulating

factor (GM-CSF), and other regulatory genes are tightly linked on chromosome 5. Irons and Stillman (1996) described a model for benzene-induced leukemia based on the disrupted functions of these genes. Young and Saha (1996) discuss several different translocations, all involving 11q23. The gene at this location has been sequenced and has been designated MLL (mixed-lineage leukemia), and while the normal function of this gene has yet to be determined, it shares homology with the *Drosophila trx* gene that regulates transcription of genes for normal development. Although many leukemias have one chromosomal rearrangement in all cells, cytogenetically unrelated clones are more frequently found in secondary leukemias than in de novo leukemias (Heim, 1996). Despite these complexities, a growing knowledge of the function and role of cytokines, their receptors, protooncogenes, and suppressor genes can provide a useful framework for analysis of the respective roles of altered cell growth and differentiation in leukemogenesis.

2.3.5. Summary

Figure 2 presents a general schematic of leukemia induced by benzene exposure as proposed by Smith (1996) that can be instructive to consider in view of the evidence in previous sections on the mode of action. As discussed in Section 2.3.1, it is generally agreed that the toxicity of inhaled benzene results from its biotransformation to reactive species. Benzene is metabolized in the liver by cytochrome P450 2E1 (CYP2E1) to its major metabolites, phenol, hydroquinone, and catechol. The intermediate benzene oxide can also undergo ring opening to trans, trans-muconic acid (Figure 1). The selective toxicity of any one of these metabolites to blood and bone marrow has been difficult to explain, so these metabolites are now viewed as proximate, with secondary activation to toxic quinones and free radicals as ultimate metabolites postulated to take place by peroxidase enzymes in the bone marrow. The hypothesis is further supported by the fact that the target organs for toxicity in rodents are rich in both peroxidase and sulfatase enzymes, i.e., the selective distribution of these two types of enzymes in the body may explain the accumulation of free phenol, hydroquinone, and catechol in the bone marrow and laboratory animal target tissues. Evidence that multiple metabolites are important in benzene toxicity has also increased in recent years. The possible mechanisms of interaction were discussed in Section 2.3.1.

Molecular targets for the action of these metabolites, whether acting alone or in concert, include tubulin, histone proteins, topoisomerase II, and other DNA-associated proteins. Damage to these proteins would potentially cause DNA strand breakage, mitotic recombination, chromosomal translocations, and malsegregation of chromosomes to produce aneuploidy. Evidence to support aspects of each of these exists to some degree; as described in Section 2.3.2. If these effects took place in stem or early progenitor cells, a leukemic clone with selective

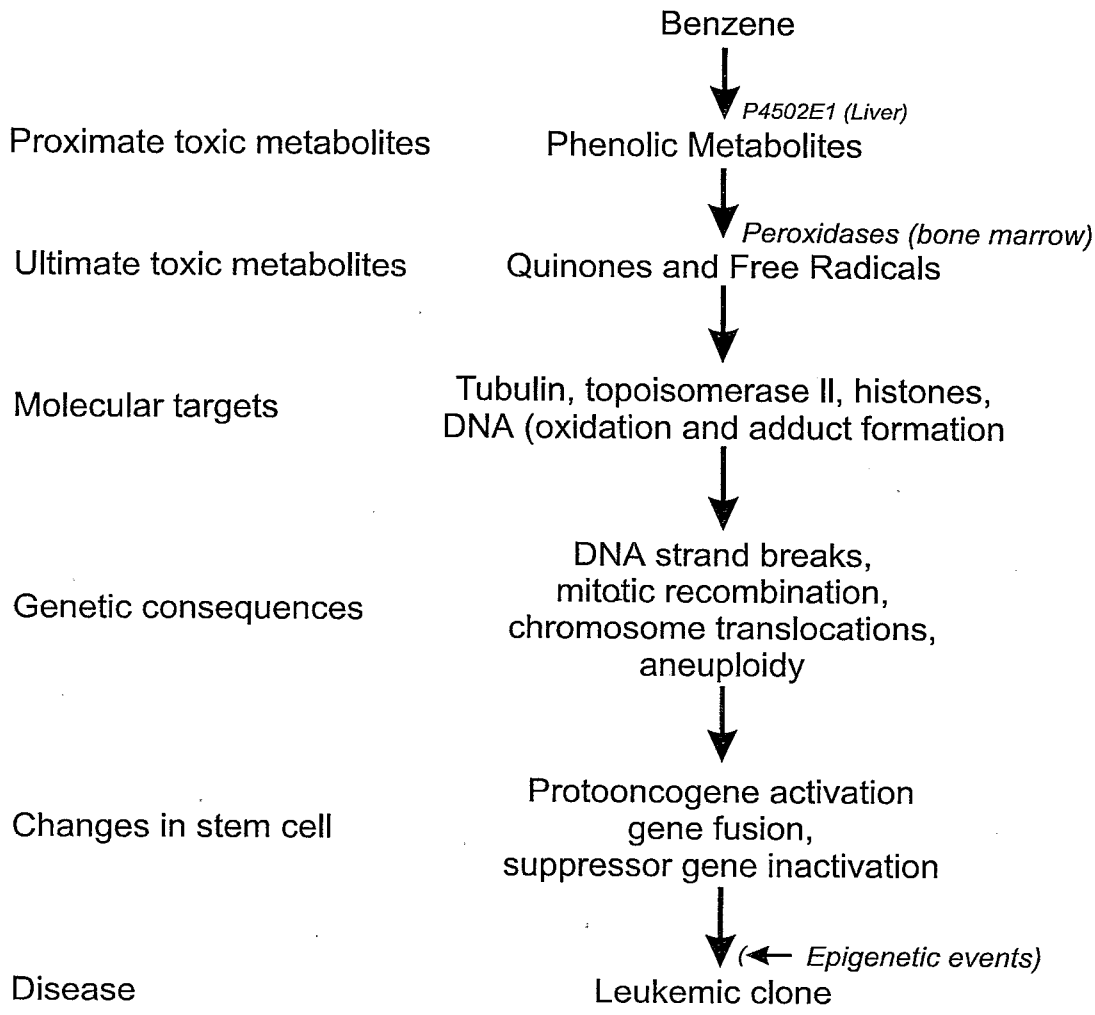

Figure 2. Schematic for mechanistic hypothesis of benzene pathogenesis (leukemognesis).

Source: Smith, 1996.

advantage to grow could arise as a result of protooncogene activation, gene fusion, and suppressor-gene inactivation. Epigenetic effects of benzene metabolites on the bone marrow stroma, and perhaps the stem cells themselves, could then foster development and survival of a leukemic clone. Data supporting a role for epigenetic effects were described in Section 2.3.3.

