PART B

HEALTH EFFECTS OF BENZENE

Department of Health Services

Epidemiological Studies Section

November 1984

PART B. A REVIEW OF THE HEALTH EFFECTS BENZENE

LIST OF TABLES

LIST OF FIGURES

SECTION B: HEALTH EFFECTS OF BENZENE

The documented human health effects of benzene have occurred mainly as the result of exposure in occupational settings. A brief exposure to 20,000 ppm can cause fatalities by producing intoxication and respiratory and circulatory collapse. Studies in humans and animals have not implicated benzene as a cause of birth defects. However, human exposures in the hundreds of parts per million range have resulted in varying degrees of depression of red and white blood cell production and in some cases have caused fatal aplastic anemia. Chromosomal abnormalities have been documented in humans years after toxic exposures in this dose range.

Exposures of tens to hundreds of parts per million have been associated epidemiologically with an increased incidence of leukemia. Chromosomal damage has occurred in animals at these levels. Recent animal cancer bioassays have shown benzene to cause leukemia and a variety of other cancers. The staff of the Department of Health Services (DHS) can find no evidence of substantial scientific disagreement about the above-mentioned effects and agrees with the International Agency for Research in Cancer (IARC) that there is sufficient evidence to consider benzene a human carcinogen.

The key issue for this evaluation is whether levels of benzene in the low parts-per-million or parts-per-billion range could cause human leukemia or other cancers at rates which are of concern, even though such rates are below the magnitude detectable by epidemiological study. DHS believes risks of adverse health outcomes other than cancer are not expected at these lower dosage levels. To address this issue, the first question which must be asked is whether benzene has a carcinogenic

threshold. To argue for a threshold, the staff of the DHS would require strong positive evidence that benzene acts only through mechanisms which ought to have a threshold. No positive evidence exists for this position. On the other hand, understanding of the mechanisms of benzene's action is not sufficient to prove definitively that there is not a threshold. Since statistical and mechanistic arguments for a benzene threshold are not compelling, DHS considers that benzene should be treated as a substance without a threshold for carcinogenesis.

The DHS has estimated the low-dose carcinogenic potency of benzene using both animal and epidemiological data. This has required extrapolation from imperfectly estimated high exposure levels in epidemiological studies or from well-measured high levels in animal studies. Such extrapolations depend on many assumptions, each with its own uncertainties. Figure A shows dose-response curves derived from these human and animal studies. The X axis in this figure shows the lifetime-averaged daily exposure in parts per billion (ppb). The Y axis shows the added lifetime risk which would theoretically result from each level of exposure. This is expressed in excess cases of cancer per million people exposed.

Each line is described below but, in summary, the very high and very low estimates of risk are provided by the Mantel-Bryan and Probit models respectively which the DHS does not advocate for reasons given in the text.

Animal and epidemiological studies provide risk estimates which are within a factor of 10 of each other, and the DHS recommends using these estimates to provide a range of risk.

The benzene risk assessment carried out by EPA's Carcinogen Assessment Group (CAG) was based on data from three epidemiological studies. More recently CAG reanalyzed data from one of these, the Rinsky re-evaluation of the Infante study. In Figure A, we present the theoretical dose response curve derived from the more recent Rinsky estimate (line 5) and the curve based on the average of the three epidemiological studies (line 6).

Line 4 shows the theoretical dose-response curve derived from lymphomas and leukemias in mice exposed to benzene by gavage. The multistage extrapolation procedure of Crump was used for this maximum likelihood estimate. This estimate for mice is similar to the human epidemiology estimates and in this particular graph overlies the Rinsky risk line. These human leukemia and the animal lymphoma/leukemia risk assessments thus suggest a theoretical added lifetime risk of around 22 to 50 cases per million for a lifetime exposure to 1 ppb.

The model that is selected to extrapolate animal high dose exposures to low dose exposures humans will encounter in ambient air can produce estimates of risk which vary by many orders of magnitude. Line 1 shows the Mantel-Bryan procedure for estimating risk for the most sensitive site in animals, the preputial gland in mice. Line 2 shows the 95% upper confidence limit for risk estimates based on the multistage model for this same site.

Line 7 shows the probit model for preputial cancers which lies far below the others. There are theoretical reasons for preferring the multistage model which are described in the text. Mice show evidence for a variety of cancers after exposure to benzene. These include mammary and ovary cancers which have not been

adequately studied in man. The theoretical risk for these cancers combined are shown by line 3.

Since epidemiology has not determined whether humans as well as animals run a risk of mammary or ovarian cancer, and since it is common practice to use the 95% upper confidence limit based on the most sensitive site and species to calculate the upper bound of risk, the staff of the DHS suggests that the ARB consider dose-response curves which lie between the EPA estimates based on the data from the Infante, Ott, and Aksoy epidemiologic studies (22×10^{-6}/1 ppb) (line 6) and the 95% upper confidence limit based on the most sensitive site in rats and mice, the preputial gland (170×10^{-6}/1 ppb) (line 2):

In summary, the DHS determines that:

(1) There is sufficient evidence to consider benzene as a human carcinogen.

(2) For lack of positive evidence to the contrary, benzene sould be treated as if it had no thresold for carcinogenic effect.

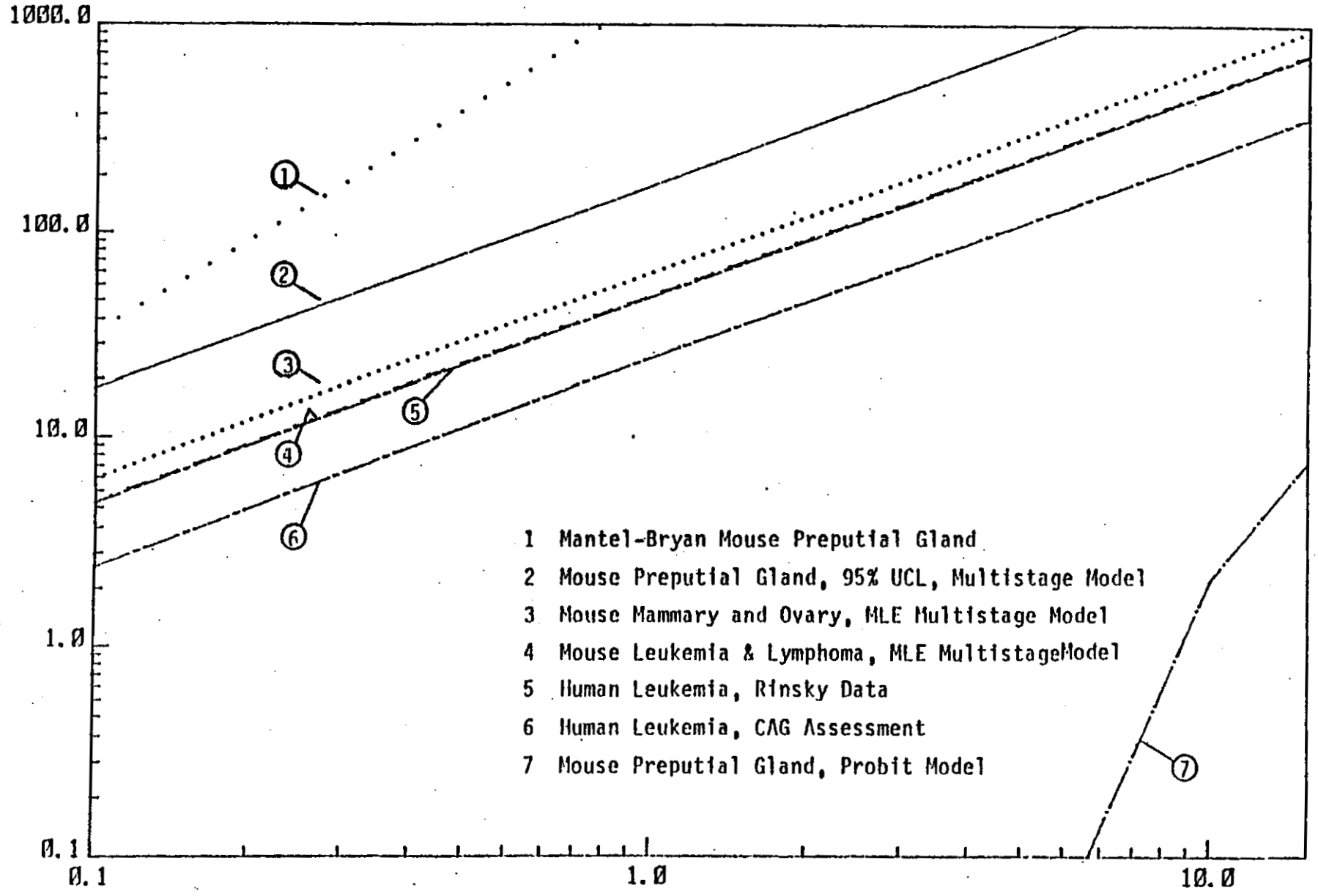
(3) Based on available evidence it is scientifically defensable to consider the lifetime risk from continuous exposure to 1 ppb of benzene in air to be between 24 and 170 excess cancer cases per million persons.

(4) Other health effects are not expected to occur at usual ambient levels.

(5) Benzene should be listed as a toxic air contaminant.

Figure A

CANCER RISK FROM BENZENE



I. Absorption, Distribution, and Metabolism

The absorption, distribution, metabolism, and excretion of benzene have been reviewed for both humans and animals test species by the IARC (1982). The purpose of the review was to identify information that would be of value in determining the human health impact of benzene exposure. This information would be particularly important for the understanding of the mechanism of benzene induced carcinogenesis and the development of a dose-response relationship. Data on the absorption and metabolism of benzene are critical for the application of pharmacokinetic models used in low dose extrapolation of cancer risks. After reviewing the available literature, the position of the staff of the DHS is as follows:

1) The existance of the proposed carcinogenic metabolite of benzene, benzene oxide, is still only speculative (Goldstein, 1983).

2) Although animal studies have demonstrated several benzene metabolic pathways, there is insufficient data with respect to pharmacokinetics (e.g., the rate determining step (rds) for the formation and inactivation of the carcinogenically responsible species, rate and equilibrium constants for the rds, information on the inducability of the critical enzymes, etc.). to model metabolism even in animals (Anderson, et al., 198).

3) There is insufficient human data with respect to the distribution or metabolism of benzene to apply any pharmacokinetic model derived from animal studies to humans risk assessment.

II. Short-Term Tests and Chromosomal Studies

1. Introduction

IARC has reviewed research on the genotoxic effects of benzene published up to 1981 (IARC, 1982). The work they reviewed includes results of studies on gene mutations or the DNA damage in bacteria and fungi, gene mutations or the DNA damage in mammalian cells in vitro and in vivo and chromosomal aberrations detected in animals and humans. The staff, in the following section on short-term tests and chromosomal studies on benzene, emphasized discussion of studies published subsequent to the 1982 IARC document. Studies reviewed by IARC were generally not discussed in our document unless:

1) The study was felt to be an important example of a specific category of short-term test; or

2) The study needed further evaluation than given by the IARC working group.

Information concerning the validation, strengths, and limitations, qualitative and quantitative characteristics of individual short-term tests will not be discussed here, but are available in review publications by Brusick, 1980; Bridges et al., 1982 and by Hsu, 1982, to cite a few examples.

Some of the studies reviewed were reported as abstracts of presentations given at recent meetings. Abstracts are difficult to evaluate and are therefore denoted in our text.

2. Gene Mutations or DNA Damage in Bacteria

IARC (1982) reported that benzene was not mutagenic in bacterial point mutation assays such as the Salmonella typhimurium microsome test of Ames et al. (1975). However, in a few of the studies cited, for example, Shahin and Fournier (1978) and Lyon (1976), a closed system to minimize evaporation of benzene during incubation was not used. This makes interpretation of the results difficult.

The study by Shahin and Fournier (1978) reviewed by IARC was basically an investigation of the mutagenicity of tar sand fractions using benzene as a solvent in the Ames assay. Benzene, however, has very limited solubility in aqueous systems such as the Ames assay, and a closed system was not used.

Benzene oxide, a proposed reactive metabolic intermediate of benzene, was tested by Kinoshita et al. (1981) in a closed-liquid incubation system. They reported an increasing mutation frequency for TA100 (increased his-positive revertants per million viable cells) with increasing doses of benzene oxide without the need for an additional metabolic activation system. The absolute number of revertants, however, increased by less than a factor of two and there was no linear dose-response relationship

demonstrated. This non-linearity may, in part, be due to toxicity of benzene oxide to bacteria. IARC noted that these studies needed to be repeated.

Jung et al. (1981) tested a variety of related oxides of benzene using the standard Ames assay or a modified assay which exposed Salmonella to the test agent in a desiccator (closed system). Twelve oxiranes out of 17 tested were weakly to moderately mutagenic in strain TA100 without the addition of metabolic enzymes. Benzene oxide, however, was not mutagenic using either of the procedures. The actual dose-response curves and numbers of bacteria surviving treatment were not reported for the oxiranes which were mutagenic. Only the slope of the line from the linear portion of the dose-response curve was reported.

3. Gene Mutation and DNA Damage in Mammalian Cells

Studies reviewed by IARC (1982) regarding the mutagenicity of benzene in mammalian cell gene mutation assays have generally been reported to be negative.

Crespi and Penman (1984, abstract) and Crespi et al. (1984) have reported that benzene at 1 mg/ml (28 hrs exposure time) was mutagenic in a new human lymphoblastoid cell assay and which has an endogenous metabolic activation system. The paper describing the assay and its validation is in press (Crespi and Thilly, 1984). The authors are currently repeating their initial work and incorporating more doses to establish dose-response curves (Crespi, personal communication). Staff believed that the data are very limited for this study because the response reported was only about two

times background and there was only one dose tested. The researchers involved believed that the results were significant (Crespi, personal communication).

4. Chromosomal Effects in Animals

4.1 Sister Chromatid Exchanges (SCEs)

Hook et al. (1984, abstract) and Erexson et al. (1984, abstract) recently reported that benzene induced SCEs in vivo in mice exposed for 4 hours at concentrations of 2815 ppm (Hook et al.) and for 6 hours at 10, 100, 1000 ppm (Erexson et al.). The DHS staff notes that these and other studies which are reported at meetings and are presently published in abstract form are difficult to evaluate.

4.2 Micronuclei

One test developed for detecting possible clastogenic (chromosome-breaking) effects of compounds in animals is the micronuclei assay (Schmid, 1975; Heddle, 1973). Micronuclei are thought to be broken fragments of chromosomes or chromatids which lag behind intact chromosomes during cell division. They are conveniently detected in polychromatic erythrocytes which generally expel their nucleus but leave behind these fragments or micronuclei. IARC has reviewed a number of these studies in detail. The studies generally consisted of treating animals (usually mice or rats) with benzene, usually by the oral route of administration for 1 or 2 days, and sacrificing the animals a few hours to days after the last treatment. Bone marrow is obtained and examined for micronuclei. In most

of the studies reviewed by IARC, there were significant increases in micronuclei. Some of the studies included dose-response relationships.

Erexson et al. (1984, abstract) have confirmed these earlier studies and reported effects at relatively low levels of brief exposure periods. They reported that there were dose-response relationships for mice exposed by inhalation to 10, 100, and 1000 ppm benzene for 6 hours. Benzene caused 0.9 $\pm$ .06, 1.03 $\pm$ .07 and 2.81 $\pm$ .08 percent micronuclei at 10, 100, and 1000 ppm, respectively. The control value was 0.21 $\pm$ .03. The effect at 10 ppm was statistically significant.

Tice et al. (1984, abstract) examined slides from inhalation studies previously conducted with C57BL/6 mice and found that mice exposed to 10, 25, 100, and 400 ppm benzene for 9 days and sacrificed 1 day later, had significantly elevated micronuclei in a dose-dependent manner.

4.3 Chromosomal Aberration

Studies reviewed by IARC (1982) on the chromosomal aberrations in bone marrow cells of animals have shown that:

1) benzene induces chromosomal aberrations in a number of animal species from different routes of administration;

2) some of these aberrations persist for days and weeks.

Styles and Richardson (1984) have recently reported results of their cytogenetic studies of rats exposed to 1,10, 100 and 1000 ppm benzene for 6 hours. Animals were sacrificed 24 hours after the end of exposure and analysis was carried out on 250 metaphases per animal from bone marrow cells. There were significant increases in the percentage of cells with abnormalities based on the group mean percentage data of a variety of aberration catagories, including or excluding chromosomal or chromatid gaps for animals exposed to 100 and 1000 ppm benzene. The categories of chromosomal damage examined were: chromosome or chromatid gaps, chromatid breaks, chromosome breaks or fragments, minutes and interchanges. There were elevated levels of chromosomal abnormalities (group mean percentage of cells with abnormalities) in rats exposed to 1 and 10 ppm, but these levels were not statistically significant. A positive dose-response relationship for most catagories of abnormalities was exhibited at benzene concentrations from 1 to 1000 ppm in this study.

5. Chromosomal Effects in Humans

Numerous investigators have reported that benzene causes DNA damage in mammalian cells in culture. For example, Morimoto (1983a) reported the induction of sister chromatid exchanges (SCEs) in human lymphocytes after the addition of benzene at 0.2, 1, or 5×10^{-3} M concentrations. Metabolic activation from an exogenous source (rat liver) was required to induce the SCEs. Reduced glutathion prevented induction of SCEs from benzene and two metabolites of benzene, catechol and hydroquinone. Morimoto and coinvestigators have published other reports on the induction

of SCEs in human lymphocytes treated with benzene (Morimoto and Wolff, 1980a, b; Morimoto et al., 1983).

Various investigators have reported that workers exposed to benzene had significant increases in chromosomal aberrations (for example, chromatid fragments and chromosome breaks) in bone marrow cells or lymphocytes. The reader is referred to review papers by Dean (1978) and IARC (1982) for discussion. Accurate data on benzene concentrations in the work environments are limited but reported to range from about 10 to a few hundred ppm. Many of the workers examined had gross clinical symptoms of benzene poisoning. However, there are reports of groups of individuals with increased chromosomal aberrations (for example, dicentric chromosomes) who were asymptomatic with respect to acute benzene toxicity (Tough and Court Brown, 1965; Tough et al., 1970; Funes-Cravieto, 1977).

Staff was aware that confounding factors such as smoking habits could affect the results of these cytogenetic studies. The reader, if interested, should evaluate these factors in each publication reviewed by Dean (1978) and by the IARC working group (1982). In the following study by Sarto et al. (1984), the investigators reported the use of matched control subjects. Matching was to the "extent possible" and included smoking habits.

Sarto et al. performed a cytogenetic study on 22 male factory workers exposed to low concentrations of benzene. Workers (mean age of 41.5$\pm$ 9.6 years) were exposed to benzene concentrations between 0.2 and 12.4 ppm during distillation of coal tar (Mean time of exposure, 11.4 $\pm$ 7 years).

