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Carcinogenic Effects of Benzene: An Update

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1. INTRODUCTION

In 1992, the U.S. Environmental Protection Agency's (EPA's) Office of Mobile Sources (OMS) requested the National Center for Environmental Assessment (NCEA) to provide an updated characterization of the cancer risk of benzene to humans. 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 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 regulatory authority (Clean Air Act Amendments, 1990) for controlling fuel emissions in vehicles resides in OMS. Before OMS exercises its regulatory authority, OMS has asked NCEA for scientific supporting documents based on health implications of continued exposure to benzene.

The scope of this report is limited to issues related to the carcinogenicity of 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 unit cancer 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 OAQPS. The previous cancer risk assessment on benzene by the Agency was completed in January 1979 (U.S. EPA, 1979). In subsequent years, this assessment became out of date as new scientific information became available.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 the three most recent 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) constituted the expanded scientific information base.

Data from the occupational cohorts of Rinsky et al. (1981) and Ott et al. (1978) 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 used 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 to the individual. 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 exposure estimate approaches assume a latency period that begins at the beginning of exposure and 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) are used to the risk estimate generated when only the Rinsky et al. (1981) and Ott et al. (1978) studies are used under the same relative risk model and cumulative exposure estimate. (Note: The Wong et al. [1983] study cannot be used under the absolute risk model because no person-year information was provided in the report.) 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 compares 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. (1994), 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 published in 1986 (U.S. EPA, 1986). The 1996 proposed guidelines include a number of changes that accommodate a more detailed understanding of the carcinogenic process (i.e., chemical and gene interactions) 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 (Wiltse and Dellarco, 1996).

The revisions are intended to provide for greater flexibility in evaluating the rapidly increasing new scientific data available on cancer research in decisions to implement the Agency's regulatory authority. Technical 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 on 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, the evaluation of the entire range of data (i.e., physical, chemical, biological, and toxicological information) allows one to arrive at a reasoned judgement of an agent's mode of action. Although cancer is a complex and diverse process, a risk assessment must operationally dissect the presumed critical events, at least those that can be measured experimentally, to derive a reasonable approximation of risk (Wiltse and Dellarco, 1996). 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 categories of 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 on agents, 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 evaluation and review of benzene health risk assessment issues is being conducted under the standing guidance of the 1986 guidelines but with a recognition of these areas of emphasis in the 1996 proposed guidelines. Thus, this review of benzene health risk assessment issues 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 studies provide the bulk of evidence of benzene's carcinogenicity, and workers are exposed at much higher levels than is the general public. The 1996 proposed 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 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 the mode of action of carcinogenicity at low exposure levels.

2. HAZARD ASSESSMENT AND CHARACTERIZATION

The "known/likely" category of the proposed 1996 cancer 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 and its metabolites causes acute nonlymphocytic leukemia and a variety of other blood-related disorders in humans (ATSDR, 1996; IARC, 1982; U.S. EPA, 1979). 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 should discuss 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 provide clear evidence of a causal association between exposure to benzene and leukemia, especially acute nonlymphocytic (myelogenous) leukemia and, to a lesser extent, chronic nonlymphocytic leukemia as well as chronic lymphocytic leukemia and multiple myeloma (Aksoy, 1976, 1977; Aksoy et al.,1974; Infante et al., 1977; Rinsky et al., 1981, 1987; Vigliani and Saita, 1964; IARC, 1982; ATSDR, 1996). Lymphocytic leukemia, a form of leukemia commonly found in children, may have a genetic component as well as an environmental exposure component (Linet, 1985). A role for benzene and other environmental chemicals cannot be ruled out. A higher risk of multiple myeloma also may be associated with exposure to benzene (DeCouflé et al., 1983; Rinsky et al., 1987).

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. Since the 1985 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 by NCEA concerning the impact of these more recent data to the present assessment of benzene and its use in a quantitative risk assessment. First, does the 1987 Rinsky et al. update lead to any substantial changes in the estimated relative risk ratios that were derived in the 1981 Rinsky et al. report? Second, 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 workplace of the Pliofilm workers were taken in the years before 1946. The first known measurements were taken in 1946, and then there were only four samples measured. The absence of definitive ambient air measurements during this time has led to a flourish of quantitative risk estimates by numerous investigators over the past several years that have differed based partially on differences in the assumptions made about what those earlier exposures to benzene were. Do the various approaches used to estimate exposure in the those early years lead to estimates that differ by a substantial amount? 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 effect would discarding the Ott et al. (1978) and Wong et al. (1983) epidemiologic studies have on 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 sometime 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 previous versions. Duration of employment in combination with personal exposure estimates during that employment was used to generate risk estimates based on grouped data. The updated version 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 added 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.