Within the bone marrow are a number of cell populations that can serve as potential targets, including the hemopoietic and lymphopoietic stem cells, committed progenitors, immature hemopoietic precursors, mature functional blood cells, and the various cells that comprise the bone marrow stromal microenvironment. As depicted in Figure 3, the altered function of different populations could result in the manifestation of different types of toxicities, e.g., immunotoxicity, aplastic anemia, or leukemia. It is already recognized that persistent cytopenias and other blood dyscrasias, including dyserythropoiesis, dysgranulopoiesis, and dysmegakaryopoiesis, frequently precede the onset of leukemia in patients developing AML secondary to exposure to benzene or alkylating agents (Irons and Stillman, 1996). Thus, although the exact mechanisms remain to be further examined and elucidated, as yet unexplored perturbations within these populations may ultimately prove to be mechanistically related and provide a unified framework to comprehensive understanding of benzene toxicity, i.e., both cancer and noncancer endpoints.

2.4. HAZARD CHARACTERIZATION SUMMARY

This document reconfirms that benzene is a known human carcinogen by all routes of exposure (U.S. EPA, 1979, 1985). This finding is supported by evidence from three different areas: human epidemiologic studies, animal data, and improvement in understanding of mechanisms of action, and numerous studies of dermal absorption in humans and animals. Human epidemiologic studies of highly exposed occupational cohorts have demonstrated unequivocally that exposure to benzene can cause acute nonlymphocytic leukemia and other blood disorders, that is, preleukemia and aplastic anemia (Aksoy, 1976, 1977; Aksoy et al., 1974; Infante et al., 1977; Rinsky et al., 1981, 1987; Vigliani and Saita, 1964; Hunting et al., 1995; IARC, 1982; ATSDR, 1997). It is also likely that exposure is associated with a higher risk of chronic lymphocytic leukemia and possibly multiple myeloma (DeCouflé et al., 1983), although the evidence for the latter has diminished with recent studies (Hayes et al., 1996, 1997). In experimental animal species, benzene exposure (both inhalation and oral routes) has been found to cause cancer in multiple target organ sites such as oral and nasal cavities, liver, forestomach, preputial gland, lung, ovary, and mammary gland (Section 2.2). It is likely that these responses are due to interactions of the metabolites of benzene (Section 2.3.1). Recent evidence suggests that there are likely multiple mechanistic pathways leading to cancer, and in particular leukemogenesis, from exposure to benzene (Section 3.2).

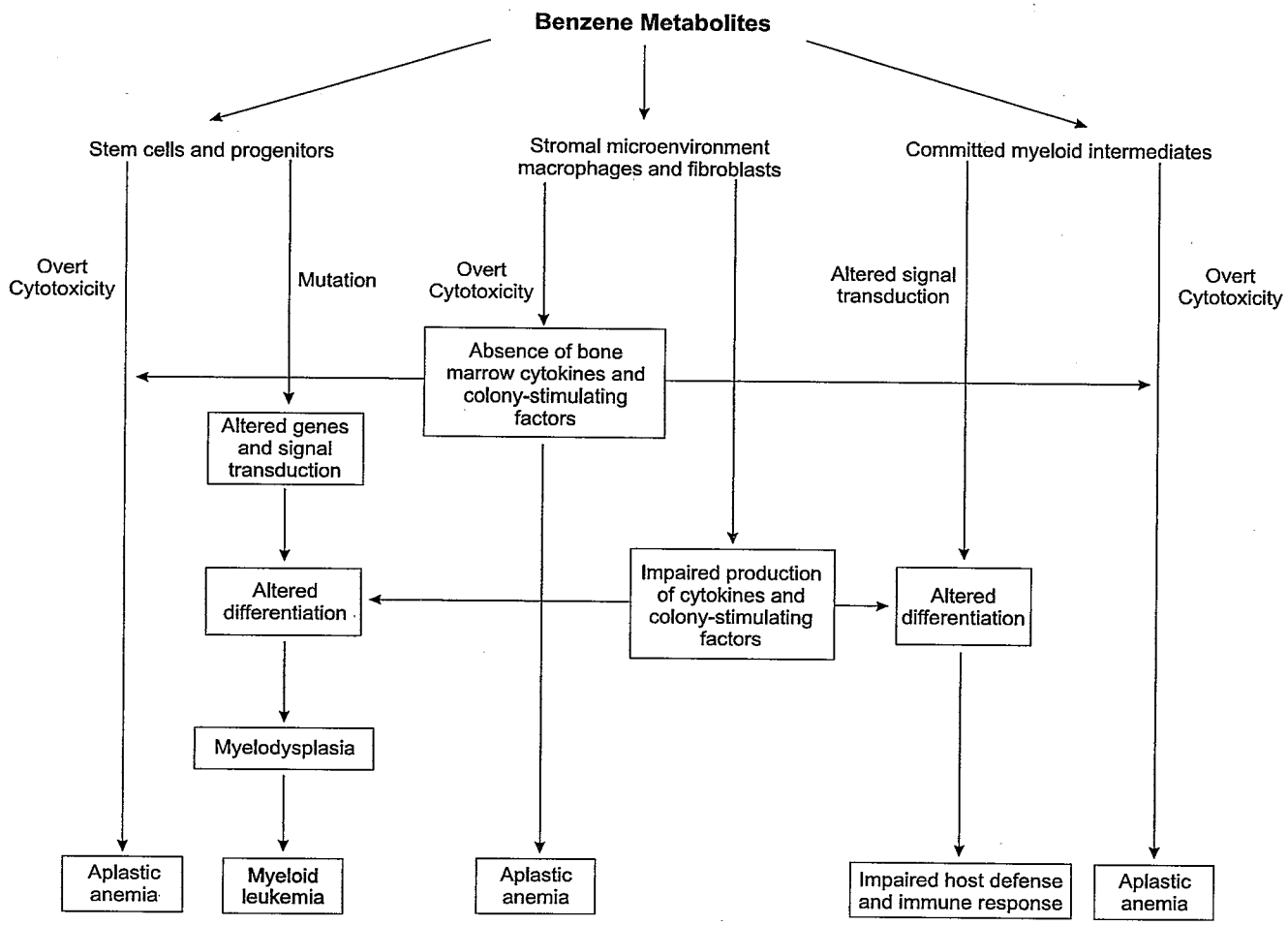

Figure 3. Schematic illustrating various options for modulation of bone marrow cell populations by benzene metabolites that could result in the induction of aplastic anemia, leukemia, and immunotoxicity.