Exposures to chemicals other than benzene may also have occurred. The occurrence in workers' lymphocytes of chromosome breaks and decentric fragments was statistically higher in workers exposed to benzene than in control subjects matched for sex, age, smoking habits and site of residence.

6. Other Tests for Genotoxicity of Benzene

6.1 DNA binding

Lutz and Schlatter (1977) reported that a benzene metabolite covalently bound to the DNA of rat liver cells in vivo. There was approximately 1.5 umoles of ^{14}C benzene bound per mole of DNA phosphate. Total benzene administered into the closed inhalation chamber of 2 liters was approximately 21 mg (average exposure time of 10 hours). Concentrations of benzene within the chamber were not measured or reported and there was no dose-response information. The number of rats used for the experiment, both for exposed and unexposed rates, appears to be limited. The authors reported that this level of binding is about 3000 times lower than the binding of N,N-dimethylnitrosamine to rat liver DNA.

Gill and Amed (1980) reported that ^{14}C benzene covalently bound to nucleic acids in bone marrow cells. This tissue is of interest since it is the site of action for benzene. The DHS staff feels that the authors do not clearly demonstrate covalent binding of ^{14}C benzene to nucleic acid fractions of bone marrow. There was very little radioactivity detected in 20 femurs tested.

Irons et al. (1980) reported that benzene metabolites covalently bound in the bone marrow of rats (bound to final residues that are left after organic solvent extraction of bone marrow). The specific binding of benzene or its metabolites to bone marrow DNA was not studied.

6.2 Drosophila

Benzene was not mutagenic in Drosophila melanogaster using a sex-linked genetically unstable system in which mutations are measured by noting changes in eye pigmentations (Nylander et al, 1978). Kale and Baum (1983) reported that there was significant induction of crossing over in the spermatogonia of male Drosophila treated with 27,000 ppm of benzene for 45 minutes. No dose-response information was presented.

6.3 Transformation

Benzene was tested for its ability to induce morphological transformations in Syrian hamster embryo cells in the absence of any exogenous source of metabolizing enzymes (Amacher and Zelljadt, 1983). Transformation is of interest because the progeny of transformed cells can ultimately produce tumors in synergenic hosts (Berwald and Sachs, 1965). Benzene induced positive morphologic transformation in the hamster transformation assay. In this study no assessments of dose-response relationships were made due to the limited number of colonies scored for any one test chemical.

7. Summary

Benzene is negative in bacterial and fungal mutagenicity assays. Whether this result is due, in part, to testing conditions needs to be further investigated. Also, the mechanism of action of benzene and its metabolites may be such that these test systems could possibly be insensitive to the reactive agent.

Benzene is not mutagenic in one published mammalian cell mutation assay. Unpublished work suggests that benzene is positive in another mammalian cell assay which used human lymphoblasts, which contain endogenous metabolizing enzymes.

Benzene is clastogenic (i.e., damages chromosomes) in animals as determined by the micronuclei test. One study reported a statistically significant induction of micronuclei at a dose of 10 ppm (6-hour exposure time). Benzene induces SCEs in mice in vivo and in human cells in culture.

Benzene causes chromosomal aberrations (chromosome and chromatid breaks, marker chromosomes [for example, dicentrics]) in animals and humans. Studies in humans and animals indicate that chromosomal damage can persist. The lowest dose at which damage occurs is difficult to determine in human studies, but in animal studies there appears to be elevated chromosomal aberrations at doses as low as 100 ppm (6-hour exposure time). Elevated levels of chromosomal aberrations occurred at benzene concentrations of 1 and 10 ppm, but the results at these dose levels were not statistically different from unexposed animals. Studies which indicate weak covalent binding of benzene or its metabolites to the DNA or other nucleic acids need to be confirmed.

III. Acute and Chronic Health Effects

1. Acute Toxicity

1.1 Experimental Animals

Inhalation exposure of relatively high doses can produce acute benzene intoxication and death in laboratory animals. Carpenter et al. (1944) described their observations of ten rabbits undergoing anesthesia with 35,000-45,000 ppm of benzene vapor in air. The average time required for light anesthesia was 3.7 minutes, 5.0 minutes for excitation and tremors, while death ensued in approximately 36 minutes. The narcotic threshold concentration is approximately 4,000 ppm, and concentrations above 10,000 ppm are usually fatal after several hours of inhalation (Leong, 1977). The lethal concentration for 50% of the animals (LC_{50}) in female Sprague-Dawley rats was reported as 13,700 ppm after a single four-hour exposure (Drew and Fouts, 1974).

The table below is a summary of oral tests to determine the lethal dose for 50% of the animals (LD_{50}) tests as reported by IARC (1982, p 109-110).

Year	Author(s)	Animal	Dose
1965	Cornish & Ryan	Male Sprague-Dawley rats	0.93g/Kg bw
		same	
1971	Kimmura	Young adults	3.4 g/kg bw
		Older adults	4.9 g/kg bw
1956	Wolf et al.	Wister rats	5.6 g/kg bw
1975	Withey & Hall	Male Sprague-Dawley	5.96 g/kg bw

Benzene is rated as moderately toxic by ingestion, inhalation, and skin absorption (Hawley, 1981).

1.2 Humans

As an acute toxin, benzene is a central nervous system depressant. The levels necessary to elicit this effect are many times higher than levels used to study chronic toxicity. One author (Flury) is cited by IARC (1982, p 116) as stating that single exposures to benzene vapor in the atmosphere at 20,000 ppm may be fatal within 5-10 minutes.

Fatal cases have occurred when workers entered enclosed spaces such as tanks where there was residual benzene. Effects observed following severe exposures are convulsive movements and paralysis followed by unconsciousness. Milder forms of acute intoxication produce an initial state of euphoria followed by giddiness, irregular heart beat, headache, dizziness, nausea, a staggering gait, and unconsciousness if the individual is not

removed from exposure. Breathlessness, nervous irritability, and unsteadiness in walking may persist, and delayed effects may arise and persist long after the acute incident (NIOSH, 1974). Autopsy findings in the case of acute benzene poisoning include extensive petechial hemorrhage in the brain, pleurae, pericardium, urinary tract, mucous membranes, and the skin. Ingestion of liquid benzene causes local irritation of the mouth, throat, esophagus, and stomach. Subsequent absorption into the blood leads to signs of systemic poisoning. Pneumonitis and bronchitis can also be present caused by the direct action of benzene as it is excreted from the lungs (NIOSH, 1974).

Liquid benzene may cause erythema and blistering of the skin, and a dry scaly dermatitis may develop on prolonged or repeated exposure. Investigations have shown that benzene is poorly absorbed through intact skin (NIOSH, 1974).

2. Hematotoxicity

2.1 Experimental Animals

Benzene causes myelotoxicity in animals and man. The severity is related to the dose, duration of treatment, and the test species. IARC (1982, p 110) cites reports which describe decreased leukocyte levels in rabbits, rats, and mice, and decreased uptake of radioactive iron into red cells (an indication of decreased erythrocyte production). Marrow aplasia, leukopenia, lymphocytopenia, anemia, and neutrophilia were also reported. Doses in these experiments ranged from 44 to 1000 ppm.

2.2 Human

It has been known since the earliest reports that benzene can cause aplastic anemia. Aplastic anemia is characterized by peripheral blood cytopenia and decreased marrow cellularity due to acquired or congenital hematopoietic progenitor cell failure. The hypocellularity varies greatly from conditions in which the marrow is completely devoid of recognizable hematopoietic precursors to those in which the precursors of only one cell line are absent or arrested in their development (Goldstein, 1977). Aplastic anemia has many causes. The etiology is uncertain in most cases. In a number of cases aplastic anemia may be preceeded by viral infections, drugs, or industrial toxins. Leukemias are said to follow benzene-induced aplastic anemia. The more serious cases of aplastic anemia succumb within three months of diagnosis due to infection or hemorrhage (Rappaport and Nathan, 1982).

Pancytopenia is the classical clinical finding in benzene hematotoxicity. This is defined as a decrease in circulating erythrocytes, granulocytes, and platelets. It is an indication that the hematopoietic marrow is damaged. Pancytopenia associated with benzene toxicity often is characterized by the presence of erythrocyte macrocytosis and leukopenia. Cytopenia of one cell line may also occur.

In addition to quantitative decreases, qualitative abnormalities in circulating cells are also produced, including macrocytic red blood cells. Shortening of red cell life span, abnormal morphology, and function of granulocytes, alterations of porphyrin pathway compounds in red cells and urine, a lower leukocyte alkaline phosphase activity, and changes in platelet morphology and function (Goldstein & Snyder, 1982).

Often the clinical and hematological findings of pancytopenia improve considerably or the blood appear normal on examination once the individual has been removed from a benzene environment.

The mechanism of action of benzene on the hematopoietic system in bone marrow is unknown. It is also important to emphasize that there is no distinguishing feature that characterizes benzene-related aplastic anemia or pancytopenia and that the evidence for benzene exposure relies upon historical data.

IV. Effects on Reproduction

1. Background

1.1 Animal

A single subcutaneous injection of 3 ml/kg bw benzene on one of days 11-15 of gestation to CFI mice produced cleft palate, agnathia, and micrognathia in 2.7 percent of fetuses (Watanabe and Yoshia, 1970). No controls were used, and IARC reported (1982, p 111) that it is likely that these effects were produced by the stress of the injection and that the relevance of the subcutaneous route to inhalation exposure is doubtful.

Other studies in pregnant mice utilizing other routes of administration, i.e., orally at 0.3-1.0 ml/kg bw (Nawrot and Staples, 1979) or by inhalation at 500 ppm for 7 hours/day (Murray et al., 1979) failed to show any teratogenic effect. Maternal and fetal toxicity and embryolethality were observed.

Inhalation studies have been done in other species. No teratogenic effect has been reported in rabbits exposed to 500 ppm for 7 hours/day on days 6-18 of pregnancy (Murray et al., 1979).

In rats inhalation studies have shown embryolethality and toxicity but only one report of teratogenicity. Kuna and Kapp, 1981, exposed Sprague-Dawley rats to 10, 50, or 500 ppm for 7 hours/day on days 6-15 of gestation. Benzene vapor was fetotoxic at concentrations of 50 and 500 ppm and manifested a teratogenic potential at 500 ppm. Offspring of dams exposed to

500 ppm demonstrated exencephaly, angulated ribs, dilatted lateral and third ventricles of the brain and lagging ossification (not considered spontaneous in this rat strain).

Negative inhalation studies in pregnant rats include exposures at 10 or 40 ppm for 6 hours/day (Murray et al., 1979); 313 ppm for 24 hours/day (Hudak and Ungvary, 1978); 313 ppm for 6 hours/day (Green et al., 1978) and 125 opm for 24 hours/day (Tatrai et al., 1980).

Tatrai et al.(1980) reported that benzene inhalation destroyed the fertilized ovum before or during implantation. Pregnancy rates for mated CFY rats exposed to 500-1000 ppm benzene were only 75 to 80 percent compared with pregnancy rates in unexposed controls of 93 percent.

Reports of teratonic effects of benzene in animals are few and the concentrations used are high. The effect on development appears to be related to fetal and maternal toxicity.

1.2 Human

Women may be more susceptible to benzene toxicity than men. Since benzene is a lipophilic solvent, women may have greater uptake and fat storage. Sato et al. (1975) suggested a slower elimination of benzene in women due to larger body fat storage. In their inhalation study, 5 male and 5 female volunteers inhaled 25 ppm benzene for 2 hours. Early blood concentrations were always higher in men. After cessation of inhalation, however, blood levels decayed faster in males. Lower blood levels in women during inhalation were felt to be due to the greater distribution volume in the fat.

Mukhametova and Vozovaya (1972) have studied menstrual function in female gluing operatives in a mechanical rubber product factory who were exposed to petroleum and chlorinated hydrocarbons, petroleum being a major source of benzene. Menstrual disorders occurred in 26.1 percent of the exposed group, compared with 15.2 percent in the control group. There was a direct relationship between length of service and disturbances of menstrual cycle.

The study by Mukhametova and Vozovaya (1972) analyzed the pregnancies of 510 pregnant women and their previous child-bearing history. Of these, 250 were gluers exposed to petroleum and chlorinated hydrocarbons and 260 served as the control group. Comparing the reproductive histories of the glue workers before and after they started work at the factory showed a 3.4-fold increase in spontaneous abortions and a 3.7-fold increase in premature births, the frequency increasing with duration of employment. In the control group there was a marked reduction in those two types of events after starting work in the factory.

Dowty et al. (1976) demonstrated that benzene crosses the human placenta. Levels in maternal blood and cord blood are similar.

Holmberg (1979) published a study of 14 children with central nervous system defects and their matched normal controls. Case mothers had been exposed to organic solvents during the first trimester of pregnancy significantly more often than controls (p 0.01).

Chronic benzene poisoning in humans is probably due to its active metabolite, benzene oxide. The evanescent benzene oxide can exert its effect mainly at the site of production, such as the liver or bone marrow, which contain the mixed function oxidases necessary for its production. Mixed function oxidases appear in the rat fetus near the end of gestation, while in the human fetus they first appear from about the ninth to the thirteenth week of pregnancy and continue. Thus the human fetus is exposed to the mutagenic and carcinogenic properties of benzene oxide for a greater part of its development gestation than the rat fetus.

2. Issues and Recommendations

Data on fertility are inadequate for complete assessment. Benzene has not been shown to be teratogenic in animals at nontoxic doses. Toxic doses may retard fetal development. There is some evidence that high and prolonged exposure to benzene may affect menstrual and reproductive function. There are no data concerning effects of benzene on male fertility. There are insufficient data on effects on pregnancy and a lack of data on transplacental carcinogenesis. If there is any reproductive effect of benzene, it would be many orders of magnitude above the highest ambient concentrations.

V. Carcinogenicity

1. The Identification of a Carcinogen

1.1 General Concepts

A comprehensive discussion of the major concepts related to carcinogenicity is beyond the scope of this report. However, three fundamental concepts relevant to carcinogenicity will be briefly addressed. These are: the definition and identification of a carcinogen, the definition of the disease cancer, and the mechanism by which the carcinogen results in the production of cancer, carcinogenesis.

A carcinogen is generally understood to be a substance or agent that increases the frequency (age-specific incidence) of cancer in humans or in other animal species. The identification of chemical substances that pose cancer risks to humans is complex and requires integration of information from several scientific disciplines.

Cancer is a malignant disease characterized by the inadequately controlled proliferation and growth of abnormal cells that compress or invade neighboring tissues or that spread to other parts of the body. Cancer actually is a collection of many different diseases because cancers in different organs of the body behave in different ways. Many factors contribute to the development of cancers, including external factors such as cigarette smoking and chemical carcinogens, internal factors such as genetic susceptibility, hormonal balance, or a decrease in the immune system's ability to recognize and destroy abnormal cells.

With respect to carcinogenesis, the mechanisms by which chemicals induce cancer and those by which chemicals cause other types of injury differ in several important ways. Most noncarcinogenic effects depend on a continuing interaction between the toxic chemical and the cells or tissues of the body. The severity and extent of the ensuing reaction is generally related to the amount of the chemical present, but there may often be a "threshold" dose below which no adverse effect takes place. Further, elimination of the toxic chemical from the body results in the cessation of additional damage. In contrast, cancer, at least in theory, can be induced by exceedingly small doses or short exposures to a carcinogen and, once initiated, the disease continues to develop in subsequent generations of cells even when the carcinogen is no longer present. To summarize the mass of data dealing with carcinogenesis, DHS agrees with and will paraphrase a portion of the consensus report issued by the Office of Science and Technology Policy (OSTP, 1984), representing 10 federal agencies, dealing with a number of important scientific issues:

1) Cancer can be induced by radiation, biological, "physical", and/or chemical agents.

2) On a biochemical and molecular level, there are important similarities among mammalian species.

3) An estimate of the potency of carcinogens may never be exact and may vary with life style, habits, age, sex, individual genetic difference, ethnic background, test strain and/or species diet, dose

rate, route of administration, vehicle or solvent used (if any) as well as the presence or absence of other agents, and the environmental conditions prior to, during, or after exposure.

4) Cancer development is a multistage process that may involve the genome, both indirectly (frequently termed epigenetic events) and directly, which may include the paticipation of chemicals or viruses, and which may be modulated by higher order functions, i.e. at the organ and organismic level. (To this statement DHS would add that the time required for the development of cancer through all stages is variable. It often takes a major part of a lifetime, though sometimes it is much more rapid. For most of this period, the partially transformed cells are likely to be undetectable. This phenomenon, i.e. the time between initiation and detection, is the latency period.)

5) Numerous factors may alter the frequency of cancer induction by altering one or more of these stages.

6) The genesis of a cancer appears to require an alteration in the ability of a cell to elaborate its appropriate genetic program i.e. in its information processing capacity, with the subsequent fixation and propagation of that alteration.

7) We still lack an in-depth understanding of the mechanisms and stages of cancer induction and expression.

8) Only by understanding the stages of tumorigenesis and carcinogenesis, the substances and processes which modulate them, and how these differ among cells, organs, individuals, strains and species will we ultimately understand the role of substances, radiations, viruses and/or life-style factors in human cancer.

1.2 Methods of Identification

1.2.1 Molecular Structure Analysis

This analysis may show that a chemical shares structural, chemical, or physical characteristics with established carcinogens. This approach can provide evidence that further testing may be required; however, at present these similarities are not a substitute for biological evidence of carcinogenic effects.

1.2.2 Short-term Tests

These tests are so named because of the relatively short time needed to conduct the experiments. An appropriate battery of these tests can show the ability of the chemical to cause mutations or damage to chromosomes or to the genetic material of cells and can provide supporting evidence that chemicals have the potential to initiate cancers. Although there is an excellent correlation between the demonstration of carcinogenicity in animals and the positive results from a series of short-term tests, short-term tests can now only augment evidence for carcinogenicity in appropriate animal bioassays.

When appropriately conducted, these tests provide unequivocal evidence that chemicals are carcinogenic in the animal species tested. The validity of using animal bioassays to identify substances that pose cancer risks to humans has both a theoretical and empirical basis. Animal bioassays with benzene have shown that benzene is carcinogenic for several organ systems (See Section VI.3)

1.2.4 Case Studies

These studies are an important source of data. Individuals with a disease are diagnosed by clinicians who in turn use the patient's historical data to establish an association between exposure and health effects. This type of study is necessary to carry out subsequent epidemiological investigations.

1.2.5 Human Epidemiological Studies

These studies offer the most direct evidence that a substance is a human carcinogen. It must be recognized that because of severe limitations of epidemiological studies, e.g. the long latency period for the development of human cancers, the difficulty of identifying a large appropriate study population, determination of past exposures, etc., epidemiological studies are of limited usefulness as a means of carcinogen identification.