To answer the second question, 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

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

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, thus giving rise to the highest risk estimates, but there is no consistent pattern among the estimates for particular years.

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 as follows: (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) installation 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. These factors tended to provide an incentive for the authors to conclude that these pliofilm workers were exposed to the highest levels during the early years of exposure.

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 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). They 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 as progressively more restrictive standards were implemented in the workplace. These estimates lie somewhere between those of Rinsky et al. (1981, 1987) and Paustenbach et al. (1993). These same estimates of Crump and Allen (1984) 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 exposure levels produced by utilizing these three sets of estimates of exposure for the employees, the cumulative standardized mortality ratios (SMRs) differed from the Crump and Allen estimates by no more than a factor of two (table 2).

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

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: Paxton, 1996.

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: Paxton et al., 1992.

More recently, a new analysis has been provided (Schnatter et al., 1996) of the Pliofilm cohort that continues to use the three main sets of exposure estimates described above and the median of the three to develop a new set of indices of exposure per person. This technique, however, differs from the standard method of measuring total exposure to benzene (i.e., cumulative exposure = length of exposure × concentration) in that an “average” total concentration per person is determined from the job with the greatest exposure (maximally) and of longest duration from the exposure estimates above. This method enables the researcher to isolate subgroups with less exposure to specified concentrations of benzene and then calculate the risk of leukemia in those subgroups. In theory, these subgroups were less likely to be exposed to concentrations greater than a specified concentration.

The results of the Schnatter et al. (1996) analysis indicate 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 appears to be instability in both these risk estimates. Interestingly, for total leukemia, the “critical” concentrations for the median are lower and appear to fall in the range of 35 to 40 ppm and the risk estimates appear somewhat less erratic.

These figures are not inconsistent with estimates from Wong (1995), who utilized the parameter cumulative exposure to estimate the risk of AML in pliofilm workers. However, the Schnatter et al. (1996) analysis suffers from the same problems that the Wong (1995) and Rinsky et al. (1987) studies suffer in utilizing pliofilm workers to estimate risks at low levels of exposure to benzene: the data lack sensitivity. To assume that a critical concentration exists at the levels indicated and from which a "threshold" could be inferred is unwarranted based solely on this data set. 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 may be a lower critical region for AML if a larger data set were available.

To answer the third question, the net result of discarding the Ott et al. (1978) and the Wong et al. (1983) studies would be to change the unit risk estimate little. 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 state that their data for risk assessment purposes should not be used because of several factors (i.e., small number of events, competing exposures to other potentially hazardous materials, and the uncertain contribution of unquantified brief exposures).

While the Wong et al. (1983) cohort has ample power to detect a risk of leukemia, and the update (Wong, 1987) includes estimates of personal exposure to benzene, the estimates apparently are not reliable. Wong (1987) states 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 decision was made to calculate a single overall unit risk estimate by obtaining the geometric mean of four maximum likelihood unit risk estimates generated from the Ott et al. (1978) and Rinsky et al. (1981, 1987) studies, 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 similar conditions, 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 numbers range from 1.1×10^{-2} to 2.5×10^{-2} and can be found in section 3 (table 4). By inspection, the inclusion of data from Ott at 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 and have questionable validity, will likely result in little change from the 1985 estimate based on the epidemiologic data alone.

2.2. LABORATORY ANIMAL DATA

Studies on the carcinogenicity of benzene in rodents include inhalation exposures in 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). 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 induced in two series of experiments in C57BL/6 mice (Snyder et al., 1980) and CBA/Ca (Cronkite et al., 1989). While the reason for the difference in lineage of hematopoetic cancers induced in the mouse and humans is not fully understood, it may be related to differences in hematopoesis. Lymphocytes are 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 the 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 have some relevance to human leukemia.

2.3. MODE-OF-ACTION INFORMATION

Much of the toxicology research summarized herein has focused on elucidating the nature of the mechanisms through which benzene exerts its leukemogenic effects. The central issue to 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 is to establish whether the mechanisms that are operative in laboratory animals are similar to mechanisms operative in humans and how to account 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. This requires an understanding and description of both physiologically and metabolically driven pharmacokinetic processes. Pharmacodynamic characterization is also necessary, i.e., description of the key mechanisms through which the dose at the target site elicits the ultimate adverse response. Processes such as altered gene regulation, cytotoxicity, and cell proliferation are processes thought to be important for benzene leukemogenesis. A quantitative understanding of the mechanisms involved along the exposure-dose-response continuum can aid in integrating the available data for risk assessment purposes.