Source: Trush et al., 1996.

Additionally, changes in blood and bone marrow consistent with hematotoxicity are recognized in humans and experimental animals. Clinical outcomes observed are leukopenia, thrombocytopenia, anemia, and aplastic anemia (ATSDR, 1997). Benzene induces peripheral blood abnormalities and disrupts hematopoiesis at separate compartments of blood cell formation (i.e., white, platelet, and red) (ATSDR, 1997). Granulocytic and erythropoietic progenitor cells are significantly depressed. Chromosomal breakage and loss are increased in mice from exposure to benzene or its metabolites, which consist of a mixture of phenol and hydroquinone (Section 2.3.2).

The metabolic studies summarized herein suggest that in both laboratory animals and humans, benzene metabolism exhibits dose-dependent behavior, with the proportion of the metabolites formed changing considerably depending on the dose of benzene administered. Benzene metabolism also has been reported to be modulated by coexposure or prior exposure to other organic chemicals (Medinsky et al., 1994).

Benzene affects bone marrow cells in several different ways. Based on our current understanding, these effects are produced by the interactive effects of multiple metabolites. Genotoxic effects are a critical component of the leukemogenic properties of benzene. As more information becomes available about the epigenetic effects of benzene and the role these effects play in the leukemogenic process in general, it is likely that these will be shown to have an important role. Evidence supports the hypothesis that more than one toxic effect contributes to the leukemogenic process, especially because benzene metabolic products may be able to cause general disruption of protein functions in bone marrow cells. Protein damage is likely to result in pleiotropic effects, including general toxicity, alteration of growth factor responses, and DNA damage. Therefore, the overall picture of benzene-induced leukemogenesis is an increased rate of genetic damage to hematopoietic cells that occurs in the context of disrupted bone marrow biology. This situation could encourage not only the production of cells with key genetic changes, but also the selection and expansion of such cells because of the abnormal marrow. However, data are not sufficient at this time to state precisely which of the various documented effects, genotoxic or otherwise, are the critical ones for benzene-induced leukemogenicity.

3. DOSE-RESPONSE ASSESSMENT AND CHARACTERIZATION

In the earlier EPA benzene risk assessment document (U.S. EPA, 1985), the lifetime leukemia risk due to 1 ppm of benzene in air was estimated to be 2.6×10^{-2}. This is the geometric mean of risk estimates that were calculated on the basis of data from one study on Pliofilm workers (Rinsky et al., 1981) and two studies of chemical workers (Wong et al., 1983;

Ott et al., 1978). On the basis of Rinsky et al.'s (1981) data alone, the risk due to 1 ppm of benzene in air was estimated to be 4.1×10^{-2} when the relative risk model was used, and 1.8×10^{-2} when the additive risk model was used.

Subsequently, several risk assessments on the basis of Rinsky et al.'s (1981) cohort have become available (Brett et al., 1989; Crump, 1992; Paxton et al., 1994). More than 100 individual risk estimates using varying assumptions and/or models have been presented, with outcomes ranging more than 6 orders of magnitude at 1 ppb exposure.

Two dose-response models, a relative and an absolute risk model, were used to calculate benzene risk estimates using epidemiologic data in the 1985 EPA document. In fitting the dose-response models, person-years of observation are divided into subgroups according to the benzene dose (ppm-year). Let O_i be the number of leukemia deaths observed in group I, E_i the expected number of leukemia deaths in the ith group based on the mortality rates in a comparison population, d_i the average benzene dose in the ith group, and Y_i the number of person-years in the ith group. The relative risk model is of the form

$$E(O_i) = aE_i(1+bd_i)$$

and the absolute risk model is of the form

$$E(O_i) = E_i + (a+bd_i)Y_i$$

where $E(O_i)$ is the expected number of leukemia deaths in the ith dose group under the respective model. The parameters a and b are estimated from cohort data under the assumption that the number of observed leukemia deaths, O_i, is a Poisson random variable with the expected value given by one of the two models above. The parameter b represents the potential of benzene to induce leukemia per unit dose (ppm-year). Once an estimate of the parameter b is obtained, it was translated into a unit risk (i.e., lifetime risk per unit of ambient air exposure in ppm or $\mu g/m^3$) by a straightforward mathematical manipulation that depends on whether the model is absolute or relative risk.

The unit risk estimate of 2.6E-2 per ppm was based on the report by Crump and Allen (1984). Because of the lack of information on exact exposure conditions for individual members of the cohort, cumulative dose (ppm-years) was used by Crump and Allen to construct dose-response models. Clearly, the use of cumulative dose is less desirable than the use of actual concentration (ppm). Its impact on risk estimates, however, is difficult to assess without knowing the exact exposure concentrations for individuals in the cohort.

3.1. DESCRIPTION OF DIFFERENT RISK ASSESSMENTS

Differences between these risk estimates largely derive from differences in the determination of the exposure estimates used in the dose-response modeling. Rinsky et al. (1981, 1987), Crump and Allen (1984), and Paustenbach et al. (1992, 1993) chiefly center on the levels that existed in the plants where the Pliofilm workers were employed before 1946. Paustenbach et al. (1992, 1993) assumed that the samples taken after 1946 underestimated actual levels chiefly because inadequate measuring devices were used; they asserted that these devices consistently underestimated exposure by as much as 50%. It was further assumed that the working week was on the average 51 h, not the 40 h usually assumed. Other assumptions are also given to justify their high exposure estimates (Paustenbach et al., 1992, 1993).

Much controversy exists concerning the levels of benzene that permeated the workplace during the early employment years of the Pliofilm workers. It has not been established what those levels were from the late 1930s until 1946. Actual measurements do not exist before 1946, when most of the Pliofilm workers were employed, including most of the leukemia victims. After 1946 and into the 1960s, few measurements of actual benzene exposure were taken, and in many instances they were taken in areas where it was known that high levels of benzene would be found. Rinsky et al. (1981) maintains that the average exposure to the workers were "within the limits considered permissible at the time of exposure." Rinsky agrees that peak exposure to high levels of benzene probably did occur but, unfortunately, there is no information regarding when these peak exposures occurred and how large they were for individual members of the cohort. Several leukemia victims were exposed to as much as 40 ppm 8-h TWA during their early years with the company. It is believed that actual levels were probably within the range of 35 to 100 ppm during those early years. These levels tended to drop in time as efforts to improve air quality in the plants were implemented.