The laboratory animal bioassay is now widely used to indicate the carcinogenic potential of a chemical. Bioassay procedures have been standardized in recent years, and (except for minor details) there is now a general acceptance of test procedures. An expert panel has recently been convened by the National Toxicology Program (NTP) to review and comment on these procedures. (NTP, 1984)

A recommended design of a cancer bioassay of a chemical includes (Sontag, 1976):

- two species of test animals (usually rats and mice of both sexes) tested at two, or preferably three, dose levels: a high-dose level (roughly the estimated maximum tolerated dose, MTD) and a lower dose level (roughly one-half the MTD) as determined in a 90-day subacute toxicity study;

- dosing and observation for most of the animals' natural lifetime, usually 104 weeks for rodents;

- adequate numbers of animals (at least 50 animals per sex) in each test group;

- adequate concurren* controls;

- detailed pathological examination of tissues; and

Evidence that can lead to a conclusion of carcinogenicity from animal experiments includes:

- statistically significant increases in malignant tumors relative to the controls at one or more of the dose levels tested;

- a statistically significant dose-related increase in malignant tumors in an analysis that makes appropriate use of data on the times at which tumors were detected;

- an increase in the occurrence of rare malignant tumors (those having a zero or low spontaneous incidence rate among historical controls); and/or

- an early appearance of cancer in the treated animals.

3. Human Carcinogenicity of Benzene

3.1 Benzene As A Leukemogen

There is evidence that benzene is a leukemogen in humans. (Goldstein, 1977; Goldstein & Snyder, 1982; van Raalte and Grasso, 1982; IARC, 1982; Maltoni, 1983b; EPA, 1984) Leukemia meets the definition

of a cancer as defined in Chapter V.1.1. The evidence is derived from accumulated case reports, epidemiological studies, and basic biological knowledge.

There are two important biological reasons why benzene might be expected to cause leukemia. The first is that benzene is believed to be an etiologic agent of aplastic anemia. Individuals with aplastic anemia due to known or unknown causes are at a greater risk of acute myelogenous leukemia (see Chapter III.2.1). The second reason is that benzene has been shown to produce chromosomal abnormalities in bone marrow and circulating lymphocytes in experimental animals and in workers with benzene-induced bone marrow toxicity. Abnormalities persist after the workers have been removed from exposure to benzene (see Chapter II.5).

3.2 Leukemias

Leukemia can be defined as the proliferation of a clone of abnormal hematopoietic cells that has the following characteristics: (1) poor responsiveness to normal regulatory mechanisms, (2) a tendency to have a diminished capacity for normal cell differentiation, (3) the ability to expand at the expense of normal myeloid or lymphoid lines, and (4) a possible ability to suppress or impair normal myeloid cell growth. Leukemias are named and grouped according to the kind of hematopoietic cell that is primarily involved. Myeloproliferative disorders affect the descendents of myeloid stem cells (platelets, erythrocytes, and granulocytes), while lymphocytic leukemias involve abnormalities in the lymphoid cell line. Without treatment even the chronic disorders are usually fatal (Schrier, 1984).

The type of leukemia most commonly associated with benzene is acute myelogenous leukemia and its variants including erythroleukemia and myelomonocytic leukemia. Other leukemias have been reported but the association is not as strong. Table V-1 summarizes case reports of hemo-lympho-reticular neoplasias (leukemias) and correlated diseases observed in individuals exposed to benzene as reported in the scientific literature until 1977.

Table V-1. Case reports of human carcinogenicity related to benzene exposure.

Type	No. of cases	No. of reports
Acute myelogenous leukemia	58	28
Erythroleukemia	16	10
Acute monocytic leukemia	3	2
Chronic myelogenous leukemia	27	7
Myelofibrosis and myeloid metaplasia	7	5
Thrombocytopenia	1	1
Acute lymphoblastic leukemia	8	4
Chronic lymphocytic leukemia	9	7
Lymphomas and correlated disorders	14	7
TOTAL	143	71

Source: Maltoni, 1983b

A detailed case-by-case listing is given by Goldstein (1977). These cases represent individuals in groups examined by hematologists who subsequently investigated the anamnestic data given by the patient. The case reports established the relationship between benzene and leukemia, principally acute myelogenous.

3.3 Lymphomas

The IARC report (1982, p 127) states, "Most case reports and case series have described the association of leukemia with exposure to benzene ... and some lymphomas have been noted." In a recent monograph, Goldstein (1983) has reviewed additional reports describing excess lymphomas in workers exposed to solvents including benzene. While there may be an association of benzene with lymphomas, the evidence is not as strong as that of the association of benzene and leukemia.

3.4 Epidemiological Studies

Case studies of workers exposed to benzene were responsible for generating the hypothesis that benzene causes leukemia. These studies resulted in a series of epidemiological studies being performed to test the hypothesis. The results of many of the epidemiological investigations has supported the causal nature of the benzene-leukemia association. A summary of some of the more salient features from 23 major epidemiologic studies appears in Table V-2.

The study by the Environmental Health Associates (1983) is another large-scale epidemiologic investigation of the association between benzene and cancer. Since it was completed following the publication of the IARC document it is briefly described here. The study was an historical prospective investigation of 4602 male chemical workers from seven plants who were occupationally exposed to benzene for at least 6 months between 1946 and 1975. Cause-specific standardized mortality ratio (SMR) analyses were conducted comparing the workers' experience to that of the general (male and female) US population. In addition, the mortality of the exposed workers was compared using both SMR and odds ratio (OR) analyses to that of a cohort of workers from the same plants during the same the period who were never occupationally exposed to benzene. Compared with those for the US population, the exposed workers' SMRs for lymphatic and hematopoietic cancers were elevated though not significantly. Their SMRs were considerably greater than those among the nonexposed workers. The OR analysis demonstrated that continuously exposed workers had a 3.20-fold increase in the risk of lymphatic or hematopoietic cancer compared with their nonexposed counterparts ($p < 0.05$). Also, the association between continuous benzene exposure and

leukemia was statistically significant ($p < 0.05$). None of the leukemic deaths, however, were of the acute myelogenous type. The study also showed a statistically significant dose-response relationship between cumulative exposure to benzene and mortality from both all lymphatic/hematopoietic cancer combined and leukemia. Thus, the staff of DHS considers that the findings of this study are not in conflict with those from other major epidemiologic investigations.

Leukemias and lymphomas are distinct and rare cancers in humans, and statistically, it is relatively easy to show a small added risk against the low background of disease. A small increase in the rate of more common cancers would be more difficult to detect epidemiologically, however, several epidemiologic investigators have looked at cancer mortality rates at sites other than the hematopoietic and lymphocytic systems. Elevated SMRs have been reported for cancers of the kidney, testis, brain, pancreas, stomach, lung/respiratory tract, bladder and uterus (Hanis, 1982; Monson and Nakano, 1976; Monson and Fine, 1978; McMichael et al., 1976; Ott et al., 1978; Environmental Health Associates, 1983). While many of these findings did not reach statistical significance at the 5% level, caution must still be exercised in the interpretation of these studies for several reasons. First, statistical significance is dependent on sample size and since many of the epidemiologic studies involved a relatively small number of exposed individuals, the studies may lack the power to detect small increases in the cancer rate. Second, data on benzene exposure levels were deficient; quantitative measurement of benzene concentrations during the period of exposure were not made on individuals but, at best, were derived from general air monitoring of the work site. Generally, qualitative assessments were used to

Table V-2. Epidemiologic studies of carcinogenicity in humans

Year	Author	Population Studied	Duration	Results
1974	Tabershaw Cooper Assoc.	Petrol indus workers		Increase in rate of lymphomas but not stat. signif. (NS)
1974	Thorpe	Petrol indus workers	1962-1972	Leukemia SMR=121 (NS) (SMR in worker controls = 60)
1974 1977	Aksoy	Shoemakers exposed to 210-650 ppm benzene	1967-1975	Annualized crude rate of acute leukemia 2-fold greater than expected
1975	McMichael et al.	Rubber indus workers		Excesses in mortality from: chronic lymphatic leukemia myelogenous leukemia lymphosarcoma
1976	Vigliani	Patients with benzene hemopathy Exposures est at 200-500 ppm	1942-1975 1959-1974	Leukemia incidence: 11/66 13/135 Estim relative risk (RR)= 20
1977	Infante et al	748 rubber indus workers followed to 7/75		1940-1949 7 cases of myeloid/monocytic leukemia. SMR= 506 (US white males) SMR= 474 (worker controls)
1978	Ott	Benzene workers (DOW) 594 workers followed to 1973	1938-1970	2 dths from anemia: one pernicious, one aplastic 3 dths from leukemia Mortality results NS but leuk rates exceed TNCS expectation ($p < .05$)
1978	Fishbeck et al.	10 chemical workers exposed to benzene	1953-1963	Changes in blood but '...no persisting significant adverse health effects.'

Table V-2 (Continued) Epidemiologic studies of carcinogenicity in humans.

Year	Author	Population Studied	Duration	Results
1978	Brandt et al.	Case-control study of 50 acute nonlympho-cytic leukemia	1969-1977	History of exposure to petroleum products among cases
1979	Vianna & Polan	Workers in NY State exposed to benzene		RR = 2.1 lymphosarcoma RR = 1 6 reticulum cell sarcoma RR = 1.6 Hodgkin's Disease For workers older than 45, the observed number of cases was stat signif (SS) greater than the expected number
1979	Greene et al.	US Gov't Printing Office workers		Significantly higher p--portion of deaths from mult myeloma, leukemia, & Hodgkin's disease and related to exposure to benzene
1980	Linos	Case-control study of 138 leukemics		4 cases found, 3 were chronic lymphocytic leukemia. RR = 3.3 (NS)
1981	Flodin et al.	Case-control study of 42 acute myeloid leukemia		Six-fold (SS) increase in risk related to exposure to solvents including petroleum products.
1981	Schottenfeld et al.	Worker cancer registry compiled by American Petrol Inst.		Incidence of lymphocytic leukemia & mult myeloma increased (SS)
1981	Rushton and Alderson	Petrol refinery workers		Risk of leukemia increased 2-fold in high & medium benzene exposed vs low exposed (p= 0.05)

Table V-2 (Continued) Epidemiologic studies of carcinogenicity in humans.

Year	Author	Population Studied	Duration	Results
1981	Rinsky et al	Continuation of follow-up of Infante study		SMR = 560 leukemia SMR = 2100 leukemia in workers with 5 or more yrs exposure
1982	Thomas et al.	Refinery workers		PMRs for mult myeloma & other lymphomas elevated (SS)
1982	Hanis et al.	Refinery & chemical workers		SMR for cancer of the lymphopoietic tissues elevated but NS
1983	Decoufle et al.	Chemical plant workers 259 men followed through 1977	1974-1960	SMR = 377 (SS) lymphocytic & hematopoietic cancer (L & H) SMR = 682 (SS) leukemia
1983	Tsai et al.	454 refinery workers		No dths observed from L & H cancer; 0.42 expected (NS)
1983	Arp et al.	Rubber indus workers		For lymphocytic leukemia: RR = 4.5 (NS) benzene exposure RR = 4.5 (NS) other solvent expos
1983	Wong et al.	Chemical workers	1946-1975	SMRs elevated (but NS)for L&H cancer leukemia, non-hodgkin's lymphoma. RR = 3.2 (SS) L & H cancer (vs worker control) Dose-response trend found SMRs for lung cancer and several other cancers elevated (NS)

categorize exposure. Further, exposure to substances other than benzene occurred in many instances. Third, the choice of the general population as the control group may not have been appropriate thereby underestimating cancer rates because of the "healthy worker effect". To summarize, better exposure measurement, larger sample sizes, and the control of potential confounding factors are necessary to confirm or refute the possible causal relationship between benzene and non-hematopoietic/lymphocytic cancers. However, the consistency of the results from different investigators studying different populations using various epidemiologic study designs strengthens the purported causal relationship between benzene and leukemia. Overall, the human data implicating benzene as a carcinogen is good.

4. Conclusions Regarding Benzene as a Human Carcinogen

In 1981, IARC met and reviewed data regarding the carcinogenic risk of benzene to humans. IARC concluded that the epidemiological studies have established the relationship between benzene exposure and the development of acute myelogenous leukemia and that there is sufficient evidence that benzene is carcinogenic to man (IARC, 1982 p 127). The staff of the DHS concurs with this conclusion.

VI. Dose-Response Assessment

1. Introduction

The goal of dose-response assessment is to determine the amount of disease that will result from a given exposure level. This goal can be achieved by reviewing the experience of humans exposed to the substance in question or by examining animal bioassay studies and making inferences about effects in man. In the case of benzene, human and animal data are available to perform a dose-response assessment, and the results of both assessments will be provided in this section. However, it should be noted that each source of information has limitations, which necessitates invoking a series of assumptions to quantify the relationship between exposure to the substance and the subsequent health effect(s). Therefore, prior to presenting the actual dose-response assessment, the major assumptions the DHS has used in performing the assessment will be discussed. The dose-response assessment based on human data will be presented first followed by the assessment based on animal data, and finally, a summary of the dose-response assessment results.

2. Dose-Response Assessment Based on Human Data

2.1 Introduction

As noted in Chapter V of this document, the epidemiologic studies have provided sufficient evidence that benzene is a human

carcinogen. However, these studies have limitations which affect their use in dose-response assessment. Since epidemiologic studies are desirable to use in quantifying the dose-response relationship, we will first summarize the problems encountered and our resolution of these issues and then present the dose-response assessment.

2.2 Assumptions Used in the Human Dose-Response Assessment

2.2.1 Exposure and Dose

The major issues related to the exposure and dose component of the assessment are the route of exposure, the period or duration of exposure, the concentration of the exposure, the amount absorbed and the amount retained.

a) Route. Epidemiologic studies on benzene completed to date are occupational studies where the major route of exposure to benzene is inhalation. Since this is the route of concern in this assessment, no assumptions need be made regarding the route of exposure.

b) Duration and Concentration. Since the mechanism of carcinogenesis is not known, it is difficult to specifically address the issues of intermittent versus continuous exposure or the effects of transient high-level exposures. Therefore, the dose-response assessments performed use the cumulative exposure

averaged over the individual's lifetime. This assumption is based on pragmatism, and it is recognized that it is not known if a one-time high exposure to a chemical carcinogen is equivalent to a time-weighted average lifetime exposure. Also, since the reported occupational concentrations of benzene range from low ppm concentrations to several hundred ppm which are several orders of magnitude greater than ambient air exposures, it is necessary to use a model to extrapolate the observed dose-response relationship to the exposure levels of concern. The model used will be discussed below (section 2.2.3). With regard to exposure levels, epidemiologic studies have not been able to directly measure benzene concentrations. Instead, estimates of historical concentrations have been used for dose-response assessment purposes. These estimates are based on such factors as employee job classification or the prevailing recommended standard for the period(s) of employment. Thus a worker's exposure profile is assumed to closely approximate the inferred levels.

c) Absorption and retention. No adjustment is made for absorption or the duration of retention of the substance; the assessment is based on the exposure dose.

2.2.2 Thresholds

As indicated in Chapter IV, acute effects and aplastic anemia have not been documented to occur at exposures below tens to hundreds of parts per million of benzene. This is thousands of times above ambient exposure levels. There is theoretical reason to believe, however, that the carcinogenic effect of benzene or indeed of any carcinogen could convey a low probability of causing cancer at very low doses. A small probability applied to a large population can produce an unacceptable number of cancers, hence the concern with the possible "no threshold" properties of carcinogens like benzene.

Traditional toxicology incorporates thresholds in the dose-response relationship. These are dose levels below which a toxicological response is not observed. This is not to imply that cellular or tissue damage does not occur below the "threshold" level but rather that the organism either has the reserve capacity to withstand damage or is able to adapt to the toxicological stress. For toxicologic effects, a threshold is said to occur at dose levels that are insufficient to cause damage. For example, if a toxic substance killed nonreplicating optical neurons, sight would not suffer until a sufficiently large number (perhaps millions) of cells had died.

But the processes of carcinogenesis appear to be qualitatively different from those in classical toxicology. In contrast to the toxic effects described above, which involve impairment of functions at the organ or organism level, the initial "target" for carcinogenic action is

believed to be extremely small. As we develop a better understanding of the mechanisms of carcinogenesis and mutagenesis, it appears likely that many carcinogens interact with DNA or other target macromolecules. In addition, there is evidence that the occurrence of such events in a single cell can produce cancer (Failkow, 1974, 1977). The chance that the critical molecules will reach the critical cell at the critical time is affected by the interplay of a variety of protective defense systems within the body. However, there is some finite probability that a few molecules would evade these defenses and produce an event that triggers carcinogenesis. This scenario, so different from classic toxicologic processes, makes a threshold less likely for carcinogenesis.

There are hypothetical mechanisms such as cytotoxicity or interference with DNA methylating enzymes which theoretically would not involve direct action on the genetic material and might display a threshold. At this point, the means for recognizing this subclass of carcinogens, if indeed it exists, is not considered by the IARC to be "exhaustive or definitive"(IARC, 1983). For this reason the staff of the DHS assumes as a general rule that an identified carcinogen has no threshold and does not distinguish between "genetic" (directly acting on DNA) and "epigenetic" (not directly acting on DNA) carcinogens for the purposes of identification or dose-response assessment (See Appendix A).

A pharmacokinetic argument has also been made for the existence of practical operational thresholds. For example, the observation of a plateau of response at the high-dose levels of the vinyl chloride dose-response curve is interpreted to mean that the enzyme system(s) that

activate vinyl chloride to its carcinogenic species are overloaded or saturated. The argument is then made by analogy that protective enzyme systems that deactivate carcinogens and are reasonably effective at low doses may likewise be saturated and hence be less protective at the high doses encountered in animal bioassays (Gehring et al., 1977, 1978, 1979; Watenabe et al., 1977; Reitz et al., 1980).

Several kinetic models which produce a threshold in the dose-response curve have been developed. These models are based on the concept that high doses of carcinogens can overcome protective systems. However, the models produce a threshold by requiring that the carcinogen be instantaneously deactivated, which is unlikely. If detoxification reactions are not instantaneous, a small amount of the agent may escape detoxification by protective enzymes and interact with the DNA. In this instance, the protective effect of detoxifying enzymes would decrease the slope of the dose-response curve but would not produce a classical threshold (Hattis, 1982).

Even if thresholds could be determined for individuals, establishing a population threshold is more difficult because of the observed variability of the human population. This variation is a consequence of extreme genetic heterogeneity and differences in physiological state associated with age, sex, reproductive activities, nutrition, and exposures to environmental and occupational stresses including other carcinogens. Even if it is assumed that each individual in the population has a threshold defined at any one time by his or her physiological state, the population is likely to be characterized by a very wide distribution of thresholds such

that there may not be an absolute lower bound or population threshold (NCR, 1977; Rall, 1979; Brown, 1976). Since the threshold dose for the human population should be the threshold dose for the most sensitive individual; this dose may be so low as to be effectively zero. By analogy, the threshold dose for an individual or organism is the threshold dose for the most sensitive cell, and this may also be extremely low (Crump et al., 1976). Operationally these variable threshold models are difficult to distinguish from nonthreshold models that are concave upward at low doses.