Benzene has been established as a human leukemogen, but the mode of action by which it produces leukemia remains unclear. This section is devoted to discussing recent data, including evidence on the role of dosimetry and toxicant-target interactions, that may help to elucidate a mode of action for benzene-induced leukemia.

2.3.1. Mutagenicity and Genotoxicity

Benzene generally has yielded negative results in gene mutations 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. 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.

Aneuploidy, the loss and gain of whole chromosomes, is common in 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, myelodysplastic syndrome, and pancytopenia (Vigliani and Forni, 1976). A recent report by Zhang et al. (1996a) showed that the induction of aneuploidy of chromosome 9 as measured by fluorescence in situ hybridization (FISH) in interphase lymphocytes from benzene-exposed workers is significantly elevated only at high levels of exposure (>31 ppm in air). However, an unpublished study has 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). The human evidence for aneuploidy induction also is supported by in vitro experiments. Hydro quinone 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 spleen cells following oral benzene exposure in mice (Chen, et al., 1994). Centromere-containing micronuclei are thought to be formed when a whole chromosome is lost during mitosis. Thus, considerable evidence supports the assertion that benzene and its metabolites are able to produce aneuploidy in a variety of systems.

In addition to causing loss and gain of whole chromosomes, benzene exposure causes clastogenicity. Recent studies using new methods have shown that benzene metabolites induce strand breaks in human cells (Plappert et al., 1994; Anderson et al., 1995). Further, experiments in rodents have provided consistent evidence from a number of studies that benzene exposure causes increased frequency of micronucleated cells (summarized in ATSDR, 1996). Micronuclei also are seen in human cells exposed in vitro to various metabolites and combinations of metabolites (Zhang et al., 1993; 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 ternary 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 (1995) corroborated the phenol and hydroquinone synergy. Using an antikinetichore-specific antibody and FISH, they demonstrated that both chromosome breakage and loss were induced and that the relative frequency of these events were indistinguishable whether mice were treated with benzene (440 mg/kg) or the binary mixture of hydroquinone and phenol (60/160 mg/kg). There is also human evidence of clastogenicity from reports of unstable chromosome aberrations in exposed humans (Aksoy, 1989; Forni, 1971; Sarto et al., 1984; Sasiadek, 1992; Tompa et al., 1994; Van den Berghe et al., 1979). For example, one report found that lymphocytes of exposed workers had increased frequency of chromosome aberrations, most of which were acentric fragments, presumably the products of double strand breakage (Sarto et al., 1984).

Growing evidence is beginning to implicate benzene in producing the chromosomal rearrangements associated with AML and myelodysplastic syndromes, such as interstitial deletion, inversion, or translocation. 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. Recent evidence that may relate to the ability of benzene to induce rearrangement comes from studies in which the glycophorin A (GPA) gene mutation assay was used to examine the type of mutations produced by benzene in human bone marrow (Rothman et al., 1995). The GPA assay measures somatic cell mutation frequency in peripheral erythrocytes. Because mature erythrocytes lack a nucleus, mutations expressed in these cells must have occurred in precursor erythroid cells or stem cells in the bone marrow. The assay detects a spectrum of mutational mechanisms, but results show that the most significant increase in benzene-exposed persons were changes that arose through gene conversion, such that heterozygous individuals became homozygous for one of their two alleles. Precisely how this type of genetic change occurs is not measured by the assay, but a likely mechanism is mitotic recombination after damage of one allele. These data do not directly address the question of whether benzene can cause translocations and other structural aberrations; however, changes at the GPA locus of this type do indicate that interchromosomal exchange occurred. Therefore, while the evidence is not currently strong, there is reason to suspect that benzene may be able to induce rearrangement in concert with its clastogenic and anueploidogenic properties.