Both Brett et al. (1989) and Paxton et al. (1994) assumed that rate ratio (RR) is related to exposure (ppm-year) by $RR(d) = \exp(b*d)$, where d is exposure in ppm-year and b is a parameter to be estimated (the two assessments differ in the way the parameter b was estimated). Only risk estimates due to occupational exposure (i.e., 8/h day, 5 days/week, 50 weeks/year) were presented. To calculate the lifetime risk due to continuous exposure of 1 ppm (i.e., d=76 ppm-years), the parameter b is multiplied by a factor of $(24/8)\times(7/5)\times(52/50)$. The resultant risks at 1 ppb and 1 ppm are given in Table 4.

Crump (1992, 1994) presented 96 dose-response analyses by considering different factors such as (1) different disease end points, (2) additive or multiplicative models, (3) linear/nonlinear exposure-response relationships, (4) two-exposure measurements (Crump and Allen [1984] vs. exposure estimates by Paustenbach eventually published in Paustenbach et al. [1993]), and (5) cumulative or weighted exposure measurements. The risk estimates range from 8.6×10^{-11} to

Table 4. Risk estimates[a] calculated on the basis of Pliofilm workers by various investigators

Source	Risk at 1 ppm	Risk at 1 ppb	Exposure and model
U.S. EPA 1985[a]	1.8E-2 (7.5E-3, 3.4E-2)	1.8E-5 (7.5E-6, 3.4E-5)	Crump and Allen, additive risk
	4.1E-2 (1.3E-2, 8.8E-2)	4.1E-5 (1.3E-5, 8.8E-5)	Crump and Allen, relative risk
Brett et al., 1989	4.0E-3 (1.0E-3, 1.2E-2) to 2.5E-2 (2.5E-3, 9.9E-2)	3.6E-6 (9.5E-7, 6.9E-6) to 1.1E-5 (2.2E-6, 1.9E-5)	Crump and Allen, conditional logistic
	2.2E-1 (1.2E-2, 1.0) to 8.4E-1 (1.5E-2, 1.0)	2.4E-5 (6.9E-6, 4.2E-5) to 3.4E-5 (8.2E-6, 5.9E-5)	Rinsky, conditional logistic
Paxton, 1992	2.2E-3 (3.8E-5, 4.9E-3)	1.9E-6 (3.7E-8, 3.7E-6)	Crump and Allen, proportional hazard
	4.6E-3 (1.3E-3, 9.0E-3)	3.5E-6 (1.2E-6, 5.8E-6)	Paustenbach, proportional hazard
	1.8E-2 (3.0E-3, 5.5E-2)	8.9E-6 (2.5E-6, 1.5E-5)	Rinsky, proportional hazard
Crump, 1992; 1994	1.1E-2 (2.2E-3, 2.0E-2) to 2.5E-2 (6.0E-3, 1.3E-1)	1.1E-5 (2.2E-6, 2.0E-5) to 2.5E-5 (6.0E-6, 1.3E-4)	Crump and Allen, linear
	5.4E-3 to 2.5E-2	4.5E-6 to 2.6E-5	Crump and Allen, nonlinear
	7.1E-3 (2.0E-3, 1.2E-2) to 1.5E-2 (3.8E-3, 2.6E-2)	7.2E-6 (2.0E-6, 1.2E-5) to 1.6E-5 (3.8E-6, 2.6E-5)	Paustenbach, linear
	8.6×10^{-5} to 6.5×10^{-3}	8.6×10^{-11} to 5.6×10^{-6}	Paustenbach, nonlinear

[a]95% confidence intervals (CI) are provided in parentheses if they can be reconstructed from the original report. For the EPA risk estimates, the same arithmetic operations (e.g., taking geometric means of several risk estimates) are applied to the derivations of CI as to the point estimates. Therefore, the statistical confidence statement for these numbers may not be precise.

[b]Not available.

[c]The author recommended that 95% upper bound be used to derive unit risk because of the instability of the maximum likelihood estimate (i.e., the linear component was estimated to be 0).

2.6×10^{-5} at 1 ppb of benzene air concentration and 8.6×10^{-5} to 2.5×10^{-2} at 1 ppm of benzene air concentration. The largest deviation from the EPA risk number (U.S. EPA, 1985) was obtained when a nonlinear model and Paustenbach et al. (1993) exposure estimates were used. When a linear model was used, risk estimates ranged from 7.1×10^{-3} to 2.5×10^{-2} at 1 ppm, regardless of which exposure measurements were used. When a linear model and Crump and Allen (1984) exposure measurements were used, the risk at 1 ppm ranged from 1.1×10^{-2} to 2.5×10^{-2}. These are close to the 1985 EPA risk estimates. As previously stated, the use of the updated Rinsky et al. (1987) cohort would not significantly alter risk estimates if the same exposure-response model and exposure estimates were used. The single factor that affects the risk estimate most is the assumption of nonlinearity. If low-dose linearity is assumed, consideration of other factors (e.g., new exposure estimates) will result in no more than a fivefold difference from the existing EPA risk number.

A need exists to further support these conclusions based on additional research on biological mechanisms of benzene-induced hematopoiesis and leukemia rather than on statistical modeling uncertainties alone.

3.2. SHAPE OF THE DOSE-RESPONSE FUNCTION AT LOW DOSES

Too many questions remain about the mode of action for benzene-induced leukemia for the shape of the dose-response function to be known with certainty. While much progress has been made in the past few years and a reasonable hypothesis can be generated for the mechanism of benzene-induced leukemia, it remains simply a hypothesis. Arguments for and against the dose-response curve being nonlinear at low doses are presented in summary form in Table 5.

Analysis of the Rinsky et al. (1987) data shows that at doses less than 40 ppm-years, the SMR for leukemia was 1.1 and is not significantly elevated. This has prompted some investigators to suggest that benzene has a threshold for leukemia induction of about 40 ppm-years. However, this analysis of leukemia dose-response is based on only nine cases of leukemia, limiting its value for dose-response analysis. In addition, only six of these cases were AML. Further, Rinsky et al. (1987) showed a clearly increased SMR for multiple myeloma at doses below 40 ppm-years, and in a larger Chinese study, involving more than 30 cases, leukemogenic effects of benzene were observed at exposures well below 200 ppm-years (Yin et al., 1989). These observations suggest, as expected, that it is difficult to determine the shape of the dose-response function based on occupational exposure studies alone.