Variable threshold models would produce absolute thresholds only under the assumptions of instantaneous deactivation and repair. Other models (Weissburger and Williams, 1983) predict nonlinearities in the dose-response curve that will lead to practical, but not absolute, thresholds. The presence, or absence, of an absolute threshold or even a practical threshold remains unconfirmable. The ED_{01} study indicated that the 2-AAF mouse exhibits an apparent threshold for bladder cancers at low doses. However, reanalysis of this low-dose data at greater resolution indicated that the threshold was more apparent than real: the incidence of bladder tumors increased with dose even at the low dose, and no threshold level could be determined (US Congress, 1981). Thus, scientists are now less concerned with the existence of thresholds than in the degree of nonlinearity of the dose-response curve in the low-dose region.

Another factor which argues against the existence of thresholds for carcinogens is the substantial "background" incidence of

cancer in humans. Unless each carcinogenic substance operates by a unique mechanism, an additional small exposure to a substance may supplement an individual's exposure to other carcinogens operating by a similar mechanism. The high incidence of cancer of unexplained etiology demonstrates that human exposure is well in excess of any possible population threshold for at least some of these mechanisms. Therefore, since we cannot know which of the possible carcinogenic mechanisms are already operating and contributing to background incidence, we will assume that no additional exposure, however small, may be considered free of risk.

There has been extensive discussion at the federal regulatory level of possible evidence relevant to a benzene carcinogenic threshold although to date this evidence is not considered conclusive. These threshold arguments are well summarized in the Federal Register (EPA, 1984, Appendix C) in which the EPA responds to public comments on the proposed regulation of benzene for the National Emission Standards for Hazardous Air Pollutants.

The public commenters have advanced three pieces of evidence to suggest that benzene has a threshold. First, they propose that certain epidemiological studies (Thorpe, 1974; Tabershaw Cooper Associates, 1974; Stallones and Syblic, 1977) fail to show a statistically significant effect, and that this must represent a threshold. The EPA concluded that these studies simply do not have the statistical power to identify a threshold. IARC was critical of the Thorpe study (1982, p 123). Next, commenters suggested that benzene works only at high doses by causing cytotoxic aplastic anemia followed by regeneration with occasional defects in the DNA

duplication which can result in leukemia (EPA, 1984). The DHS agrees with the EPA that although this is possible, there is no convincing positive evidence to show that this has happened. The epidemiological studies do not provide the information to determine if all leukemia cases were preceded by aplastic anemia or not (Goldstein, 1977). The EPA points out that studies in workers suggest chromosomal effects of benzene at 1 to 25 parts per million (Killian and Daniel, 1978; Picciano, 1979). These exposure levels are below those considered to be associated with clinical symptoms of toxicity. They (EPA, 1984) further point out that chromosomal damage and cancer have occurred after radiation (Bloom et al., 1970) and after certain chemical exposures (Mulvihill, 1975), and that chromosomal damage may lead to cancer. If this were true, chromosomal damage due to low-level exposures to benzene could cause cancer.

The staff of the DHS additionally notes that benzene causes cancer at a variety of sites in rats and mice in addition to leukemia (Chapter VI.4). These other cancers do not involve an aplastia anemia stage in their natural history.

Commenters to the EPA have also pointed out that bacterial, fungal, and other in vitro tests have not proven that benzene or its metabolites interact with the DNA and suggest that this is evidence that benzene ought to have a threshold The DHS staff agrees with the EPA (1984) that in general the failure to detect an effect with insensitive tests is not positive evidence of no effect on DNA (See also Appendix A of the present the DHS document). Additionally, for benzene some of these tests were done in open systems where benzene might evaporate. As pointed out in

Chapter II, more recent experiments on benzene oxide in bacterial assays (Kinoshita et al., 1981 and Jung et al., 1981) and benzene with human cells (Crespi et al., 1984) and with DNA binding (Lutz and Schlatter, 1977) provide weakly positive results which, while not definitive, suggest that further research is needed before any degree of certainty about benzene's carcinogenic mechanism can be achieved.

After reviewing the literature and its extensive discussion at the federal level, the staff of DHS agrees with EPA staff in concluding that there is no strong positive experimental or epidemiological evidence that benzene has a carcinogenic threshold and that it should be treated as a substance without a threshold.

2.3 Dose-Response Assessment Based on Human Studies

2.3.1 Available Data

Few epidemiologic studies have been able to sufficiently document benzene exposure levels for quantifying a dose-response relationship, although, as pointed out in Chapter V, numerous studies have qualitatively demonstrated an association between benzene and leukemia. The initial Carcinogen Assessment Group (CAG) assessment identified three studies which it felt provided adequate data. These were the studies conducted by Infante (1977), Aksoy (1976), and Ott (1978). Exposure measurements and the choice of control group were heavily criticised in the Aksoy study while multiple chemical exposures among the leukemic cases in

initial Carcinogen Assessment Group (CAG) assessment identified three studies which it felt provided adequate data. These were the studies conducted by Infante (1977), Aksoy (1976), and Ott (1978). Exposure measurements and the choice of control group were heavily criticised in the Aksoy study while multiple chemical exposures among the leukemic cases in the Ott study obscured the interpretation of these data. In 1981 Rinsky et al (1981) provided additional follow-up information on the workers in the Infante study as well as a detailed profile of benzene exposure levels. The results of both the Infante-Aksoy-Ott studies and the Rinsky re-evaluation of the Infante data have been used by CAG in its benzene dose-response assessment (EPA, 1983; EPA, 1984).

2.3.2 EPA Dose-Response Assessment Model

The CAG risk assessment for benzene (EPA,CAG, 1979, Appendix D) used a linear nonthreshold model to estimate the leukemia risk that would result from exposure to the low ambient benzene levels that the general population is exposed to. The model also assumes that the relative risk of leukemia from benzene exposure is identical in workers and the general population and is independent of the length of exposure or age at which exposure occurred. To use this model, estimates for the background rate of leukemia, the relative risk of leukemia in an exposed group of people, and the level of benzene in the exposed group must be obtained. The background leukemia rate was based on vital statistics data for the entire U.S. population (Infante and Rinsky studies) or on a general Western population (Aksoy) while specific data from each of the studies were used to estimate the relative risk and exposure parameters of the model. These data were used to

derive the slope parameter of the model, i.e., the increased risk of leukemia per unit concentration of benzene. Algebraically, the estimate of the slope of the CAG model can be expressed as

Slope = (Background Disease Rate)(Relative Risk - 1)/(Exposure Level)

The DHS did not modify the model for purposes of this assessment and therefore uses the potency estimate for benzene as derived by CAG based on the Rinsky re-evaluation (EPA, 1983, Ch 13, p 165) of

$$\text{slope} = 5.2 \times 10^{-2} (\text{mg/kg-day})^{-1}$$

This corresponds to a lifetime (70-year) risk from a lifetime average exposure to 1 ppb benzene <u>in air</u> of:

$$48 \times 10^{-6} \text{ (see VII.2).}$$

The following values were substituted in the equation above to calculate this slope estimate: background rate of leukemia = 0.006732, relative risk = 21 (based on workers with at least 5 years of benzene exposure), and a lifetime average exposure level of 2.81 ppm (taken as the geometric mean of the estimated high and low exposure levels based on an 8 hour day 40 hour week time weighted averages of 40.36 ppm for 35 years and 23.7 ppm for 25 years).

By contrast, the slope based on the three studies was calculated by CAG as the geometric mean of each study's estimated slope. This yielded an increase in risk of 22×10^{-6} for a continuous lifetime exposure to 1 ppb benzene.

It should be noted that these slope estimates respresent point estimates of the increase in leukemic risk associated with benzene; they do not reflect the statistical uncertainty related to the relative risk parameter found in the worker populations. (The range of risk associated with these epidemiologic data could be determined by using the upper and lower confidence limits of the relative risk estimate in the model. For example, the data from the Rinsky re-evaluation are compatible with a risk of 32×10^{-6} to 120×10^{-6} based on a 95% confidence interval.)

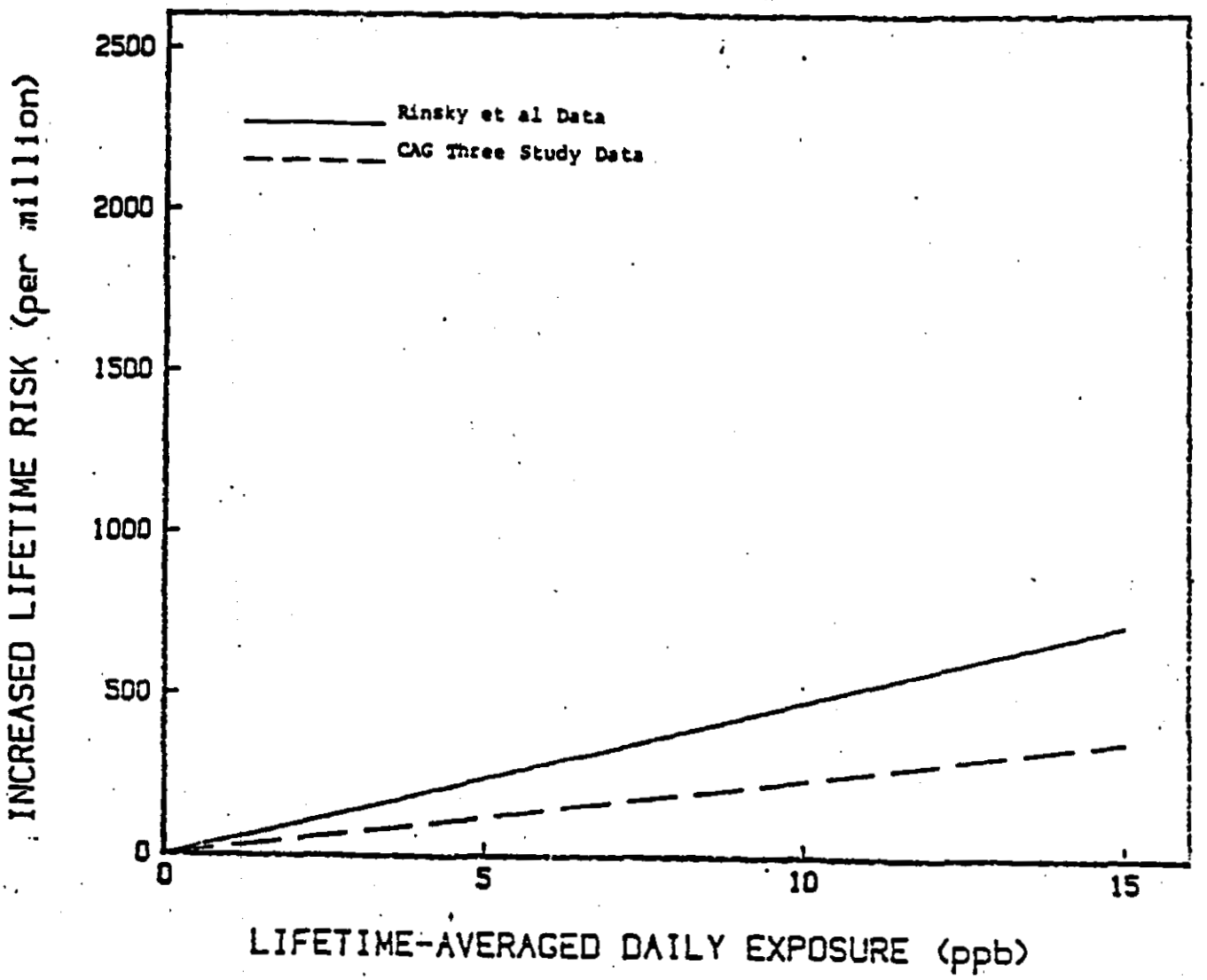


Figure VI-1. Dose-Response Curve For Benzene Risk Assessment based on the CAG model applied to the Rinsky reevaluation of Infante's epidemiologic study and the CAG assessment using the Infante, Aksoy and Ott epidemiologic studies.

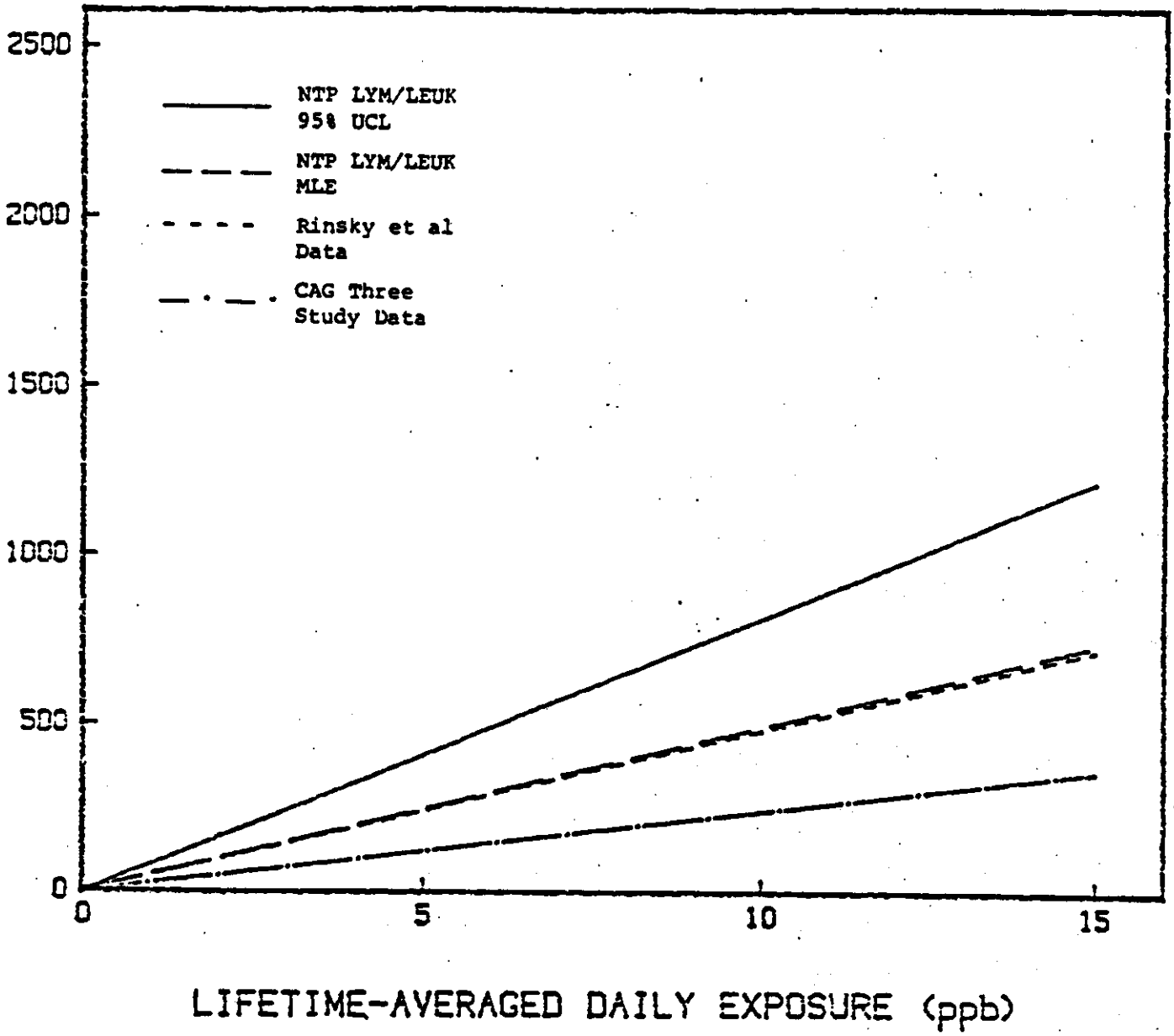


Figure VI-2. Dose-Response Curves for Benzene Comparing Bioassay and Epidemiologic Studies of Leukemia

Shown are the curves for the 95% upper confidence limit of risk (UCL) for the National Toxicity Program (NTP), lymphoma and leukemia (LYM/LEUK) data, the maximum likelihood estimate (MLE) of risk from the same NTP study, and EPA-CAG estimate of risk based on Rinsky's data and the original study using the data of Infante, Aksoy, and OTT. The NTP curves are based on the unadjusted attack rates.

2.3.3 Review of Critiques of the CAG Dose-Response Assessment

Criticism of the CAG risk assessments has tended to focus on three areas: the model and some of its assumptions, the appropriateness of the data used to estimate parameters, and an inadequate discussion of the uncertainty surrounding the leukemia risk conveyed by benzene (Environ Corporation, 1983; Hattis and Mendez, 1980). The assumptions of the model which have been challenged are those of no-threshold and low-dose linearity. The staff of DHS sees no reason to question these assumptions for the case of benzene. The model assumes that the benzene-induced leukemia is independent of age and that the risk associated with a given dose is a function of the cumulative dose were also questioned. Reviewers of the CAG document tended to accept these assumptions but noted that they cannot readily be tested, and the direction of the bias on the estimate of risk, if the assumptions are wrong, is not simple to determine. Since there are other reasonable assumptions which could be substituted in the model, it is argued that the CAG risk assessment contains more uncertainty than the document conveys. Most of the criticism of the CAG document, however, focuses on the data used to estimate model parameters.

* In addition to the criticisms noted above for the Aksoy and Ott studies, the use of the entire U.S. population to calculat the background rate of leukemia was questioned in the Rinsky re-evaluation. The point of contention is that the relative risk estimate comes from a population of white working males while the expected number is based on the entire U.S. population and the two are not compatible. It is argued, for example,

that since women experience a leukemia rate that is 57% of the male rate, the background rate is too low. This will be reflected in a slope parameter that is also too low. Another criticism focuses on estimating the slope parameter from all types of leukemia rather than non-lymphatic leukemia. Had CAG consistently based the slope calculations on non-lymphatic leukemias, as many of the epidemiologic studies suggest is appropriate, the slope would be slightly lower than CAG reported.

Much of the criticism of the CAG risk assessment related directly to the quality of the benzene exposure data; specifically, the exposure period and the exposure levels. For example, the exposure period in the Rinsky study is taken to be 35 years, an estimate some consider to be longer than the actual exposure time. The use of this exposure period has the effect of increasing the cumulative exposure dose and hence yields a slope that is lower than the actual. Further, since no routine monitoring of the work place was performed during the periods workers in this study were exposed, much controversy has arisen as to the level of benzene workers were exposed to. Here it is argued that exposure level used for the assessment substantially underestimated the true exposure level thereby resulting in an overestimate of the slope.