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 experiments using concentrations ranging from 25 to 880 mg/kg and treatments ranging from a single i.p. injection to daily injections for 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). More recently, data obtained using accelerated mass spectrometry, which has tremendous sensitivity, show that the formation of protein and DNA adducts in mouse bone marrow is linear over 8 orders of magnitude to doses as low as 700 pg/kg (Turtletaub et al., 1996). Indeed, the dose range that can be studied by this technique is remarkable, and benzene was shown to produce a linear dose-response curve for adduct formation between a dose equivalent to one cigarette to doses equivalent to high occupational levels of exposure. Using the "comet assay," which detects strand breaks and alkaline labile sites in DNA, Plappert et al. (1994) observed damage in bone marrow with 100 ppm benzene in mice exposed 6 h/day for 5 days.

2.3.2. Metabolism

The first critical event in benzene carcinogenicity is conversion of benzene to active metabolites (figure 1). Benzene is first metabolized in the liver, mainly via cytochrome P4502E1. The major oxidation product is phenol, which is either conjugated, primarily to phenyl sulfate in humans, or further hydroxylated by P4502E1 to hydro quinone (Snyder and Kalf, 1994). Hydroquinone is then secondarily converted to other highly toxic products (discussed below). Other major products of primary benzene metabolism include catechol and *trans, trans*-muconic acid (Witz et al., 1990a). The latter is presumed to be formed from the ring opening of benzene epoxide via benzene oxepin, or perhaps benzene dihydrodiol. The intermediate product *trans*, *trans*-muconaldehyde has genotoxic properties and could play a role in benzene toxicity (Witz et al., 1990b). However, the selective toxicity of benzene to blood and bone marrow is unlikely to be explained by *trans, trans*-muconaldehyde alone. Further, it is unlikely that significant quantities of *trans, trans*-muconaldehyde escape hepatic glutathione and are transported to the bone marrow. Studies have shown that little or no *trans, trans*-muconaldehyde is likely to leave the liver, bringing its role in benzene toxicity into question (Brodfuehrer et al., 1990).

Secondary metabolism of the phenolic products of benzene generally is regarded as a critical aspect of benzene toxicity. All of the phenolic metabolites of benzene can be oxidized by myeloperoxidase and other peroxidase enzymes to their active quinones and semiquinone radicals (Smith et al., 1989; Subrahmanyam et al., 1991). These species are highly toxic by directly binding to cellular macromolecules and/or generating oxygen radicals through redox cycling. There is now strong evidence that the quinone products of secondary metabolism and related free radicals are the ultimate toxic metabolites of benzene. Specifically, 1,4-benzoquinone and its semiquinone radical, derived from hydroquinone, are likely to be the most critical toxic intermediates. The conversion of phenol to diphenoquinone and radical intermediates also could play an important role, as could oxidation products of 1,2,4-benzenetriol. Although formed in small amounts, 1,2,4-benzenetriol has potent effects (Zhang et al., 1993, 1994). The role, if any, of 1,2-benzoquinone derived from catechol remains unclear at this time.