Table 5. Arguments for and against benzene-induced leukemia linearity at low doses

Pro	Con
Micronucleus assay is relatively insensitive and may not show effects at low doses.	Micronucleus induction by benzene and its metabolites in mouse bone marrow and in human cells in vitro is nonlinear.
Induction in aneuploidy of other chromosomes (e.g., 7) occurs at lower doses, and effect of benzene on hyperdiploidy of chromosomes 7, 8, and 9 shows a significant linear trend.	The induction of aneuploidy of chromosome 9 is nonlinear and is significant only at high levels of exposure (>31 ppm in air) (Zhang et al., 1996a).
Data obtained using accelerator mass spectrometry shows that the formation of DNA adducts in mouse bone marrow is linear to very low doses.	DNA adduct formation is observed by P^{32}-postlabeling only at high doses.
Errors during repair may cause point mutations.	Oxidative DNA damage may contribute to benzene genotoxicity (Kolachana et al., 1993) but has a high rate of repair.
Hematotoxicity may increase risk of malignancy but has not been shown to be a prerequisite.	Hematotoxicity is required for leukemia induction, and this will have a threshold.
There is a high background of exposure to benzene and its metabolites. Additional environmental exposure will simply add to this and be linear. There are also numerous mechanisms of aneuploidy induction, and aneuploidy is not the only mechanism of suppressor gene loss and oncogeny activation.	If aneuploidy is critical, then leukemia induction is likely to have a threshold. (Numerous molecules of benzene metabolites will be required to disrupt microtubules.)
There is a high background exposure to benzene and its metabolites, so additional exposure could escape defenses.	The cells in the bone marrow have numerous defense mechanisms.

As indicated previously, benzene is not a classic carcinogen; that is, its metabolites are not genotoxic in simple mutation assays. It most likely produces leukemia by chromosomal damage rather than simple point mutations. An argument can be made for nonlinearity on the basis that the induction of chromosome damage by benzene and its metabolites is nonlinear and in some instances shows a threshold. However, it should be pointed out that the micronucleus assay of chromosomal damage is relatively insensitive and may not show effects at low doses, even though some chromosomal damage is occurring. A recent report by Zhang et al. (1996a) showed that the induction of aneuploidy of chromosome 9 as measured by FISH in interphase lymphocytes from benzene-exposed workers is significantly elevated only at high levels of exposure (>31 ppm in air). However, as yet unpublished studies have shown that the induction of aneuploidy of other chromosomes (e.g., chromosome 7) occurs at lower doses and that the

effect of benzene on hyperdiploidy of chromosomes 7, 8, and 9 shows a significant linear trend (Zhang et al., 1996b).

As discussed earlier, bone marrow DNA adducts as detected by P^{32} postlabeling after in vivo exposure to benzene correspond with adducts formed by in vitro treatment with hydroquinone or 1,2,4-benzentriol (Pathak et al., 1995).

It also has been demonstrated that oxidative DNA damage may contribute to benzene genotoxicity and thus benzene-induced leukemia (Kolachana et al., 1993; Lagorio et al., 1994). Because oxidative damage has a high rate of repair and studies in benzene-exposed mice and human cells in vitro showed that the oxidative DNA damage was rapidly repaired, it could be argued that this high level of repair will produce a threshold or nonlinearity at low doses. However, it is errors during this repair process that cause point mutations from oxidative DNA damage. Further, because there is already a considerable background level of oxidative damage (Ames and Shigenaga, 1992), additional damage caused by benzene exposure may induce a linear increase in point mutations.

It also could be proposed that hematotoxicity is required for leukemia induction. Because hematotoxicity is likely to have a threshold, it is therefore possible that benzene-induced leukemia will have a threshold and be nonlinear at low doses. In theory, hematotoxicity may increase the risk of benzene-induced leukemia, because it could cause quiescent stem cells to enter the cycling feeder cell stage, thereby expressing any genetic damage. However, there is no evidence that hematotoxicity is a prerequisite for leukemia induction. Cases of leukemia following benzene exposure without previous hematotoxicity have been reported, but the thoroughness of monitoring for hematological effects is always a question. Benzene recently also has been shown to have hematological effects below 10 ppm (Ward et al., 1996), and thus the relevance of a threshold for hematotoxicity has decreased in most investigators' estimation.

Irons, Subrahmanyam, Eastmond, and their co-workers have argued that the induction of aneuploidy is a component of leukemia induction by benzene (Irons and Neptune, 1980; Subrahmanyam et al., 1991; Eastmond, 1993). If this is true, then it could be argued that leukemia induction has a threshold because numerous molecules of benzene metabolites would be required to disrupt microtubules and cause aneuploidy. However, it should be pointed out that there is a high level of background exposure to benzene and its metabolites. Benzene and its metabolites are present in our diet and in cigarette smoke. Additional environmental exposure will simply add to this background. Indeed, McDonald and co-workers have shown that proteins in both the blood and bone marrow of humans and animals contain high levels of benzene metabolite adducts and that the exposure of animals to benzene causes a linear increase in 1,4-benzoquinone adducts on top of this background (McDonald et al., 1993, 1994). This additional benzene exposure from the environment is likely to have a linear additional effect on the

background. Further, there are numerous mechanisms of aneuploidy induction that do not necessarily involve binding to microtubules, and aneuploidy is not the only genetic mechanism of suppressor gene loss and oncogeny activation. Care must therefore be exercised in claiming that benzene is nonlinear on the basis of aneuploidy involvement.

Another theoretical argument is that the cells in the bone marrow have numerous defense mechanisms to cope with toxic benzene metabolites. However, as discussed above, there is a high background exposure and so additional exposures could actually escape defenses. Indeed, we have calculated that there are approximately 10,000 benzene molecules per bone marrow cell following normal environmental background exposures to benzene. The addition of further molecules from environmental or occupational exposures will simply add to this and may easily overwhelm or escape defense mechanisms.

Even if there are threshold levels at which each individual experiences increased leukemia risk, population variability will almost certainly dictate that there is no one threshold dose that applies across the population of people exposed to benzene. The data on susceptibility factors for benzene toxicity and leukemogenicity are growing and will likely shed some light on population variability in sensitivity to benzene's adverse effects.

3.3. DOSE-RESPONSE CHARACTERIZATION

The major result of this update is a reaffirmation of the benzene interim unit risk estimates derived in EPA's 1985 interim risk assessment (U.S. EPA, 1985), which established the probability of humans developing cancer from exposure to 1 ppm of benzene. Review of the 1985 interim risk assessment required addressing two main concerns. The first was use of the updated epidemiologic data from Rinsky et al.'s (1987) cohort of Pliofilm workers and selection of appropriate estimates of their exposure to benzene for the derivation of the unit risk estimate. The second major concern was continued application of the low-dose linearity concept to the model used to generate estimates of unit risk. It was concluded that at present there is insufficient evidence to reject this concept.