3. Animal Dose-Response Assessment

3.1 Introduction

In general, lack of opportunity, ethical considerations, confounding factors, sample size, measurement of exposure, and latency period are among the problems related to conducting and using epidemiologic data to quantify a dose-response relationship. Notwithstanding the difficulties of extrapolating animal health effects to humans, bioassays can provide support for epidemiologic studies or offer further insight into a chemical's potential human health effects because animals can be useful predictors of human cancers. Review of data on carcinogenic effects in rodents demonstrate that most chemicals which increase the cancer incidence in one species also increase the incidence in a second species (Ames et al, 1975; Purchase, 1980; Tomatis et al., 1973; Griesemer and Cueto, 1980; Chu et al., 1981). Thus, in its review of all the data related to benzene's carcinogenicity, the DHS will provide dose-response assessments based on animal data.

In this section we will first discuss the major assumptions DHS has invoked to perform the dose-response assessment using animal data. This will be followed by a brief description of the animal studies and the results of the assessments.

3.2 Assumptions Used in the Animal Dose-Response Assessment

3.2.1 Use of Most Sensitive Species and Site.

To reduce the influence of extraneous factors in the bioassay, genetically homogeneous animals are used and external environmental factors are minimized. This is in direct contrast to the genetically diverse human population which is exposed to numerous environmental contaminants some of which may greatly increase susceptibility to the incremental action of carcinogens. DHS concurs with IARC (1978) and U.S. Interagency Regulatory Liason Group (IRLG) (1979) in that it is appropriate to use the most sensitive species, sex, and tumor site in its assessments because there is often little correlation between tumor types or target organs between species which may in part be due to both physiologic differences between species and differences in the conditions of the bioassay and actual human exposure. Additionally, if two or more tumor sites show a statistically significant increased tumor rate, DHS may combine the number of animals with tumors at each of the specific sites under consideration and use this in the risk model.

3.2.2 Exposure and Dose

As with the human dose-response assessment, several assumptions regarding the dose component of the assessment warrant discussion.

a) Use of Studies with High Dosage Schedules. Due to the high cost of animal bioassays, studies are conducted with relatively few animals (usually 50) per dose group. This results in a very low statistical power to detect small increases in disease rates. To compensate for this, high dose schedules are used. This assumes that chemically induced carcinogenic responses at high doses will also result in similar responses at low doses.

b) Use of Lifetime Cumulative Average Daily Dose. The daily dose of benzene averaged over the animals lifetime is used in the assessment. This assumes that the cancer risks from short-term high dose schedules are equivalent to cumulative lifetime doses. As knowledge of the mechanisms of carcinogenesis become known and specific biochemical, intermediate metabolism, and pharmacokinetics are established for both humans and test animals, this assumption will no longer be necessary. However, at present this is the state-of-the-art in risk assessment and DHS is consistent with major federal and state agencies in maling this assumption.

c) Absorption. In the absence of evidence to the contrary, it is assumed that the test species absorbs the same percentage of benzene as do humans.

d) Route of exposure. DHS may include assessments based on routes of exposure other than inhalation recognizing that the carcinogenic response may differ according to the mode of administration. DHS believes this is less likely to be a problem for dosages providing systemic exposure (e.g. ingestion, gavage, inhalation, and intravenous or intraperitoneal injection) and therefore some non-inhalation animal studies can provide information applicable to human exposures. Moreover, unless there is specific or compelling information that metabolic and pharmacokinetic differences occur between humans and rodents for either the same or different routes of exposure, alternate systemic routes will be considered equivalent.

3.2.3 Thresholds

The assumptions regarding thresholds in animals are identical to those presented in the human dose-response assessment section.

3.2.4 Use of Benign Tumors

Where both benign and malignant tumors are induced at the same site and the malignant tumors are significantly increased, DHS may combine the data on both types of tumors as the basis for dose-response assessment. This is consistent with IARC (IARC, 1980a) and NTP (NTP, 1984).

The rationale is that the induction of benign tumors in the experimental animals reflects the biological activity of the carcinogen, which may well be manifested as the induction of malignant tumors in other species. In addition, benign tumors in several tissues may progress to malignancies (Tomatis et al., 1973; Tomatis et al., 1978; Griesemer and Cueto, 1980; Chu et al., 1981; Tomatis, 1979; IARC, 1980a; IARC, 1972-1983; IARC, 1980b).

3.2.5 Interspecies Scaling Factors

There are three generally used methods for relating animal dosages to humans: mg/kg-day, mg/surface area-day, and mg/lifetime. To date no study has been explicitly undertaken with the objective of determining what unit best expresses equivalence of carcinogenic potency across mammalian species. Studies which have looked at this problem have shown that the choice of scaling factor will directly affect risk calculations with the risk increasing in the following order: mg/kg-day < mg/surface area-day < mg/lifetime. Following the suggestion of Mantel and Schneiderman, 1975; EPA, 1980; and Crump, 1981, the DHS believes the mg/surface area-day factor is most appropriate because it falls near the middle of the range of measures that have been proposed.

3.2.6 Low Dose Extrapolation Models

Because dose levels in bioassays are generally much higher than the levels to which the human population is exposed it is necessary to extrapolate downward to estimate the health effects in the range of

exposure that we are interested in. Several mathematical models are available and newer models continue to be developed. The models fall into three broad categories: 1) quantal or dichotomous response models which base their risk estimate strictly on whether or not the animal acquired the tumor of interest by the end of its "natural" lifetime--they are not corrected for competing risk or independent background cancer incidence, 2) time-to-tumor models which are sensitive to the time of tumor onset (in practice, the time to tumor detection is used), and pharmacokinetic models which relate the carcinogenic response to biochemical interactions between the exposure substance and components of the body. Time-to-tumor and pharmacokinetic models are believed to provide more valid estimates of risk but it is often not possible to apply these models because the cancer onset data has not been reported in sufficient detail or metabolic pathways and rates are not known.

Although all models tend to fit the dose-response data equally well in the observable range of exposure, low dose estimates of risk can vary by orders of magnitude. It is not possible to validate any of these models in the low dose range with either animal or epidemiologic data so selection of a particular model is somewhat arbitrary. However, in selecting a model, DHS is guided by the generally accepted understanding of carcinogenesis in which it is assumed that chemical carcinogens contribute to the already existing carcinogenic mechanisms. This implies that an exposure will incrementally add to the existing rate of cancer which in turn is consistent with a linear response at low doses (Crump et al., 1976). (If the carcinogen acts by an independent mechanism, the response will not necessarily be linear). With this basis, a brief description the more salient features of the major models and our reasons for either using the model or not recommending it

follows. It should also be noted that here, as in the case with the epidemiologic based assessment, the models yield a point estimate of risk based on the data at hand. While this value may be interpreted as the "best" estimate of risk, statistical theory tells us that the "true" estimate of risk compatible with the data at hand is contained within the range of a lower and upper confidence limit around the point estimate. Public health prudence dictates that we be concerned with the highest amount of risk a substance may pose so in addition to the point estimate, the upper 95% confidence limit of this risk estimate will also be given.

a) Quantal or dichotomous response models.

i One-Hit or linear Model (Krewski et al., 1981) - The basis of this quantal model is the concept that the response (cancer) can be induced after a single susceptible target has been hit by a single biologically effective unit of dose. This model is originally derived and received validation from radiation theory. The form of this model is:

$$P(d) = 1 - e^{-(d_o + \beta d)}$$

Where:

P(d) is the probability of response at a provided dosage,

β is the slope of the dose response curve,

d is the provided dose, and

d_o is the background rate, if one exists, when d=0.

At low doses (i.e., $\beta d < 0.02$) the dose response curve becomes linear with dose.

ii Multistage Model (quantal) (Armitage and Doll, 1961) - This model assumes that the carcinogenic response is the result of an ordered series of biological events and that the occurrence of each event is dose related. This theory was derive to account for the fact that in many types of cancer, the logarithm of the cancer mortality rate increases in direct proportion to the logarithm of age. This suggests that a cell may go through a sequence of specific changes (stages) in order to become malignant. The transition between stages (i) is dependent on two constants: a constant term a_i which is related to the background rate and a term b_i which indicates the potency of the agent at the ith stage. The total response, P(d), is an exponential product of each stage:

$$P(d) = 1 - \exp-[(\prod_{i=1}^{k} a_i + b_i d)]$$

where $a_i \geq 0$ and $b_i \geq 0$ and k is the number of stages or events required before cancer is observed. More generally,

$$P(d) = 1 - \exp-\{ \sum_{i=0}^{k} q_i d^i \} \quad q_i \geq 0,$$

where the exponential term is a polynomial function of dose with zero or nonnegative coefficients. The model is fitted using maximum likelihood theory to estimate q_i and the number of stages that best fit the data. A restriction is placed on k such that it is not greater than the number of dose levels in the bioassay. DHS used a version of the multistage model developed by Crump and Watson (1979). This procedure assumes the number of stages is equal to the number of dose groups minus one. In estimating the upper confidence limit of risk, either a linear term is forced into the model if one does not exist or the existing linear term is maximized which assures that the model will be linear at low doses.

iii Gamma Multi-hit Model (Rai and Van Ryzin, 1981) - This model can be derived from the assumption that there is a discrete change, called a hit, which has to occur several times in order to produce a response. The expected number

of hits is proportional to dose and is bd. The probability of k or more hits occurring is given by a Poisson distribution:

$$P(d) = \sum_{x=k}^{\infty} \frac{(bd)^x \exp(-bd)}{x!}$$

This can be shown to equal:

$$P(d) = \int_0^{bd} \frac{x^{(k-1)} \exp(-x)}{(k-1)!} dx$$

More generally, for arbitrary k:

$$P(d) = \int_0^{bd} \frac{x^{k-1} \exp(-x)}{\Gamma(k)} dx$$

where $\Gamma(k)$ is the gamma function which satisfies

$$\Gamma(k) = (k-1)!$$

for integer values of $k \geq 1$.

iv Probit Model Results of toxicity tests have often shown that the proportion of responders monotonically increases with dose and exhibits a sigmoid relationship with the

logarithm of the exposure level. This led to the development of the probit or log normal model. The dose-response function is given by the cumulative normal probability:

$$P(D) = \phi[(\log(D) - \mu)/\sigma]$$

where μ and σ represent the mean and standard deviation of the distribution of the log tolerances (Bliss, 1935)

v Mantel-Bryan Model (1961)- This model assumes a log-normal distribution of individual sensitivities to a carcinogen in a population. That is, if a population is exposed to a given dose rate d, then response to the log of the dose will follow the normal (Gaussian) distribution function:

$$P(d) = \phi\ (a + b \log d)^*$$

where ϕ is the standard normal density evaluated at a + b log d. In this formulation, a ($a \geq 0$) is the intercept (background incidence) while the parameter, b ($b \geq 0$), is the slope of log-probit distribution. The slope is assumed to be 1.0 in the Mantel-Bryan model though other values are possible. Mantel and Bryan proposed this "conservative"

---

*The normal density is given by: $\phi(x) = \int_{-\infty}^{x} (2\pi)^{-1/2} \exp(-u^2/2)\, du$.

slope because it was less steep than slope estimates they had observed in toxicity testing. The purpose of the model was to estimate a safe dose for a given level of risk rather than to estimate the true dose-response relationship. However, susequent research has shown that in the low-dose region the use of the lognormal distribution tends to produce relatively high "safe dose" estimates (Crump et al., 1976).

vi Log Logistic (Logit)(Worcester and Wilson 1943, Berkson 1944) - This model is also based on an assumed tolerance distribution which is sigmoidal in shape. The dose-response function is given by:

$$P(D) = [1 + \exp(a + b\log_{10}(D))]^{-1}$$

where a and b are parameters estimated from the data. The curve is symmetric about the 50% response level but approaches the extremes, 0% and 100% response, more slowly than does the probit model.

b) Time-to-Tumor Models

Many of the previously described dichotomous response models can be modified to express cancer risk as a function of both dose and time (duration of exposure). A simple model which considers risk only as a function of time only is described here It can be shown that in many situations the probability of occurrence of cancer by time t can be given by:

$$P(t) = 1 - \exp\{- b(t - a)^k\}$$

for $t \geq a$, $r > 0$, $b > 0$, and $k \geq 1$ (Pike, 1966).

The coefficient b represents the potency of the carcinogen; a is related to the latency period of the carcinogen; and k in some applications refers to the number of stages of carcinogenesis. This model has been shown to represent human mortality data for certain cancers where t represents age (Armitage and Doll, 1961). Furthermore, this function has been incorporated into multi-event models in order to describe the relationship between cancer occurrence and both dose and time.

An empirical relationship between time and dose (Druckrey, 1967) and two time-to-tumor models which incorporate dose (Weibull [Pike, 1966]; Hartley and Sielken, 1977) follow:

i Druckery: An empirical relationship between dose (d) and median time of tumor appearance (t) was found in data from animal cancer studies of the form

$$dt^n = \text{constant}$$

where n is usually between 2 and 6 (Druckery, 1967). The response times were assumed to be a lognormally distributed in a manner analogous to the population tolerance models discussed earlier. This relationship has been applied to several data sets from both animal experiments and epidemiologic studies (Albert and Altschuler, 1973).

Use of this relationship for low dose extrapolation purposes was proposed by Jones and Grendon (1975). After examining data of their own and of Druckrey, they proposed the generalization that the median time-to-tumor appearance is proportional to the one-third power of the dose, and suggested that this relation reflects the average time needed for two affected cells to coalesce to form a cancerous clone. However, several studies have indicated that Jones and Grendon's model is not appropriate for low-dose extrapolation.

ii Weibull Model (Pike, 1966) - In this model, response can be related to both time and dose by the expression

$$P(t,d) = 1 - \exp\{g(d)t^k\}$$

where $g(d)$ is some function of dose (i.e., $g(d) = a + bd^m$).

One study indicated that the Weibull model yielded a better fit to various sets of animal cancer data than did other models (Peto et al, 1972). Furthermore the relationship observed by Druckrey can be derived from the Weibull model (Carlborg, 1981).

iii Hartley and Sielken (1977) - A general model was developed which has the expression:

$$P(t,d) = 1 - \exp-[(\sum_{j=0}^{k} q_j d^j)\, h(t)].$$

The Weibull model is a special case of the general product model in which

$$h(t) = kt^{k-1}.$$

Although it is of theoretical interest, use of the general product model would require prior selection of three of its parameters; therefore, the effect that these essentially arbitrary choices have on estimated risks needs to be studied (Crump, 1981).

3.2.7 Rationale for Selection of a Low Dose Extrapolation Model

The probit, Mantel-Bryan, and logit models are not based on mechanisms of carcinogenesis. The first two models are based on the cumulative distribution that arises assuming a lognormal distribution of tolerances in the exposed population. The logit model is derived from chemical kinetic theory. Since these models are not based on the generally accepted current understanding of carcinogenic processes and noting that these models are not linear at low doses, the DHS does not recommend their routine application for dose-response assessment. The other models discussed in the previous section are based on mechanistic arguments but some have limitations that are discussed below.

The one-hit model is a special case of the multi-hit and multi-stage models. By itself, the one-hit model has only one parameter to estimate and therefore may not fit experimental data as well as other models. In addition, since it assumes a linear response in the observed exposure range, this model will produce very high estimates of risk for a given exposure relative to other models.

A major problem with the gamma multi-hit model is the interpretation of the parameter reflecting the number of hits, k. Since k is determined by the data and is not restricted to integer values, it is not biologically interpretable. Moreover, depending on its value, the model is not necessarily linear at low doses. Lastly, the DHS does not recommend this model because it has been demonstrated that the model will "produce" background rates of cancer where none exist (Haseman et al., 1981).

The DHS will preferentially use time-to-tumor and pharmacokinetic models given adequate data. Such data were not available for the benzene assessment. In general, a constraint on the use of time-to-tumor models is the difficulty of determining the actual response times in an experiment. In most cases, internal tumors are difficult to observe in live animals and their presence is usually detected only at necropsy. In addition, the application of these models frequently requires making a distinction between whether the tumor was the cause of death or was found only coincidentally at necropsy where death was due to some other cause; pathologists are reluctant to make such distinctions.

Thus, of the models generally used today, the DHS agrees with the EPA (1980) in the use of the multistage model. This model is consistent with biologic theories of carcinogenesis and has been shown to fit several sets of experimental data for animals (Brown, 1978; Krewski, 1983) and humans (Peto, 1977). It is a flexible model in that the form of the model is not determined apriori, thus it may take on some of the characteristics of the other models depending on the number of stages used and the corresponding parameter values.

Assessment

Additivity and linearity at low dose are assumed as part of the mechanism of carcinogenesis.

Animal data are applicable to humans.

There need not be an exact correspondance between the histiopathological distribution of animal and human cancer; the use of most sensitive animal species, sex, and tumor site to predict human effects is justified.

High dose bioassays are appropriate for determining low dose responses.

Lifetime cumulative average daily dose is the appropriate dose to use for dose-response assessments.

The route of exposure need not be identical between animal and man if the tumors of interest appear distally to the point of exposure contact.

A threshold for benzene's carcinogenic effect is not established.

Benign and malignant tumors may be combined for dose-response assessment.

Doses on a surface area basis are equivalent between species.

The multistage theory most appropriately describes the phenomenon of carcinogenesis and the low dose extrapolation procedure developed by Crump based on the multistage theory is an appropriate method for dose-response assessment.

3.3 Dose-Response Assessment Based on Animal Data

3.3.1 Long-term Animal Bioassays Available for Dose-Response Assessment

a) Historical Experimental Data. The available experimental data prior to 1976 has been summarized by Maltoni et al. (1983a) and is shown in Table VI-1. These earlier works did not provide evidence for carcinogenicity in animals. The IARC (1974) concluded, "Benzene has been tested only in mice by subcutaneous injection and skin application. The data reported do not permit the conclusion that carcinogenic activity (in animal bioassays) has been demonstrated." In an updated version when Maltoni's early bioassays became

TABLE VI-1. Long-term Carcinogenicity Bioassays on Benzene: Available Data up to the Mid-1970's*

Authors	Animals: Species	Strain	Sex	No.[a]	Treatment and other experimental details	Results	Observations
Lignac [1932]	Mice	"Albino"	M,F	33 T	Subcutaneous injection of 0.001 ml of benzene in 0.1 ml of olive oil, once weekly for 17-21 weeks (total dose, about 1 mg/kg bw)	8 leukemias (from 4 to 8 months from the start of treatment)	No control group
Kirschbaum and Strong [1942]	Mice	F	?	30 T	Subcutaneous injection of 0.001 ml of benzene in 0.1 ml of sesame oil	6 leukemias (30%) (from 200 to 300 days of age)	Leukemia increase in treated animals is not statistically significant
				212 C		29 leukemias (14%) (before 300 days of age)	
Amiel [1960]	Mice	DBA2	M	30 T	Subcutaneous injection of 0.001 ml of benzene in 0.1 ml of olive oil, for the whole life span	0	Maximum survival of DBA2, C3H and C57BL6 mice: 730 days
		C3H		30 T		0	
		C57BL6		30 T		0	
		AKR	M	30 T		16 leukemias (between the 7th and the 16th month of treatment); 8 animals died before the 9th month of treatment	Major survival of animals of control group
				35 C		30 leukemias	
Hiraki et al [1963]	Mice	Swiss	M,F	10 T	Subcutaneous injection of 0.001 ml of benzene in 0.1 ml of olive oil, for 10 weeks	5 subcutaneous sarcomas (at autopsy, performed between the 162nd and the 253rd day from start of treatment)	2 animals died within the first 8 weeks of treatment No control group
Many	Mice Rats Rabbits	Various	M,F	A great many	Skin applications	No effects	Nonsystematic and non ad hoc planned experiments

*Maltoni et al. (1983) from Goldstein, B: Hematotoxicity in humans. In: Benzene toxicity, A critical evaluation. Eds. S. Laskin and B. Goldstein. J Toxicol & Env Hlth Supplement 2:69-105, 1977.