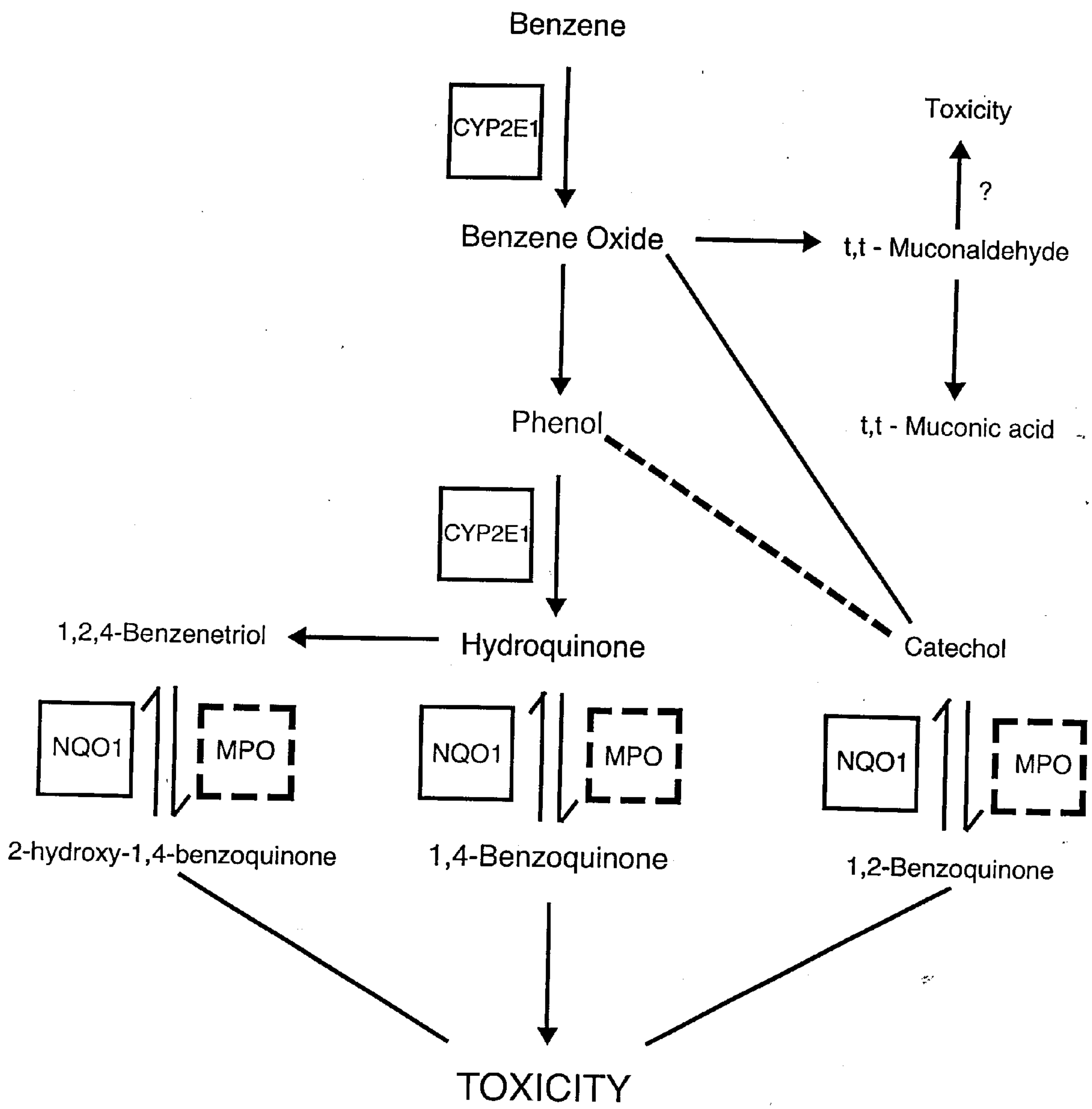

Figure 1. Key metabolic activation pathways in benzene toxicity.

Recent human studies show that the dose-response curve for benzene-induced leukemia has been shown to be supralinear because the formation of toxic metabolites plateaus above 25 ppm benzene in air (Rothman et al., 1996; Bechtold et al., 1996). The risk ratios for hematopoietic malignancies tend to remain somewhat constant albeit significantly elevated at exposure levels ranging from less than 10 ppm-years to over 400 or more ppm-years based on Chinese cohort data (table 2 in Hayes et al., 1996). On the other hand, the Rinsky et al. (1981, 1987) data show a definite dose-response relationship in pliofilm workers (table 1) from 40 to over 400 ppm-years of exposure. The Chinese data are not inconsistent with the knowledge that massive exposure to benzene suppresses the hematopoietic system. However, with respect to levels of benzene below 10 ppm, the dose is predicted to be linear with the risk of leukemia (Bois et al., 1996). This is based on three cases, however, and there are insufficient human data at such low levels to validate the supposition. Most human studies deal with subjects exposed to much greater levels of benzene.

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 is the result of subtle interplay of oxidation and conjugation pathways and 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.

2.3.3. 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 acute myelogenous leukemia (AML). Myelodysplastic syndromes (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. Irons and Stillman (1996) have summarized much of the extensive literature that exists relating to secondary leukemia involving either therapy or occupational exposures. It is generally recognized that chromosomal aberrations or deletions can alter the regulation and function of protooncogenes and other growth-promoting genes. 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 are found in the great majority, while 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.

2.4. 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. 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; IARC, 1982; ATSDR, 1996). It is also likely that exposure is associated with a higher risk of chronic lymphocytic leukemia and multiple myeloma (DeCouflé et al., 1983; Rinsky et al., 1987). 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).

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, 1996). Benzene induces peripheral blood abnormalities and disrupts hematopoiesis at separate compartments of blood cell formation (i.e., white, platelet, and red) (ATSDR, 1996). Granulocytic and erythropoietic progenitor cells are significantly depressed. Chromosomal breakage and loss are increased in mice from exposure to benzene or its metabolites, 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. These effects are produced by synergistic interaction 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 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.