Use of the update of Rinsky et al.'s (1987) cohort could have only a limited impact on the EPA (1985) interim risk estimates if the same exposure-response (linear) model and the Crump and Allen (1984) exposure measurements were used. When the higher estimated exposure measurements by Paustenbach et al. (1993) were substituted for those of Crump and Allen, the corresponding risk estimates were reduced by only a factor of, at most, 2. None of the approaches for estimating exposure has greater scientific support than any other because there was no ambient air benzene exposure data for the Pliofilm workers prior to 1946. Thus, there is no clear basis for choosing a single best estimate. Rather, these sets of risk estimates reflect both

the inherent uncertainties in the applied model and the limitations of the exposure characterization and response information in the epidemiologic data.

Without conducting extensive analyses of the raw data in Rinsky et al. (1987), only theoretical analyses of the impact of various exposure assumptions and presumed etiologic mechanisms on risk estimates for benzene are possible. There are two approaches for estimating this impact. One is assuming a biological mechanism of benzene-induced leukemia (e.g., assuming that benzene-induced leukemia involves a sequence of genetic and epigenetic changes, and that some of these steps are effected by benzene exposure), and the other is assuming a linear model, like that used in the EPA (1985) assessment. In the linear model, use of cumulative exposure would have less impact on the resultant risk estimate if the concentration were roughly constant during the work history of the cohort. However, if exposure concentrations in early work history were higher than in the later years, the unit risk could be overestimated. A theoretical discussion about this subject can be found in Hoel (1984), which concludes that true lifetime cancer risk can be significantly under- or overestimated when using epidemiological data with short exposure.

While the risk estimates would be significantly different if a nonlinear exposure response model were found to be more plausible, characterizing the shape (i.e., the nonlinearity) of the exposure-response curve still would require a better understanding of the biological mechanisms of benzene-induced leukemia. Some recent evidence suggests the possibility that the low-dose curve could be supralinear because the formation of toxic metabolites plateaus above 25 ppm benzene in air (Rothman et al., 1996b). This pattern is similar to that seen in laboratory animals (Sabourin et al., 1989), where the effect per unit dose of benzene is less at high doses than at low doses. Thus, it is possible that the unit risk is underestimated if linearity is assumed at low doses. Arguments made in favor of benzene-induced leukemia being nonlinear at low doses can be matched by arguments opposing this viewpoint. Currently, there is insufficient evidence either to reject a linear dose-response curve for benzene in the low-dose region or to demonstrate that benzene is, in fact, nonlinear in its effects. Even if the dose-response relationship were nonlinear, the shape remains to be determined. Because of current lack of knowledge, continuing the Agency's previous approach of using a model with low-dose linearity is recommended. Of the various approaches employing a linear assumption, the risk at 1 ppm ranges from 7.1×10^{-3} to 2.5×10^{-2} (Table 4).

Based on the Rinsky et al. (1987) study, the risk of leukemia is significantly elevated (SMR = 1,186; 95% C.I. = 133-4,285) at a dose of 200 to 400 ppm-years (e.g., a person exposed to a level of 5 to 10 ppm for 40 years). This assumes that exposure occurred for only 8 h each day. However, Rinsky et al.'s (1987) data suggest that a rise in the SMR may begin at levels under 40 ppm-years, although the trend does not attain statistical significance until a dose of 200

to 400 ppm-years is reached. We, therefore, are less confident that the risk begins to rise below 40 ppm-years (1 ppm for 40 years) than we are of increased risk at doses above 200 ppm-years. Failure to find elevated risk at exposures to benzene below 40 ppm-years may, however, be a matter of the lack of power to detect risk as significant below this level rather than an absence of increased risk. Wong (1995), in a separate analysis of the risk of only AML in the Rinsky et al. (1981) cohort, calculated an SMR of 0.91 (1 observed, 1.09 expected) in the exposure category under 200 ppm-years. However, because AML is a subtype within the leukemia category, the sensitivity for detecting a significant risk at that level of exposure is much lower.

On the other hand, several recent studies appear to support the possibility of elevated risk at low exposures. Recent data from the Chinese cohort (Yin et al., 1989) suggest that the risk of AML might be well below 200 ppm-years, although the data analysis still is incomplete and, as discussed above, exposure estimates may have been biased toward unrealistically low values. Out of 30 identified leukemia cases reported in that study, 11 reported cumulative exposure of under 200 ppm-years, and of these 11, 7 were subject to average levels of under 5 ppm-years during the time that they were exposed. In fact, Hayes et al. (1996) added 12 leukemias to this total in an update of the Yin et al. (1989) study. Interestingly, dosimetry data were calculated on selected causes of death in this same cohort. Hayes et al. (1996) reported that excess risks of death from hematopoietic malignancies were found at the level of 10 ppm-years cumulative exposure (9 observed vs. 3.6 expected). Unfortunately, length of employment was not provided in the Chinese cohort. In addition, Bond et al. (1986a) reported five cases of myelogenous leukemia, four with cumulative doses of benzene exposure between 1.5 ppm-years and 54 ppm-years. Their average yearly exposure ranged from 1.0 ppm to 18 ppm. The Wong study (1987) reported that six of seven cases of leukemia had cumulative benzene exposures of between 0.6 ppm-years and 113.4 ppm-years. Their average yearly exposure ranged from 0.5 ppm to 7.6 ppm. The seventh case had no measured cumulative dose. Although it is possible that peak exposures could have occurred at any time during employment, such information is unavailable. However, although the authors of these studies developed dose-response data for some members of their respective cohorts, the results of their studies cannot be used in unit risk calculations. In addition to the questionable exposure estimates, it is clear that these workers were subject to concomitant exposures to other toxics that also were present in the workplace and that might affect the risk of cancer. Furthermore, methodological problems are present in these studies.