[a] T = treated: C = control.

available, IARC (1982) reported, "Benzene has been tested in rats by intragastric administration and inhalation exposure, and in mice by skin application, inhalation exposure and subcutaneous injection. Oral administration to rats resulted in an increase in the incidence of Zymbal gland carcinomas ... and an increased incidence of lymphoid tumors occurred in male mice exposed by inhalation to benzene ...." IARC concluded, "There is limited evidence that benzene is carcinogenic in experimental animals." (IARC 1982). These were studies that were available through 1981; subsequently two significant series of bioassay studies have been reported, those of Maltoni et al. (1983a) and the National Toxicology Program (NTP, 1983).

b) Recent Bioassay Studies. Maltoni et al. (1983a) conducted carcinogenesis bioassays of benzene (99.93% pure, with 0.06% paraffin and 0.01% toluene) administering the test chemical either by inhalation or orally via stomach tube (gavage) using extra-virgin olive oil as the dose vehicle. Two different gavage studies were done. In one study (Experiment 1, #BT901) groups of 35 male and 35 female Sprague-Dawley rats were administered doses of 250 mg benzene/kg body weight; groups of 30 male and 30 female Sprague-Dawley rats were administered doses of

50 mg benzene/kg body weight; and groups of 30 male and 30 female Sprague-Daley rats were administered olive oil alone (controls). Animals were dosed once daily for 4-5 days per week for 52 weeks. All animals were to be kept under observation until spontaneous death. Mortality was higher in the benzene treated groups and was correlated to dosage. The authors stated that the increase in mortality correlated to the direct effects of the treatment in the first period of the experiment, and later both to the effects of treatment and to the higher incidence of malignant tumors caused by the compound. The body weights of the test animals generally were lower than the control group, and this appears to be dose related. The results of the gavage study are shown in Table VI-2.

In the second gavage study (Experiment 3, #BT902-906) the authors studied benzene, toluene, xylene and, ethylbenzene, also administered in olive oil. Groups of 40 male and 40 female 7-week-old Sprague-Dawley rats were given doses of 500 mg/kg for 4-5 days weekly for 104 weeks. The control group consisted of 50 male and 50 female rats that were given olive oil alone on the same dosing schedule. This study was still in progress at the date of publication (1983), but the authors provided the 92-week interim results.

Only benzene resulted in a statistically significant increased number of tumors (Zymbal gland of the oral cavity; see Table VI-2).

The authors also studied the effects of benzene inhalation on Sprague-Dawley rats using 13-week-old female breeder rats and rats first exposed in utero as 12-day embryos and then exposed immediately after birth (Experiment 2, #BT4004 and #BT4006). The inhalation studies used two different exposure periods of 15 and 104 weeks. Groups of 140 12-day embryo rats and 54 13-week-old breeder rats were used for the 104-week study. The animals were exposed to inhalation by benzene at concentrations of 200-300 ppm, 4-7 hours daily. The 15-week study used 129 rats exposed as embryos from the 12th day of pregnancy and exposures of 200 ppm benzene for 4-7 hours daily. The control group consisted of 218 12-day embryo-exposed rats and 60 breeder rats kept in inhalation chambers with no benzene. All animals were to be kept under observation until spontaneous death. The results of the inhalation studies are also shown in Table VI-2. This study begun in 1979 was still in progress as of the date of publication (1983).

Although Zymbal gland carcinomas, "leukemias", oral cavity carcinomas, and mammary gland carcinomas

Table VI-2

Maltoni et al. Benzene Rat Bioassay Summary

Organ (site)	Tumor Type	Route of Exposure	Sex	Dosage	Cochran-Armitage Linear Trend Test	Fisher Exact Test	Difference in Cancer Attack Rate per 100 (Dose - Control)
Zymbal Gland	Carcinomas	Gavage[1]	Female		P < 0.001		
				13.9 mg/kg-day	----	P = 0.25 (NS)	6.7
				66.7 " " "	----	P = 0.003	25.0
Hemolympho-reticular	"Leukemias"	Gavage[1]	Male		P = 0.005		
				13.9 mg/kg-day	----	P = 1.0 (NS)	0.0
				66.7 " " "	----	P = 0 078	12.1
Mammary	Carcinoma	Gavage[1]	Female		P = 0.091 (NS)		
				13.9 mg/kg-day	----	P = 0.50 (NS)	3.3
				66 7 " " "	----	P = 0.178 (NS)	11.9
Zymbal Gland	Carcinomas	Gavage[2]	Female	321.4 mg/kg-day	N/A (single dose level)	P = 0.007	15.0
" "	"	"	M & F		N/A (single dose level)		
				321.4 mg/kg-day		P < 0.001	14.0
Oral Cavity	Carcinomas	Gavage[2]	Male	321 4 mg/kg-day	N/A (single dose level)	P = 0.003	17.5
" "	"	"	M & F		N/A (single dose level)		
				321 4 mg/kg-day		P < 0.001	13.7

Table VI-2 (Continued)

Maltoni et al Benzene Rat Bioassay Summary

Organ (site)	Tumor Type	Route of Exposure	Sex	Dosage	Cochran-Armitage Linear Trend Test	Fisher Exact Test	Difference in Cancer Attack Rate per 100 (Dose - Control)
Zymbal Gland	Carcinoma	Inhalation[3]	Female	17 mg/kg-day	N/A (single dose level)	P = 0.27 (NS)	3.9
Zymbal Gland	Carcinoma	Inhalation[4]	Male and Female	16.4 mg/kg-day	N/A (single dose level)	P = 0.002	5.1
Liver	Hepatomas	Inhalation[5]	Female	1.42 mg/kg-day	N/A (single dose level)	P = 0.022	5.1

NS - Not Statistically Significant, P > 0.05.
N/A - Not Applicable.

[1] Experiment 1:(#BT901), animals dosed for 52 weeks.

[2] Experiment 3:(#BT902), 92-week interim results, dosing to be carried out for 104 weeks, 118-week interim results.

[3] Experiment 2.(#BT4004), inhalation exposure of 13-week old breeder rats for 104 weeks, 118-week interim results

[4] Experiment 3:(#BT4004), inhalation exposure of 12-day old embryos for 104 weeks. Dose in utero not considered, 118 week interim results.

[5] Experiment 3:(#BT4006), inhalation exposure of 12-day old embryos for 15-weeks. Dose in utero not considered, 118 week interim results.

demonstrated positive trends with respect to dose, only the Zymbal gland carcinomas (one-tailed p < 0 001), the "leukemias (one-tailed P = 0.005), and the oral cavity carcinomas (p = 0.028) reached statistical significance by the Cochran-Armitage linear trend test (see Table VI-2).

The Zymbal glands (auditory sebaceous glands) are large modified sebaceous glands which surround the external ear canal of rats. Neoplasms of the Zymbal gland usually begin at the base of the glands then often invade the ear canal. Humans have no glands homologous to the rodent's Zymbal gland (Altman and Goodman, 1979).

Maltoni et al. (1983a) concluded that:

"1) Benzene is carcinogenic in rats when given by gavage and by inhalation.

2) Benzene produces different types of tumors in different organs, and therefore it must be considered a multipotential carcinogen.

3) Benzene is a potent carcinogen, since it not only enhances the incidence of tumors frequently occurring in untreated animals of the tested colony but

also produces infrequent or unusual tumors in the animals used.

4) There appears to be a direct relationship between the dose of benzene (concentration or length of treatment) and tumor response."

The National Toxicology Program (NTP) conducted a 2-year toxicology and carcinogenesis gavage study of benzene on 50 rats (F344/N) and 50 mice ($B6C3F_1$) of both sexes per dose group (NTP, 1983). The NTP established dosages for the chronic study based on a series of subchronic (single-dose, 2-week, and 17-week) toxicity studies. Doses used for the 2-year studies were selected based on clinical observations (tremors in higher dosed mice) and on clinical pathology (lymphoid depletion in rats and leukopenia in mice). The study design used four dose levels: a zero dose vehicle control, a low dose at 20-30% of the maximum tolerated dosage (MTD), a middle dosage of 50% of the MTD, and a high dosage at the MTD. Male and female mice and female rats in the 2-year study were administered 0, 25, 50 or 100 mg/kg body weight of benzene (purity of > 99.7%) in a corn oil vehicle by gavage 5 days per week for 103 weeks. Male rats were administered doses of 0, 50, 100, or 200 mg/kg body weight of benzene by gavage in corn oil for 5 days a

week for 103 weeks. Mean body weights of the 200mg/kg male rats which survived to the end of the study were 23% lower than those of the vehicle controls, the 100 mg/kg dose males were 17% lower, and females, 14%. This study established a statistically significant dose-related increase (one-tailed $P < 0.05$) in the incidence of neoplasms at multiple sites for male and female rats and for male and female mice (see Table VI-3). Of particular significance is the induction of Zymbal gland squamous cell carcinomas in male and female mice and rats and preputial gland carcinomas in male mice. Both the Zymbal and preputial gland carcinomas are rare in untreated mice and provide convincing evidence for benzene's carcinogenic effect (see Table VI-4).

The preputial glands in the male are slender, flattened glands that lie just beneath the skin of the prepuce and open into its cavity. In the female the preputial glands (bulbi vestibuli) are located in the prepuce of the clitoris (Altman and Goodman, 1979). The are both believed to be lubricating glands.

NTP concluded, "Under the conditions of these studies, there was c ear evidence of carcinogenicity of benzene for male F344/N rats, female F344/N rats, male $B6C3F_1$ mice, and female $B6C3F_1$ mice."

Table VI-3

Summary of NTP Bioassay for Significant Neoplasms

Organ (site)	Tumor Type	Species	Sex	Cochran-Armitage Trend Test	Fisher Exact Test*	Difference in Cancer Attack Rate per 100 (High Dose - Control)
Zymbal Gland	Squamous Cell Carcinoma	Rat	Male	P < 0.001	P < 0.001	30
" "	"	"	Female	P < 0.001	P < 0.001	28
" "	"	Mouse	Male	P < 0.001	P < 0.001	43
" "	"	"	Female	P = 0.022	P = 0.121 (NS)	6
Skin	Squamous Cell Carcinoma	Rat	Male	P = 0.007	P = 0.003	16
		Mouse	Male	P = 0.028	P = 0.121 (NS)	6
Lip	Squamous Cell Carcinoma	Rat	Male	P = 0.012	P = 0.003	16
Tongue	Squamous Cell Carcinoma	Rat	Male	P = 0.078	P = 0.059	8
		"	Female	P = 0.078	P = 0.059	8
Oral Cavity	Squamous Cell Carcinoma	Rat	Male	P = 0.006	P = 0.006	14
		"	Female	P = 0.011	P = 0.028	10
Hematopoietic System	Malignant Lymphomas or Leukemia	Mouse	Male	P = 0.006	P = 0.005	23
Harderian Gland	Carcinoma	Mouse	Female	P = 0.004	P = 0.059	8

Table VI-3 (Continued)

Summary of NTP Bioassay for Significant Neoplasms

Organ (site)	Tumor Type	Species	Sex	Cochran-Armitage Trend Test	Fisher Exact Test*	Difference in Cancer Attack Rate per 100 (High Dose - Control)
Lung	Alveolar/Bronchiolar Carcinoma	Mouse	Male	P = 0.028	P = 0.020	19
		"	Female	P = 0.021	P = 0.013	12
Preputial Gland	All Carcinomas	Mouse	Male	P < 0.001	P < 0.001	63
Mammary Gland	Carcinomas	Mouse	Female	P < 0.001	P < 0.001	20
" "	Carcinosarcoma	"	"	P = 0.006	P = 0.059 (NS)	8
Harderian Gland	Adenoma or Carcinoma	Mouse	Male	P = 0.001	P < 0.001	27
" "	Carcinoma	"	Female	P = 0.004	P = 0.059 (NS)	8
Ovary	Granulosa cell tumor or Carcinoma	Mouse	Female	P = 0.003	P = 0.017	15

* Comparison of highest dose group with control, one-tailed test. Mice of both sexes and female rats dosed at 71.4 mg/kg-day, male rats dosed at 143 mg/kg-day.

Table VI-4

Rare Tumors

A. Historical Incidence of Preputial Gland Tumors in Male $B6C3F_1$ Mice Administered Corn Oil by Gavage:

1. Historical Incidence at Battelle Columbus Laboratories*:

No tumors observed in 100 animals.

2. Overall Historical Incidence:

Number of Animals at Risk	Number of Tumors	Type	Percentage
1,090	1	Adenoma, NOS	0.09 %

B. Historical Incidence of Zymbal Gland Tumors in $B6C3F_1$ Male Mice Administered Corn Oil by Gavage:

1. Historical Incidence at Battelle Columbus Laboratories*:

No tumors observed in 100 animals.

2. Overall Historical Incidence.

Number of Animals at Risk	Number of Tumors	Type	Percentage
1,090	0	----	< 0.09 %

---

* The Laboratory that conducted the benzene study for the NTP.

3.3.2 Discussion of Bioassay Results

Benzene administered by gavage and by inhalation in the Maltoni studies resulted in Zymbal gland cancers in both sexes. Both the Maltoni and the NTP studies demonstrated that the female rat is less sensitive to benzene than the male for Zymbal gland carcinomas. In the Maltoni inhalation studies a statistically significant increased incidence of Zymbal gland cancers could only be demonstrated by combining results in male and female rats that were first exposed as embryos on the twelfth day of pregnancy and treated for 104 weeks after birth. The only gavage study resulting in statistically significant female rat Zymbal gland carcinomas was Maltoni's in the high dose experiment 3, #BT902. In this study the combined male and female attack rate was 14/100 at a dose of 321.4 mg/kg-day. The attack rate for the combined male and female inhalation study was 5.8/100 at a dose of 15.7 mg/kg-day.

Both Maltoni studies are interim results of lifetime bioassays. The inhalation study went for 118 weeks, while the gavage study for only 92 weeks. These studies will continue until spontaneous death of all animals. The observed 8-fold higher attack rate in the inhalation studies versus the gavage studies (response per mg/kg-day: inhalation = 0.369/100, gavage = 0.0436/100) may be an artifact of the shorter period of

follow-up and exposure for the gavage studies.[1] Maltoni does not provide data on the lifetime of the average rat; however, the literature value for the average lifetime for rats is 2.5 to 3 years with a mean of 2.75 years (Baker et al., 1979).[2]

---

[1] Nonfatal, incidental tumors that may have already developed will not become apparent until death and the early incidence rate will underestimate the true risk.

[2] See Table VII-1 for assumptions of rodents' lifetime for the purposes of risk assessments.

It is possible to estimate the lifetime cancer rate using the method in the EPA report (1983)[1] which incorporates a factor into the model to reflect the length of observation relative to the species average lifetime:

Let:

L_t = the average rat lifetime, in weeks.

L_o = the observational period, in weeks.

Then:

$$\text{adjustment factor} = \left(\frac{L_t}{L_o}\right)^3$$

Hence for these studies:

		Corrected Attack Rates
$\text{Factor}_{\text{gavage}}$	$= \left(\frac{143}{92}\right)^3 = 3.76$	0.16/100
$\text{Factor}_{\text{inhalation}}$	$= \left(\frac{143}{118}\right)^3 = 1.78$	0.66/100

---

[1] Since these were single-dose studies, the provided rates are essentially low dose slopes and since if $x < 0.1$, values of $(1 - e^{-x})$ are equal to x. Thus, these values are equivalent to the exponent q that CAG discusses for adjustment for nonlifetime observational periods.

The corrected rates suggest a possible 4-fold higher Zymbal gland carcinoma rate for benzene exposures by inhalation. Thus, it would be fair to say that benzene exposure via inhalation is at least as, if not more potent than gavage exposure.

The rat may provide an effective model for human breast cancer. Mammary gland tumors, both benign and malignant, occur as spontaneous and induced lesions in both male and female rats. The incidence of mammary tumors varies greatly within and among different strains. Female Sprague-Dawley rats have been reported to spontaneously develop mammary tumors at rates ranging from 14/100 to 57/100. Eighty-eight percent of them are benign fibroadenomas (Baker et al., 1979). The historical incidence of mammary gland carcinomas or adenocarcinomas in female $B6C3F_1$ mice that were administered corn oil by gavage at Battelle Columbus Laboratories (the institution that performed the bioassy for the NTP) is 1.3% with a standard deviation of 1.55% (NTP, 1983).

The NTP and Maltoni studies both resulted in significant noncarcinogenic adverse toxicological responses. Animals in both studies had dose-related increased mortalities and dose-related weight losses. Although it could be argued that these chronic toxicological insults from high doses of benzene could be responsible for the carcinogenic response to benzene, the staff of DHS believe that the evidence supporting this theory (cytotoxicity) is insufficient and at present there does not appear to be convincing scientific or public health grounds to justify incorporating the

cytotoxicity theory in the risk assessment process. (See Appendix A for a more detailed discussion of cytotoxicity.)

3.3.3 Dose-Response Assessment Based on Recent Bioassays

A summary of the calculated low-dose risk assessments is shown in Table VI-5. The table presents results for mouse and rat data including several target sites and gavage and inhalation routes of exposure. The results of the CAG epidemiologic based assessments are also included in the table for comparative purposes.

The excess risk of cancer associated with exposure to benzene is estimated to be in the range of 20-340 X 10^{-6} for a lifetime exposure to 1 ppb benzene in air. These estimates are given in the last column in the table, entitled "Multistage Model for Human Equivalent Cancer Risk/ppb Benzene," which may require some explanation. These values represent the lifetime (70-year) theoretical excess cancer risk to a human population, based on the incidence of specified animal cancer, from a lifetime (continuous) exposure to a time-weighted average of one part per billion (ppb) of benzene in the ambient air. Thus, if a cohort of one million individuals were exposed to an average concentration of 1 ppb of benzene in their respired air from birth to death, one might expect to see the stated the cancer rate in excess of the "normal" background rate. The theoretical yearly risk from this lifetime exposure to 1 ppb is approximately 1/70 of that number. Since the risk model is linear at low

doses, the theoretical risk is directly proportional to the benzene concentration; e.g., a 10 ppb lifetime exposure to benzene would convey ten times the risk listed in the last column. The risk estimates provided represent the maximum likelihood estimate (MLE) of risk which is the best point estimate and the 95% upper confidence level (95% UCL) for this point estimate.