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 risk number 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 (Thorslund 1988, 1993; Brett et al., 1989; Crump 1992; Paxton et al., 1994; Cox, 1996). 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, relative and absolute risk models, 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 i^{th} group based on the mortality rates in a comparison population, d_i the average benzene dose in the i^{th} group, and Y_i the number of person-years in the i^{th} group. The relative risk model is of the form

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

and absolute risk model 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 i^{th} 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, asserting that these devices consistently underestimated exposure by as much as 50%. It was further assumed that the working week was on the average 51 hours for the Pliofilm workers, not the 40 hours usually assumed. Other assumptions are also given to justify his 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 in the period 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 are listed as being exposed to as much as 40 ppm 8-hr 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 the course of their employment during those early years. These levels tended to drop in time as efforts to improve air quality in the plants were implemented.

Using a simplified version of a model developed by Moolgavkar and Knudson (1981), Thorslund (1988) presented several risk estimates, all of which are at least an order of magnitude smaller than the EPA risk numbers.

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)×(7/5)×(52/50). The resultant risks at 1 ppb and 1 ppm are given in table 4.

Crump (1992) 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 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 are 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 subset of calculations of Crump (1992) appear in Crump (1994).

Thorslund (1993), departing from his 1988 risk assessment, later used a more conventional approach (i.e., additive risk model) to calculate risks. The newer risk estimate at 1 ppm (Thorslund, 1993) with the linear-quadratic model was increased about eight times from his previous report (Thorslund, 1988) of 1.0×10^{-3} to 7.8×10^{-3}, utilizing the same (Crump and Allen, 1984) exposure data. Also, the newer estimate, 7.8×10^{-3} per ppm, is one-half that of the 1985 EPA estimate of 1.8×10^{-2} (based only on the Rinsky et al. [1981] cohort and Crump and Allen [1984] exposure estimates) when the same (additive) model was used. Only AML was used in Thorslund's (1993) calculations, although other leukemia cell types may be associated with benzene exposure. When the linear-quadratic model was used with the Paustenbach et al. (1993) exposure estimates, the risk at 1 ppm was estimated to be 4.7×10^{-3} (note that a 95%

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

Source	Risk at 1 ppm	Risk at 1 ppb	Exposure	Model
U.S. EPA, 1985	1.8×10^{-2}	1.8×10^{-5}		Additive risk
	4.1×10^{-2}	4.1×10^{-5}		Relative risk
Thorslund, 1988	1.4×10^{-4}	1.4×10^{-10}	Crump and Allen, 1984	Quadratic
	1.0×10^{-3}	1.0×10^{-6}	Crump and Allen, 1984	Linear quadratic
	3.2×10^{-3}	3.2×10^{-6}	Crump and Allen, 1984	One stage/one hit
	3.5×10^{-3}	3.5×10^{-6}	Crump and Allen, 1984	Two stage/two hit
Brett et al., 1989	5.2×10^{-3} to 2.5×10^{-2}	3.9×10^{-6} to 1.1×10^{-5}	Rinsky et al., 1984	Conditional logistic
	4.3×10^{-1} to 8.1×10^{-1}	2.9×10^{-5} to 3.4×10^{-5}	Rinsky et al., 1984	Conditional logistic
Paxton et al., 1994	2.2×10^{-3}	1.9×10^{-6}	Crump and Allen, 1984	
	4.6×10^{-3}	3.5×10^{-6}	Paustenbach et al., 1993	
	1.8×10^{-2}	8.9×10^{-6}	Rinsky et al., 1984	
Crump, 1992	1.1×10^{-2} to 2.5×10^{-2}	1.1×10^{-5} to 2.5×10^{-5}	Crump and Allen, 1984	Linear
	5.4×10^{-3} to 2.5×10^{-2}	4.5×10^{-6} to 2.6×10^{-5}	Crump and Allen, 1984	Nonlinear
	7.1×10^{-3} to 1.5×10^{-2}	7.2×10^{-6} to1.6×10^{-5}	Paustenbach et al., 1993	Linear
	8.6×10^{-5} to 6.5×10^{-3}	8.6×10^{-11} to 5.6×10^{-6}	Paustenbach et al., 1993	Nonlinear
Thorslund, 1993	1.2×10^{-2}	1.2×10^{-5}	Crump and Allen, 1984	Linear, additive
	7.8×10^{-3}	7.8×10^{-6}	Crump and Allen, 1984	Linear quadratic, additive
	5.5×10^{-3}	5.5×10^{-6}	Paustenbach et al., 1993	Linear, additive
	4.7×10^{-3}[a]	4.7×10^{-6}	Paustenbach et al., 1993	Linear quadratic, additive

[a]95% upper bound is provided because of instability of the maximum likelihood estimate (linear coefficient was estimated to be 0).

upper bound must be used for the later risk number because of the instability of the point estimate; the linear coefficient was estimated to be 0). These risk estimates are about two to four times smaller than the corresponding EPA additive risk estimate of 1.8×10^{-2}, depending on whether the Crump and Allen (1984) or the Paustenbach et al. (1993) exposure estimates were used.