Based on observations from Rinsky et al. (1981, 1987) and recent studies, the Agency is fairly confident that exposure to benzene increases the risk of leukemia at the level of 40 ppm-years of cumulative exposure. However, below 40 ppm-years, the shape of the dose-response curve cannot be determined on the basis of the current epidemiologic data. Benzene exposure at 40 ppm-years of occupational exposure (8 h/day, 5 days/week, 50 weeks/year) would be

equivalent to a lifetime (76 years) environmental exposure of 120 ppb. Hence, 120 ppb would be a reasonable point of departure (POD) below which the shape of the dose-response curve is uncertain. To put this POD of 120 ppb into perspective, it is necessary to examine information on ambient benzene concentrations. Environmental surveys completed around the United States have provided a variety of information on monitored levels of benzene, using both ambient measurements and personal exposure measurements. (ATSDR [1997] provides a convenient summary of much of the data.) Ambient measurements have been made both outdoors and indoors. Shah and Singh (1988) report that the Volatile Organic Compound National Ambient Database (1975-1985) contains the following daily median benzene air concentrations: workplace air (2.1 ppb), indoor air (1.8 ppb), urban ambient (1.8 ppb), suburban ambient (1.8 ppb), rural ambient (0.47 ppb), and remote (0.16 ppb). The EPA (1987) reports data from 44 sites in 39 cities of the United States, taken during the 6 to 9 a.m. "morning rush hour" periods during June-September of 1984, 1985, and 1986. The median concentrations at these sites ranged from 4.8 to 35 ppb, with the authors noting that mobile sources (motor vehicles) were the major source of ambient benzene in these samples. In industrialized areas, Pellizzari (1982) reports outdoor levels of 0.13 to 5 ppb in Iberville Parish, LA, and Cohen et al. (1989) report median outdoor levels in the Kanawha Valley region of West Virginia as 0.78 ppb. Cohen et al. (1989) also report that mean indoor levels in the study were 2.1 ppb (median = 0.64 ppb, maximum = 14.9 ppb).

The EPA's Total Exposure Assessment Methodology (TEAM) studies showed consistently that personal exposures to benzene were higher than ambient indoor levels, and that indoor levels, in turn, were higher than outdoor levels. Wallace (1989) reported that the overall mean personal benzene exposure (smokers and nonsmokers) from the TEAM data was 4.7 ppb, compared with an overall mean outdoor ambient level of 1.9 ppb. Median levels of benzene indoors were broken out by those homes without smokers (mean = 2.2 ppb) and those where one or more smokers were present (mean = 3.3 ppb). The TEAM authors frequently suggested smoking as a source for indoor benzene concentrations (Wallace, 1987; Wallace et al., 1989). Brunnemann et al. (1989) reported that indoor air samples at a smoke-filled bar ranged from 8.1 to 11.3 ppb of benzene. Wester et al. (1986) noted that benzene in the breath of smokers was higher than that of nonsmokers, and that both were higher than the concentrations in outdoor ambient air.

Other measurements of benzene concentrations include transient levels of benzene approaching 1 ppm outside a vehicle while refueling (Bond et al., 1986b). Within a parking garage, Flachsbart (1992) found a maximum level of 21 ppb. The estimated maximum level in a basement during the Love Canal situation was about 160 ppb, with levels of about 60 ppb estimated around an uncontrolled hazardous waste site (Bennett, 1987; Pellizzari, 1982). The

maximum single personal monitoring sample, representing one night's exposure, during the 1981 New Jersey TEAM study was 159.6 ppb.

Using 120 ppb as the POD, the margin of exposure (MOE) can be calculated for several of these levels. Since the 120 ppb is a 76-year lifetime value, this should be compared with average levels in whichever exposure scenario is used. For example, if one assumes that 4.7 ppb is the long-term average exposure for the general population, the MOE would be 120/4.7, or about 26. If one assumes that the ambient indoor levels of 2.2 ppb cited above represent actual exposures to nonsmokers, their MOE would be 120/2.2, or 55. If one were to construct a hypothetical scenario where a person spent their entire life in a smoke-filled bar, the MOE would drop to a range of 10 to 15.

The purpose of the margin of exposure discussion is to provide the risk manager with a public health perspective on the adequacy of the difference between an environmental exposure of interest and human equivalent exposures at the point of departure. Since the mode of action of benzene exposure-induced leukemia is not well enough understood to support a nonlinear dose-response analysis approach as is discussed in sections 2.3. and 3., the MOE serves as a default approach. There are several factors which are not easily accounted for in the benzene data base and which may impact on interpretation of the MOE and an acceptable risk to the population, i.e., the slope of the dose-response curve at or below the calculated POD, the nature and magnitude of temporal exposure scenarios, extent of human variability and sensitivity within the general population, use of a precursor effect other than leukemogenesis and the role of benzene and/or its metabolites in leukemogenesis. These issues must be discussed with the decision-maker if he/she deems the MOE approach to be a useful alternative to the linear approach which has been recommended in this document.

4. CHILDREN'S RISK CONSIDERATIONS

The effects from exposure to benzene can be quite different among subpopulations. Children may have a higher unit body weight exposure because of their heightened activity patterns which can increase their exposures, as well as different ventilation tidal volumes and frequencies, factors that influence uptake. This could entail a greater risk of leukemia and other toxic effects to children if they are exposed to benzene at similar levels as adults. Infants and children may be more vulnerable to leukemogenesis because their hematopoietic cell populations are differentiating and undergoing maturation. Many confounding factors may affect the susceptibility of children to leukemia (e.g., nutritional status, lifestyle, ethnicity, and place of residence). Furthermore, in children, the predominant type of leukemia is lymphatic, while in

adults it is a combination of myeloid and lymphatic. Leukemia formerly classified as a single disease now has been recognized as several different distinct malignancies that are characterized by varying patterns in terms of age, race, sex, ethnic group, different secular trends, and different etiologic factors (Linet, 1985).

Some recent research has shown, with limited consistency, that parental occupational exposure to benzene plays a role in causing childhood leukemia. Shu et al. (1988) conducted a case-control study of acute childhood leukemia in Shanghai, China, and found a significant association between acute nonlymphocytic leukemia (ANNL) and maternal occupational exposures to benzene during pregnancy (OR = 4.0). These excesses occurred among second- or laterborn children rather than firstborn children. In addition, Mckinney et al. (1991) conducted a case-control study to determine whether parental occupational, chemical, and other specific exposures are risk factors for childhood leukemia. They found a significant association between childhood leukemia and reported preconceptional exposures of fathers to benzene (OR = 5.81, 95% confidence intervals 1.67 to 26.44) and concluded that the results should be interpreted cautiously because of the small numbers, overlap with another study, and multiple exposures of some parents. Furthermore, Buckley et al. (1989) conducted a case-control study of occupational exposures of parents of 204 children (under 18 years of age) with ANNL. They found a significant association between ANNL and maternal exposure to pesticides, petroleum products, and solvents. Among many chemicals, benzene was identified as one of the solvents. These studies, however, have not provided data to indicate how the occupational exposures might affect offspring. Some possible mechanisms include a germ-cell mutation prior to conception, transplacental fetal exposures, exposures through breast milk, or direct exposures postnatally to benzene from the environment.