Two types of tumor rate analyses are presented for the (NTP) studies, the attack rate (unadjusted rate) and a life table adjusted rate. The attack rate is the simple percentage of the number of animals with the stated site-specific tumor divided by the number of animals in which that site was examined. The life table adjusted rate attempts to correct for animals which die during the 2-year course of the experiment either due to benzene toxicity or due to natural causes, and thus are not available to develop cancer. Accounting for this loss would increase the cancer risk. As can be seen from Table VI-5, the implication of this correction is a 2-4 fold difference in the estimated risk from a 1 ppb exposure to benzene. However, since DHS now only has an attack-rate-based Crump model, attack rates will be used. Since the epidemiologic data were not adjusted for competing causes of death, the risk levels based on these data would also be underestimates.

It should be noted that although the male mice Zymbal gland tumors provide the highest cancer attack rates in the experimental studies, the low-dose extrapolation rates for both the male mice preputial gland carcinomas and lymphomas or leukemias are greater than the Zymbal

Table VI-5

Summary of Benzene Low Dose Risk Assessments

Study	Route of Exposure	Lifetime[a] TWA Dosage	Tumor Type	Species	Sex	Type of Analysis	Multi-Stage Model for Human Equivalent Cancer Risk/ppb benzene
NTP (1983)	Gavage	17.9 mg/kg-day[b]	Zymbal gland Carcinomas	Mouse	M	Crude Attack Rate	MLE - 7.4 X 10^{-6} 95% UCL - 34 X "
						Lifetable Adj. Rate	MLE - 6.9 X 10^{-6} 95% UCL - 47 X "
NTP (1983)	"	" " "	Preputial gland Carcinomas	"	M	Crude Attack Rate	MLE - 78 X 10^{-6} 95% UCL - 170 X "
						Lifetable Adj. Rate	MLE - 140 X 10-6 95% UCL - 340 X "
NTP (1983)	"	" " "	Lymphoma or Leukemia	"	M	Crude Attack Rate	MLE - 49 X 10^{-6} 95% UCL - 81 X "
						Lifetable Adj. Rate	MLE - 170 X 10-6 95% UCL - 230 X "
NTP (1983)	"	" " "	Mammary Carcinomas	"	F	Crude Attack Rate	MLE - 32 X 10^{-6} 95% UCL - 57 X "
						Lifetable Adj. Rate	MLE - 61 X 10-6 95% UCL - 92 X "

Table VI-5 (Continued)

Study	Route of Exposure	Lifetime[a] TWA Dosage	Tumor Type	Species	Sex	Type of Analysis	Multi-Stage Model for Human Equivalent Cancer Risk/ppb benzene
Maltoni et al (1983)	Gavage	13.9 mg/kg-day[c]	Zymbal Gland Carcinomas	Rat	F	Crude Attack Rate	MLE - 26 X 10^{-6} 95% UCL - 42 X "
Maltoni et al (1983)	Inhalati...[d]	16.45 mg/kg-day[e]	Zymbal Gland Carcinomas	Rat	M & F Combined	Crude Attack Rate	MLE - 6.4 X 10^{-6} 95% UCL - 12 X "
Infante et al (1981)	Inhalation	2.81 ppm[f] (2.99 mg/kg-day)	Leukemia (Myelocytic or Monocytic)	Human	M	Fatal Tumor Life Table	15 X 10^{-6}
Rinsky et al (1981)	Inhalation	2.81 ppm[f] (2.99 mg/kg-day)	Leukemia (Myelocytic or Monocytic)	Human	M	Fatal Tumor Life Table	48 X 10^{-6}
Aksoy et al (1974,76,77)	Inhalation	4.22 ppm[f] (4.49 mg/kg-day)	Leukemia	Human	M	Fatal Tumor	20 X 10^{-6}
Ott et al (1977)	Inhalation	0.171 ppm[f] (0.182 mg/kg-day)	Leukemia	Human	M	Fatal Tumor	46 X 10^{-6}
CAG (EPA, 1984)	Inhalation		Leukemia	Human	M	Fatal Tumor	22 X 10^{-6}

TABLE 5 ASSUMPTIONS

Assumptions: 60 kg person, human inhalation at 20 m^3/day

MLE - Maximum likelihood estimate
95% UCL - 95% upper confidence limit on risk for provided dose.

[a] Dosages provided without scaling factors.

[b] Lowest dose used in three dose risk assessment, Cochran-Armitage linear trend test for these tumors: Preputial gla
P < 0.001, Zymbal Gland, P < 0.001; Lymphoma or Leukemia, P = 0.035; Mammary Carcinoma, P < 0.001.

[c] Lowest dose used in two point risk assessment, Cochran-Armitage linear trend test for these tumors P < 0.001.

[d] Pregnant Sprague-Dawley rats from the twelfth day of pregnancy at a concentration of 200 ppm, 4 hr/day, 5 day/week
delivery, then offspring assumed to be exposed to 200-300 ppm 4-7 hr/day, 5 days/wk for 104 weeks. Exposure in ute
not calculated for total lifetime dosage

[e] Provided for comparative purposes.

[f] Estimated lifetime dosage by the EPA-CAG (EPA, 1979).

gland low-dose extrapolations. This is because the Zymbal gland dose-response curve drops off more rapidly than those of the other tumors. Hence the dose-response curves cross at moderate doses 0.1-2 mg/kg-days.

Figure VI-2 illustrates that the MLE and the 95% UCL unadjusted leukemia incidence rates, based on the most sensitive species, strain, and sex, are of a similar magnitude as those based on the observed human occupational exposure rates despite the numerous different assumptions made in the assessments from each source of data. This rough agreement between the human and animal bioassay estimates suggest to the staff that it would be inappropriate to choose an animal bioassay that was less sensitive than the EPA leukemia estimate.

As noted previously, the results of animal bioassays indicate that benzene causes cancer at sites other than the hematopoietic system. The multistage model was applied to mouse data from the NTP bioassay and yielded the following human equivalent excess cancer risks for a lifetime exposure to 1 ppb benzene:

Data Used (Sex and Site)	Risk[1] X 10^{-6}	
	MLE	95% UCL
Male-Lung Cancer	42	76
Female-		
Mammary Carcinomas	32	57
Male-Oral Cavity Tumors	100	130

Female-Ovarian Tumors	19	77
Female-Mammary or Ovarian Tummors	63	92

[1] Dose-response curves are linear in the low dose range so risk estimates for different doses would be multiples of the values given for 1 ppb in air.

Thus, these assessments yield risk estimates which are similar (i.e., differing by less than an order of magnitude) to the estimates derived from both the epidemiologic data for leukemia and the most sensitive species, sex, and site animal data.

Table VI-6 shows the results of the assessments using different extrapolation models. The results are as expected: the multistage models provide a linear dose-response relationship in the low dose range and risk estimates that are intermediate relative to the other models. The Mantel-Bryan and the logistic models yield risk estimates similar to that of the multistage model though the Mantel-Bryan is slightly more conservative while the logistic shows slightly less risk. The probit model is non-linear and predicts a risk that drops off quite dramatically in the range of ambient air concentrations.

For illustrative purposes three different interspecies scaling factors were applied to the male mouse preputial gland data. The estimates of human risk per exposure to 1 ppb benzene in air are as follows:

mg/kg-day	14×10^{-6}
mg/surface area-day	170×10^{-6}
mg/lifetime	580×10^{-6}

Table VI-6

BENZENE RISK ESTIMATES USING SEVERAL EXTRAPOLATION MODELS[1]

Benzene Concentration in Air	Crump 95% UCL of M-S[2]	M-S[3]	M-B[4]	Logit[5]	Probit[6]
0.1 ppb	1.7×10^{-5}	7.8×10^{-6}	3.2×10^{-5}	4.7×10^{-6}	-
0.5 ppb	8.5×10^{-5}	3.9×10^{-5}	4.8×10^{-4}	2.4×10^{-5}	-
1.0 ppb	1.7×10^{-4}	7.8×10^{-5}	1.4×10^{-3}	4.7×10^{-5}	1.3×10^{-13}
5.0 ppb	8.5×10^{-4}	3.9×10^{-4}	1.1×10^{-2}	2.4×10^{-4}	2.9×10^{-8}
10.0 ppb	1.7×10^{-3}	7.8×10^{-4}	-	4.7×10^{-4}	2.1×10^{-6}
50.0 ppb	8.5×10^{-3}	3.9×10^{-3}	-	2.4×10^{-3}	3.4×10^{-4}

(1) Lifetime excess cancer risk from a lifetime exposure to benzene at the stated concentrations, based on NTP bioassay results for male mice preputial gland cancers.

(2) Crump estimate of 95% upper confidence level of risk for multistage (M-S) model.

(3) Multistage model

(4) Classical Mantel-Bryan model using 99% UCL for response for lowest dose and assuming a probit slope of 1.0. This model was developed to find a virtually safe dose (assmued to be a risk level of 1×10^{-8}). The model uses a plot of the log dose versus the normal equivalent deviate (NED) to establish this level.

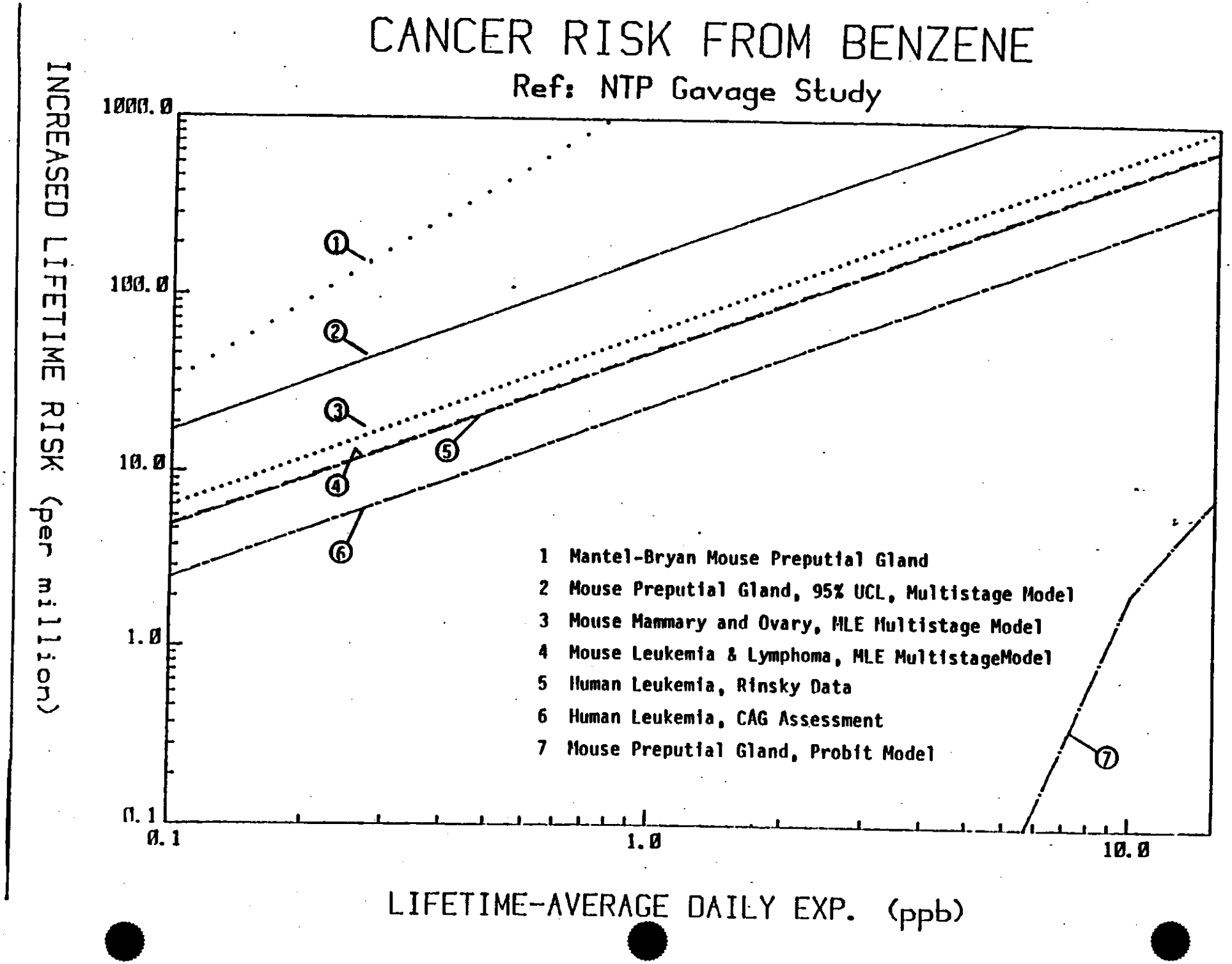
(5) Log of the logistic model weighted by the inverse of the variances:

$$\text{Incidence} = 1 + \exp-(a + bd)$$

Corrected for model generated background cancer incidence by use of Abbott's correction.

(6) Unweighted Probit model for log dose versus the NED

Figure VI-3


CANCER RISK FROM BENZENE
Ref: NTP Gavage Study
INCREASED LIFETIME RISK (per million)
1000.0
100.0
10.0
1.0
0.1
0.1
1.0
10.0
LIFETIME-AVERAGE DAILY EXP. (ppb)
1 Mantel-Bryan Mouse Preputial Gland
2 Mouse Preputial Gland, 95% UCL, Multistage Model
3 Mouse Mammary and Ovary, MLE Multistage Model
4 Mouse Leukemia & Lymphoma, MLE MultistageModel
5 Human Leukemia, Rinsky Data
6 Human Leukemia, CAG Assessment
7 Mouse Preputial Gland, Probit Model

Legend for Figure VI-3

Cancer Risk From Benzene Using Different Low-Dose Extrapolation Models

Animal data are based on the National Toxicity Program (NTP) gavage study in mice.

Line 1: Mantel-Bryan procedure applied to male preputial gland cancers.

Line 2: 95% upper confidence limit (UCL) from the Crump procedure for the multistage model also for male preputial cancers.

Line 3: Maximum likelihood estimate (MLE) using Crump procedure with mouse mammary and ovarian cancers.

Line 4: MLE from Crump procedure with mouse leukemia and lymphoma.

Line 5: A linear extrapolation based on the Rinsky reevaluation of Infante's epidemiologic study. This line has been cited by EPA-CAG.

Line 6: A linear extrapolation model using epidemiologic data from three studies. This line has also been cited by CAG.

Line 7: The probit model applied to mouse preputial gland cancer.

4. Summary of Dose-Response Assessments

Epidemiologic studies are intuitively the best source of information for use in establishing dose-response relationships since the species of concern is being studied and, in this particular assesment where exposure via inhalation is the paramount concern, the route of exposure is also the route of concern. However, the studies reported to date are not without problems. Exposure levels and exposure periods are poorly documented, mortality rather than incidence is reported, the number of exposed individuals tends to be small, appropriate control groups are not always used, results are only directly applicable to white employed males--effects in women and children have not been sufficiently studied, and few confounding factors are controlled for. However, using the available data and making reasonable assumptions with regard to the unknown factors, a risk estimate of 22×10^{-6} per ppb benzene can be derived.

Animal studies also have advantages and disadvantages for use in estimating human dose-response relationship. Experiments tend to be performed under controlled conditions and exposure levels are known. However, the dosage used is typically very high, the route of exposure may not directly correspond to that in humans, and, to over simplify, animals used in bioassays are different from human . Again, making reasonable assumptions it is possible to use animal data to predict effects in man. The human risk associated with the most sensitive species, sex and site yielded a value of 170×10^{-6} per ppb in air which is only 3.5-7 times as great as the risk estimated from human leukemia mortality data. Given that this risk value is

the expectation of the highest risk and is a surrogate for all cancers that might result from exposure, the DHS considers this value to be comparable to the risk estimate based on the epidemiologic data. Since women and children are exposed to benzene in ambient air and since there have not been adequate epidemiological studies to establish if there are risks for mammary, ovarian, or other cancers, the DHS recommends that the risk of benzene induced cancer from ambient air exposure be taken as falling in the interval with the current epidemiologic studies as a lower bound and the animal estimate above as the upper bound.

VII. Computations for Risk Assessment

1. General Assumptions for Risk Assessments for Animal Bioassays

1.1 The average weight of a person from birth until death for both sexes combined is assumed to be 60 kg.

1.2 The average human lifetime is assumed to be 70 years.

1.3 The average adult inhales 20 m^3 of air per day.

1.4 It is assumed that all benzene given by gavage in the animal bioassays is absorbed.

1.5 The absorption of inhaled benzene is assumed to be the same for all species.

1.6 It is assumed that the lifetimes of both rats and mice are 104 weeks. This is consistent with the terminal sacrifice period for lifetime studies used by the NTP and the EPA-CAG calculations.

1.7 A scaling factor based on surface area provides the best estimation of equivalent doses between species. Since the surface area is approximately proportional to the two-thirds power of the weight, exposures expressed in mg/kg-day in one species are assumed to be equal to exposures in other species when expressed as $mg/(kg)^{2/3}$ per day.

1.8 In experiments in which dosing is not given for the entire lifetime of the animal, the total amoung given is averaged over the lifetime of the animal.

1.9 The Crump multistage risk model (Crump and Watson, 1979), which assumes no practical threshold, is used. Dosages were entered using surface area corrected doses for the gavage studies and mg/m^3 for inhalation studies.

Both the maximum likelihood estimates and linearized 95% upper confidence limit of risk for a specified dose are presented.

2. Human Epidemiological Studies

The EPA (EPA, 1979) has updated their risk assessment of benzene using the Rinsky et al. re-evaluation (1981) of the Infante et al. occupational study (1977). CAG provides a potency estimate for benzene as a low dose slope (EPA, 1983, Ch 13, p 165):

$$\text{slope} = 5.2 \times 10^{-2} \text{ (mg/kg-day)}^{-1}$$

This slope can be converted into a lifetime risk from a lifetime exposure to 1 ppb benzene in air as follows:

$$1 \text{ ppb benzene} = 3.195 \times 10^{-3} \text{ mg/m}^3$$

CAG used an average adult weight of 70 kg for workers and if the average adult inhales 20 m^3/day, the average dose is:

$$\text{dose} = (3.195 \times 10^{-3} \text{ mg/m}^3) \times (20 \text{ m}^3\text{/day}) \times (1/70 \text{ kg})$$

$$= 9.129 \times 10^{-4} \text{ mg/kg-day}$$

Hence the lifetime human excess cancer risk from a lifetime exposure to 1 ppb of benzene in the ambient atmosphere is:

$$\text{Risk/ppb benzene} = (9.129 \times 10^{-4}) \times [5.2 \times 10^{-2} \text{ (mg/kg-day)}^{-1}]$$

$$= 48 \times 10^{-6}$$

3. Animal Bioassays

3.1 Statistical Methods

3.1.1 Fisher Exact Test

a. NTP supplied values for the Fisher exact test, comparing the number of tumors in each dose group with those in the vehicle control group.

b. The pairwise comparison of each dose group with the controls to establish a one-tailed P value was calculated by DHS for the Maltoni et al study (Sokal and Rohlf, 1969).