Recently, Cox (1996) reassessed benzene risks using internal doses and Monte-Carlo uncertainty analysis. He reexamined the physiologically based pharmacokinetic models of benzene metabolism in animals and humans, and a Monte-Carlo uncertainty analysis based on maximum-entropy probabilities. Bayesian conditioning was used to develop an entire probability distribution for the true but unknown dose-response function. He concluded that the excess risk due to benzene exposure may be nonexistent (or even negative) at sufficiently low doses.

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 studies alone.

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

Table 5. Evidence that benzene-induced leukemia is nonlinear at low doses

Category	Pro	Con
Genotoxicity	Micronucleus induction by benzene and its metabolites in the mouse bone marrow and in human cells in vitro is nonlinear.	Micronucleus assay is relatively insensitive and may not show effects at low doses.
	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).	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.
	DNA adduct formation is observed by P^{32}-postlabelling only at high doses.	Data obtained using accelerator mass spectrometry shows that the formation of DNA adducts in mouse bone marrow is linear to very low doses.
	Oxidative DNA damage may contribute to benzene genotoxicity (Kolachana et al., 1993) but has a high rate of repair.	Errors during repair may cause point mutations.
Theoretical	Hematotoxicity is required for leukemia induction, and this will have a threshold.	Hematotoxicity may increase risk of malignancy but has not been shown to be a prerequisite.
	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 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.
	The cells in the bone marrow have numerous defense mechanisms.	There is a high background exposure to benzene and its metabolites, so additional exposure could escape defenses.

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). Also, recent data obtained using accelerated mass spectrometry show that the formation of protein and DNA adducts in mouse bone marrow is linear over 8 orders of magnitude to doses as low as 700 pg/kg (Turtletaub et al., 1996). Indeed, the dose range that can be studied by this technique is remarkable, and benzene was shown to produce a linear dose-response curve for adduct formation between a dose equivalent to one cigarette to doses equivalent to high occupational levels of exposure.

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 that can be made is the fact 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 to benzene and its metabolites 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 finding from this update is that it reaffirms the benzene interim unit risk estimates derived in EPA's 1985 interim risk assessment (U.S. EPA, 1985). The 1985 interim risk assessment established the probability of humans developing cancer from exposure to 1 ppm of benzene. Two main concerns had to be addressed before this conclusion could be reached. The first involves using the updated epidemiologic data from Rinsky et al.'s (1987) cohort of Pliofilm workers as well as selecting the appropriate estimates of exposure for the derivation of the unit risk estimate. The second major issue involves the continued application of the low-dose linearity concept to the model from which the unit risk estimates are generated. At present, there is insufficient evidence to reject this concept.

The update of Rinsky et al.'s (1987) cohort could have only a limited impact on the existing EPA (1985) interim risk estimates if the same exposure-response (linear) model and Crump and Allen (1984) exposure measurements were used. When the Paustenbach et al. (1993) higher estimated exposure measurements were substituted, the corresponding risk estimates (SMRs) were reduced by only a factor of, at most, two. The linear-quadratic exposure-response model used by Thorslund (1993) deals with the concept of low-dose linearity, and the resultant risk estimates also are not markedly different from the 1985 interim risk EPA estimate. None of the approaches toward estimating exposure have greater scientific support than any other because ambient air benzene exposure data did not exist before 1946 in the Pliofilm workers. 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 as well as the limitations of the exposure characterization and response information in the epidemiologic data.

Without extensive analyses of the raw data, only theoretical discussions of the possible impact on risk estimates under various exposure assumptions and presumed etiologic mechanisms can be provided. There are two approaches to address this issue; one is to assume a biological mechanism of benzene-induced leukemia (e.g. to assume that benzene-induced leukemia involves a sequence of genetic and epigenic changes, and that these steps are effected by benzene exposure). Another approach is to assume a linear model, as was used in this assessment. Using the linear model, the use of cumulative exposure would have less impact on the resultant risk estimate if the concentration was roughly constant during the work history of the cohort. However, if exposure concentrations in early work history were higher than the later years, the unit risk could be over-estimated.