Data on children exposed to benzene in the environment are very limited. Weaver et al. (1996) conducted a pilot study that evaluated the feasibility of using trans, trans-muconic acid as a biomarker of environmental benzene exposure in urban children. Although the authors concluded that muconic acid could be used as a biomarker in children for environmental exposure, no studies have been found that used this biomarker to determine actual benzene exposure in children.

In summary, children may represent a subpopulation at increased risk due to factors that could increase their susceptibility to effects of benzene exposure (e.g., activity patterns), on key pharmacokinetic processes (e.g., ventilation rates, metabolism rates, and capacities), or on key pharmacodynamic processes (e.g., toxicant-target interactions in the immature hematopoietic system). In addition, parental occupational exposures to benzene have been associated with their increased risk. However, the data to make quantitative adjustments for these factors do not exist at this time.

5. FUTURE RESEARCH NEEDS

Data insufficiencies in several areas have been noted, and research in these areas ultimately should provide a better understanding of how benzene causes cancer, particularly the mechanism of benzene-induced leukemia. Several classes of data are needed on humans, i.e., more extensive epidemiologic data with good exposure estimation, to permit verification and validation of the prediction models. Additional research on exposure metrics that incorporates cumulative exposures and occurrence of various effects inferring risk to general as well as sensitive subpopulations is needed. More complete data on the preleukemic hematology of benzene-exposed persons, such as the abnormal monoclonality and blood cell counts seen in such persons, would be a significant contribution. Specific measures of early genetic damage in humans with known exposure to benzene will help define the biological events leading up to the disease by providing internal markers of its progression. This could be potentially useful in risk prediction and assist in the identification of the steps leading to leukemia induced by exposure to benzene. Such information may be forthcoming in the near future from a large cohort of benzene-exposed workers under study in China. Investigators from the National Cancer Institute in the United States, the Chinese Academy of Preventive Medicine, and the University of California at Berkeley are currently developing such biomarker information as well as gathering clinical data on hematologic abnormalities.

A need exists to further validate toxicokinetic models and to assess metabolic susceptibility factors in human subjects. The collection of such information is problematic at best because it requires exposure of human volunteers to a known carcinogen. However, data now being collected in the Chinese cohort on the urinary metabolites of benzene, as well as in vitro studies of cell-specific metabolism and toxicity in defined human bone marrow cell populations, may be of use.

Research is needed to reduce some uncertainties in risk assessment of benzene, including better understanding of the role of specific metabolites of benzene in toxic effects (cancer, leukemia, hematotoxicity), the shape of the dose-response curve at low levels of benzene exposure, and the role of DNA adducts and chromosomal aberrations in the development of leukemia.

Continued basic research in hematopoiesis and leukemia biology is critical for identifying the mechanisms of leukemogenesis. There remain important unanswered questions about the cell population that contains targets for leukemic transformation, such as cell number and rate of division, quiescence patterns, maturation, regulation, and apoptotic behavior. Future understanding of the phenotypic consequences of common genetic aberration in MDS and AML also is needed to assist in identifying the stages of leukemic transformation. There still exist

some uncertainties as to the exact classification of leukemias and leukemic subtypes in the epidemiological studies of benzene exposures.

Current uncertainties limit the ability of modeling to explicitly consider all relevant mechanisms, such as the formation of several types of genetic aberrations; disruption of proliferation, differentiation, or apoptotic behaviors through genetic change or epigenetic chemical interference; and the extremely complex and subtle regulation of hematopoietic processes under normal feedback systems. Future research would be able to quantitatively describe benzene pharmacokinetics in humans, relate dose measures to the above pharmacodynamic mechanisms, and account for observed epidemiologic features of benzene-induced leukemia, such as patterns of latency and susceptibility. For any mechanistic model of leukemogenesis to be validated, it must be applied to existing data that relate known human exposures to the probability of contracting MDS/AML. While there are epidemiologic data for benzene, estimation of exposure is a complex task with considerable uncertainty. Therefore, a suggested approach is to first develop a biologically based risk model for AML. It should be recognized that in modeling benzene-induced leukemia in the general population there is considerable interindividual variability that may influence risk. Some of the genetic factors important in metabolic variability are becoming known, but other aspects of susceptibility remain less well characterized. For example, the factors are unknown that control whether patients who suffer benzene-induced myelosuppression progress to AML or recover after exposure is reduced or removed. To what extent susceptibility factors will dictate leukemia risk and to what extent leukemia is a manifestation of stochastic processes are not known.

There are a number of potential subgroups in the general population that may be at increased risk from benzene exposure. These include but are not limited to indoor house painters using oil paints, people with hobbies involving glues and solvents, do-it-yourself automobile repairers working at home, alcoholic individuals, and smokers. With the exception of alcoholics and smokers, a potential for benzene exposure exists for these individuals via both inhalation and dermal routes.

Particular emphasis should be placed on research on those sensitive subpopulations who are believed to be at increased risks (e.g., infants and children, the elderly). Given the high proliferative rates and rapid rate of development of organ systems in the fetus and the prevalence of leukemia in children, research directed at determining if the developing fetus is at increased risk for cancer and noncarcinogenic effects is warranted. Research is needed to show how growth, development, and aging affect the risk to humans. In addition, environmental and epidemiological studies are needed to better determine the environmental benzene exposure levels that sensitive subpopulations such as pregnant women, infants and children, and the elderly are likely to encounter. Studies are needed to better understand how the absorption,

distribution, metabolism, and elimination of benzene varies with age, gender, race, or ethnicity and how this information can be modeled to predict risk to sensitive subpopulations.

Current understanding of metabolism of benzene and preliminary findings of genetic heterogeneity suggest that future research should emphasize subpopulations with particular sensitivity to benzene toxicity and genetic polymorphism of enzymes involved in benzene metabolism. Although this document focuses on benzene exposure via inhalation, the major route of exposure to the general population, the potential contribution of dermal exposure among individuals who use gasoline and other cleaning solvents containing benzene remains unknown. Therefore, an additional potentially important area is the role and contribution of dermal absorption, metabolism, and toxicity of benzene in the presence of other solvents including gasoline. The effects of dermal absorption are an issue of public health concern because individuals in occupations such as automobile repair and home painting, or who have hobbies involving solvents, are likely to have higher than average benzene exposure. Another needed area of research includes an understanding of how the sensitivity of individuals to benzene poisoning is affected by various disease conditions or abnormalities of hematopoiesis.

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