While the risk estimates would be significantly different if a nonlinear exposure response model was found to be more plausible, characterizing the shape (i.e., the nonlinearity) of the exposure-response curve would still 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 supra-linear since the formation of toxic metabolites plateaus above 25 ppm benzene in air (Rothman et al., 1996; Bechtold et al., 1996). 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. The 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 to reject a linear dose-response curve for benzene in the low-dose region, and there is insufficient evidence 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 this knowledge is not available at the present time, the Agency's 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 4.7×10^{-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 (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 begins at levels under 40 ppm-years, although the trend does not attain statistical significance until the dose of 200 to 400 ppm-years is reached. Therefore, we are not as confident that the risk begins to rise below 40 ppm-years (1 ppm for 40 years). However, this may be a matter of the lack of power to detect a risk as significant below this level. 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, recent data from the Chinese cohort (Yin et al., 1989) implies that the risk of AML is well below 200 ppm-years, although the data analysis is still incomplete. 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 very 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. 2.5 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. It is possible that peak exposures could have occurred at any time during employment, however, that information is unavailable.

Although the authors of these studies developed dose-response data for some members of their respective cohorts, it was clear that these same workers were subject to other concomitant exposures that were also present in the workplace. These also might affect the risk of cancer. Methodological problems also existed in these studies. These considerations precluded the use of these studies in the unit risk calculations.

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 based on the current epidemiologic data. Because of this lack of data at low levels, the Agency could not confidently say that the risk begins to increase at exposures below 40 ppm-years, although some data seem to suggest that. Based on the above discussion, the point of departure (POD) is determined at a level of 40 ppm-years in the occupational environment, which assumes 8 h of exposure per working day. Assuming that a 24 h exposure would incur a threefold risk increase, then from an 8 h exposure, the POD is likely to be one-third of 40 ppm-years, or about 13 ppm-years.

To put this POD of 190 ppb into perspective, 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 [1996] 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, Louisiana, 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 to 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 suggest smoking as a source for indoor benzene concentrations (Wallace, 1987, 1989). Brunnemann et al. (1989) report 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, but 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 190 ppb as the POD, the margin of exposure (MOE) can be calculated for several of these levels. Since the 190 ppb is a 70-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 190/4.7, or about 40. If one assumes that the ambient indoor levels cited above of 2.2 ppb represent actual exposures to nonsmokers, their MOE would be 190/2.2, or 86. 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 17 to 23. The MOE for other exposure scenarios can be similarly calculated.

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, for example, 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 age, race, sex, and ethnic group patterns; different secular trends; and different etiologic factors (Linet, 1985).

There exist very limited data on children from environmental exposure to benzene. 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. The authors concluded that muconic acid could be used as a biomarker in children for environmental exposure, but there have been no studies found that used this biomarker to determine actual benzene exposure to children.

In summary, children may represent a subpopulation at increased risk due to factors that could increase their susceptibility to effects on 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). 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. Additional research into these areas will promote a better understanding of how benzene causes cancer, particularly the mechanism of benzene-induced leukemia. Several classes of data are needed on humans, that is, more extensive epidemiologic data with good exposure estimation, to permit verification and validation of the prediction models. Additionally, 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 and in vitro studies of cell-specific metabolism and toxicity in defined human bone marrow cell populations may be of use.

Continued basic research in hematopoiesis and leukemia biology is critical. Issues of importance are 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 myelodysplastic syndromes (MDS) and AML also is needed to assist in identifying the stages of leukemic transformation.

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. The 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 (t-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 are less well characterized. For example, the factors controlling whether patients who suffer benzene-induced myelosuppression progress to AML or recover after exposure is reduced or removed are unknown. To what extent susceptibility factors will dictate leukemia risk and to what extent leukemia is a manifestation of stochastic processes are not known.

Particular emphasis should be placed on research on those subpopulations who are believed to be at increased risks (e.g., infants and children, the elderly). Research is needed to show how growth, development, and aging affects the risk to humans. In addition, environmental and epidemiological studies are needed to better determine the environmental exposure levels that sensitive subpopulations such as pregnant women, infants and children, and the elderly are likely to encounter on potential benzene risks. 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.

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