3.1.2 Cochran-Armitage Linear Trend Test

a. NTP supplied values for the Cochran-Armitage Linear Trend Test for each tumor type for each target organ.

b. For the Maltoni studies the Cochran-Armitage Linear Trend Test was calculated by DHS according to the procedure of Peto et al. (1980).

3.2 Maltoni et al Bioassay Studies

These bioassay studies provide scant information on the experimental methods and protocol employed, and thus to quantitate this data a number of assumptions must be made. These will be discussed in detail for each route of benzene administration.

3.2.1 Dose Calculations

a. Gavage (Experiment 1, #BT 901) - female rats.

(1). It is assumed that the animals were dosed 4.5 times/week.

(2). The authors only provide average weights at 26 and 52 weeks during the dosing period. The best estimate for the animal's average weight during this period should include the animal's initial (13-week) weight so as not to overestimate the dosage. The weight of a 13-week old Sprague-Dawley rat was estimated from the average weight of rats supplied by two separate laboratories (see Table VII-1). Sensitivity analysis of this method suggests that using only the 26-week average weight of the animals as a best estimate will result in an approximate 1% difference in dose at a specified risk level.

Table VII-1

Weights of Sprague-Dawley Rats

1. Simonsens Albino Rats Sprague-Dawley derived:

Week	Females
10	240 - 260 g
12	270 - 280 g
13	280 - 290 g

Simonsens Laboratories Inc.
1180-C Day Road
Gilroy, CA 95020

2. Outbred Sprague-Dawley Rats

Weeks	Females
12	250 - 275 g
13	275 - 300 g

Hilltop Lab Animals Inc.
Hilltop Drive
Scottdale, PA 15683

Average 13-week age:

Simonsens:	280 - 290 g	av. 285 g
Hilltop:	275 - 300 g	" 287.5 g
	overall average:	286.2 g

If: Wt_1 - The average weight for 13-week old female rats from two different laboratory animal suppliers.

Wt_2 - Average weight at 26 weeks.

Wt_3 - Average weight at 56 weeks

D - Dose rate (mg/kg).

Then:

$$\text{Total dose} = 1/2\left[\frac{(Wt_1 + Wt_3)}{2} + Wt_2\right] \times D \times 4.5 \text{ times/week} \times 52 \text{ weeks}$$

(3). The average lifetime weight for each dose group was calculated from the mean of the average weights for 26, 52, 78, and 104 weeks. The average lifetime weights are:

Low Dose = 0.3584 kg

High Dose = 0.3689 kg

(4). The lifetime average daily dose was calculated by dividing the total lifetime dose by 728 days/lifetime and the lifetime average weight:

Low Dose = 13.9 mg/kg-day

High Dose = 66.7 mg/kg-day

(5). The calculated lifetime surface area (SA) corrected dosages are:

Low dose = 9.87 mg/SA-day

High dose = 47.9 mg/SA-day

b. Inhalation (Experiment 2, #BT4004 and #BT4006)

The risk assessment was based on male and female rats the exposure of which began in utero on the twelfth day of pregnancy and continued after birth for 104 weeks. It is assumed that the total exposure period does not include the approximately 1.5 weeks of in utero exposure.

(1). The dose in mg/kg of partially soluble vapors [the octanol/water partition coefficient of benzene is 135 (Chiow et al, 1977)] is proportional to oxygen consumption, which in turn is proportional to $Wt^{2/3}$ and is also proportional to the solubility of the gas in body fluids, which in turn can be expressed as an absorption coefficient, r, for the gas. Therefore, expressing the O_2 consumption as $O_2 = (k)(W^{2/3})$, where k is a constant independent of species, it follows that:

If:

m - the average dose/day in mg during administration of the agent.

v - the average lifetime concentration of benzene in the inhalation chambers.

Then:

$$m = (k) \times (W^{2/3}) \times (mg/m^3) \times r$$

$$dose = \frac{m}{W^{2/3}} = kvr$$

In the absence of experimental information or a sound theoretical argument to the contrary, the absorption fraction, r, is assumed to be the same for all species. Therefore, for these substances a certain concentration in ppm or in mg/m^3 in experimental animals is equivalent to the same concentration in humans.(EPA, 1983)

(2) The calculated time-weighted average daily benzene exposure is 26.24 mg/m^3 (8.21 ppm).

(3) The lifetime average body weight for each sex and the lifetime average body weight for both sexes combined (weighting by the initial number of male and female animals) is shown below:

Males average lifetime weight = 0.50780 kg

Female average lifetime weight = 0.32072 kg

Lifetime average body weight
for both sexes combined = 0.42094 kg

(4) The daily lifetime average dose for both sexes combined is 15.73 mg/kg-day.

3.2.2 Attack Rate Calculations

The attack rate is the ratio of the number of those animals with a specified tumor divided by the number of animals at risk. The numerator of the attack rate is the number of animals with the specified tumor. The denominator (animals at risk), which Maltoni calls the corrected number, was provided by the authors and is the surviving number of animals when the first tumor of any type was observed. These times were:

a. Gavage (Experiment 1, #BT901) - Number of animals alive at 20 weeks (type of tumor not specified).

b. Gavage (Experiment 3, #BT902) - Number of animals alive at 52 weeks following first appearence of Zymbal gland tumor.

. Inhalation (Experiment 2, #BT4004 and BT4006) - Number of animals alive at 22 weeks following first appearance of mammary tumor.

3.3 NTP Bioassay Studies

3.3.1 Dose Calculations.

a. The average lifetime weight of mice are shown below and were calculated by summing the provided individual 34-35 weekly average weights for each dose group.

(1) Male mice.

Low Dose Group	= 37.1 g	std. deviation	= 5.8 g
Mid Dose Group	= 37.2 g	"	= 5.3 g
High Dose Group	= 35.1 g	"	= 3.9 g

(2) Female mice.

Low Dose Group	= 31.8 g	std. deviation	= 7.9 g
Mid Dose Group	= 33.8 g	"	= 9.2 g
High Dose Group	= 32.2 g	"	= 7.6 g

b. The average lifetime daily dose was calculated by dividing the lifetime average weight of the animals by the provided concentrations and then by multiplying by 5/7 (the weekly dosing schedule). These values are shown below:

	Male and Female
Low Dose Group	= 17.9 mg/kg-day
Mid Dose Group	= 35.2 " " "
High Dose Group	= 71.4 " " "

c. The average surface area corrected daily lifetime dosages are shown below:

	Male	Female
Low Dose Group	= 5.96 mg/SA-day	5.66 mg/SA-day
Mid Dose Group	= 11.9 " " "	11.6 " " "
High Dose Group	= 23.4 " " "	22.7 " " "

3.3.2 Calculation of Adjusted Lifetable Rates

The NTP provides the lifetable adjusted rate as a decimal fraction while the Crump Global 79 program that the staff of DHS uses to calculate the low dose risk requires data input as a simple tumor ratio, i.e. for 7 tumors out of 50 animals the Crump input is 7,50. In order to utilize the program, the total animals in each dose group (50) were multiplied by the fractional lifetable adjusted incidence rate and rounded to the closest whole number. Confidence intervals thus derived by the Crump model will more directly reflect the statistical uncertainty for the number of animals per dose group.

Essentially the Crump program is merely used to generate the maximum likelihood polynomial that best fits the data. Since the 95% UCL is linear, this use of the program should correctly estimate the response. However this is not a standard use of the program and thus the lifetable rates derived by this method are included for comparative, illustrative purposes only.

4. Multistage Model

The multistage theory of carcinogenesis was derived to account for the fact that in many types of cancer, the logarithm of the cancer mortality rate increases in direct proportion to the logarithm of age. This suggests that a cell may go through a sequence of specific changes (stages) in order to become malignant (Brown, 1978; Peto, 1977).

Multistage models assume that a carcinogen can act to increase any of the event rates (the rate at which a cell passes from one stage to another). Further, each transition (i) is dependent on two constants, a constant term a_i, dependent on the background rate, and a term b_i, which indicates the potency of the agent at the ith stage. The total response, P(d), is an exponential product of each stage:

$$P(d) = 1 - \exp -\left(\sum_{i=1}^{k} a_i + b_i d^i\right)$$

where $a_i > 0$ and $b_i > 0$ and k is the number of stages or events required before cancer is observed. More generally,

$$P(d) = 1 - \exp -(\sum_{i=0}^{k} q_i d^i), \text{ where: } q_i > 0.$$

Thus, the response, P(d), is a polynomial function of dose with nonnegative coefficients. The model is fitted using maximum likelihood theory and the coefficients and k (number of stages) is established by the best fit to the data. Alternatively, k can be assumed to be no more than the number of dose levels.

The staff of DHS used a version of the multistage model developed by Crump and Watson (1979). This version sets the number of stages, k, to one less than the total number of dosage groups used in the bioassay. It also forces a linear term in the estimation of the upper confidence limits of the coefficients. For most data sets, therefore, P(d) based on the upper confidence limits for the Crump multistage model will give approximately the same low dose extrapolation as the one-hit model. In Crump's program, coefficients for the model and its upper confidence limits of risk are obtained using maximum likelihood estimation. The presence of a linear term in the model insures near linearity for this confidence limit in the low dose range.

VIII. References

Aksoy M, Erden S, Dincol G. (1974) Leukemia in shoe-workers chronically exposed to benzene. Blood 44:837-41.

Aksoy M, Erdem S, Dincol G. (1976) Types of leukemia in chronic benzene poisoning. A study in thirty-four parts. Acta Haematol 55:65-72

Albert R, Altshuler B. (1973) Considerations relating to the formulation of limits for unavoidable population exposures to environmental carcinogens. In: Ballou J, Busch R, Mahlum D, Sanders C. Eds. Radionuclide carcinogenesis, proceedngs of the 12th annual Hanford Biological Symposium at Richland Wasington, May 10-12, 1973, sponsored by Battelle Memorial Institute, Pacific Northwest Laboratories, and the Atomic Energy Commission. Springfield, VA: NTIS, 1973, AEC symposium series, conference 720505, pp 232-253.

Altman NH, Goodman DG. (1979) Neoplastic diseases. In: Baker HJ, Lindsey JR, Weisbroth SH., eds., The laboratory rat, volume I, biology and diseases. New York: Academic Press, pp 337-8.

Amacher DE, Zelljadt I. (1983) The morphological transformation of Syrian hamster embryo cells by chemicals reportedly non-mutagenic to Salmonella typhimurium. Carcinogenesis 4:291-5.

Ames BN, Gold LS, Havender WR, et al. (1981) Carcinogenic potency, California Policy Seminar, Final Report No. 3. Berkeley, CA: University of California, Institute of Governmental Studies.

Ames BN, McCann J, Yamasaki E. (1975) Methods of detecting carcinogens and mutagens with Salmonella/mammalian microsome mutagenicity tests. Mutat Res 31:347-64.

Anderson D, Richardson CR. (1981) Issues relevant to the assessment of chemically induced chromosome damage in vivo and their relationship to chemical mutagenesis. Mutat Res. 90:261-72.

Anderson MW, Hoe DG, Kapland NL. A general scheme for the incorporation of pharmacokinetics in low-dose risk estimation for chemical carcinogensis. Toxicol Appl Pharmacol 1980;55:154-61

Armitage P, Doll R. Stochastic models for carcinogenesis. In: Neyman J., ed. Proceedings of the Fourth Berkeley Symposium on Mathematical Statistics and Probability: Biology and Problems of Health, Berkeley, California, June 20-July 30, 1960. Berkeley, CA: University of California Press, 1961, vol 4, pp 1403-1438.

Arp EW, Wolf PH, Checkoway H. (1983) Lymphocytic leukemia and exposures to benzene and other solvents in the rubber industry. JOM 25:598-602.

Baker HJ, Lindsey JR, Weisbroth SH (1979) Appendix I. Selected normative data. In; Baker HJ, Lindsey JR, Weisbroth SH, eds. The laboratory rat, volume I, biology and diseases. New York: Academic Press.

Bergman K. (1979) Whole-body autoradiography and allied tracer techniques in distribution and elimination studies of some orgainc solvents. Scand J Work Environ Health 5(suppl 1):5-92.

Berkson J. (1944) Application of logistic function to bioassay. J Amer Stat Assoc 39:134-167

Berwald Y, Sachs L. (1965) In vitro transformation of normal cells to tumor cells by carcinogenic hydrocarbons. J Natl Cancer Inst 35:641-61.

Bliss CI. (1935) The calculation of the dosage-mortality curve. Ann Appl Biol 22:134-167.

Bloom AD, Nakagone Y, Awa A, Neriishi S (1970) Chromosome abberations and malignant disease among A-bomb survivors. Am J Public Health 60:641-4.

Brandt L, Nilsson PG, and Mitelman F. (1978) Occupational exposure to petroleum products in men with acute non-lymphocytic leukaemia. Br Med J 1:553-4.

Bridges, BA, Butterworth, BE, Weinstein, IB (Eds) (1982) Indicators of Genotoxic Exosures, Banbury Report 13. Cold Spring Harbor. NY: Cold Spring Harbor Laboratory.

Brown CC. (1976) Mathematical aspects of dose-response studies in carcinogenesis - the concept of threshold. Oncology 33:62-5.

Brown CC. (1978) Statistical aspects of extrapolation of dicotomous dose-response data. J Natl Cancer Inst 60:101-8.

Browning E. (1965) Toxicity and metabolism of industrial solvents. Amsterdam: Elsevier Publishing Company.

Brusick DB. (1980) Principles of Genetic Toxicology, Plenum Press, New York.

Carlborg FW. (1981) Multistage dose-response models in carcinogenesis. Food Cosmet Toxicol 19:361-5.

Chiow CT, Freed VH, Schmeddling DW, Kohnert RL. (1977) Partition coefficient and bioaccumulation of selected organic chemicals. Environ Sci Tecnol 11:475-8.

Chu KC, Cueto C Jr, Ward JM. (1981) Factors in the evaluation of 200 National Cancer Institute carcinogen bioassays. J Toxicol Environ Health 8:251-80

Cotruvo JA, Simmon VF, Spanggord RJ (1977) Investigation of mutagenic effects of products of ozonation reactions in water. Ann NY Acad Sci 298:124-140.

Crespi CL, Penman BW. (1984) The mutagenicity of benzene in a metabolically competent human lymphoblastoid cell line (Abstract No. Fa-10). Environ Mutagen 6:425.

Crespi CL, Ryan CG, Seixas GM, et al. (1984) Mutagenic activity of ten compounds using a gene-locus mutation assay in human lymphoblasts. Report of the Collaborative Study on Short-term tests. In press.

Crespi CL, Tilly WG. (1984) Assay for gene mutation in a human lymphoblast line, AHH-1, competent for xenobiotic metabolism. Mutat Res (in press).

Crump KS, Hoel D, Langley C, Peto R. (1976) Fundamental carcinogenic processes and their implications for low-dose risk assessment. Cancer Res 36:2973-9.

Crump K. (1981) An improved procedure for low-dose carcinogenic risk assessment from animal data. J Environ Pathol Toxicol 5:675-89.

Crump KS, Watson WN (1979) Global 79, a fortran program to extrapolate dichotomous animal carcinogenicity data to low dose. US National Institute of Environmental health Sciences, contract No. 1-ES-2123. Ruston, LA: Science Research Systems.

Dabney BJ. (1981) The role of genetic monitoring in the workplace. JOM 23:626-31.

Dean BJ. (1978) Genetic toxicology of benzene, toluene, xylenes and phenols. Mutat Res 47:75-97.

Decoufle P, Blattner WA, Blair A. (1983) Mortality among chemical workers exposed to benzene and other agents. Environ Res 30:16-25.

Dowty BJ, Laseter JL, Storer J. (1976) The transplacental migration and accumulation in blood of volatile orgainc constituents. Pediatr Res 10:696-701.

Drew RT, Fouts JR. (1974) The lack of effects of pretreatment with phenobarbital and chlorpromazine on the acute toxicity of benzene in rats. Toxicol Appl Pharmacol 27:183-193.

Druckrey H. (1967) Quantitative Aspects in Chemial Carcinogenesis. In: Truhaut R, ed. Potential carcinogenic hazards from drugs, evaluation of risks, UICC Monograph Series volume 7. Berlin and New York: Springer-Verlag, pp 60-78

Environ Corporation. (1983) Review of benzene risk assessments. Prepared for the American Petroleum Institute. Washington, DC: Environ Corporation.

Environmental Health Associates. (1983) An industry-wide mortality study of workers exposed to benzene. Submitted to the Chemical Manufacturers Association. Wong O, principal investigator. Berkeley, CA: Environmental Health Associates.

Environmental Protection Agency. (1979) Carcinogen Assessment Group's final report on population risk to ambient benzene exposures, EPA-450/5-80-004. Research Triangle Park, NC: US EPA, Office of Air Quality Planning and Standards.

Environmental Protection Agency. (1980) Water Quality Criteria Documents; Availability. Federal Register 45: 79318-79357.

Environmental Protection Agency (EPA) (1983) Health assessment document for acrylonitrile, final report, EPA-600/8-82-007F. Washington, DC: USEPA, Ofice of Health and Environmental Assessment, pp 13-143 - 13-167.

Environmental Protection Agency. (1984) National Emission Standards for Hazardous Air Pollutants; Regulation of Benzene; Response to Public Comments. Part II. Federal Register 40 (June 6): 23478-23493.

Erexson GL, Wilmer JL, Auman CD, Kligerman AL. (1984) Induction of sister chromatid exchanges and micronuclei in male DBA/2 mice by the inhalation of benzene (Abstract No. Cb-19). Environ Mutagen 6:408

Fialkow PJ. (1974) The origin and development of human tumors studied with cell markers. N. Engl J Med 291:26-35.

Fialkow PJ. (1977) Clonal origin and stem cell evolution of human tumors. In: Mulvihill JJ, Miller RW, Fraumeni JF Jr. Genetics of human cancer. New York: Raven Press, pp 439-53.

Fishbeck WA, Townsend JC, Swank MG. (1978) Effects of chronic occupational exposure to measured concentrations of benzene. J Occup Med 20:539-42.

Flodin U, Anderson L, Anjou CG, et al. (1981) A case-referent study on acute myeloid leukemia, background radiation and exposures to solvents and other agents. Scand J Work Environ Health 7:169-178.

Funes-Cravioto F, Komodin-Hedman B, Lindsten J, et al. (1977) Chromosome aberrations and sister-chromatid exchange in workers in chemical laboratories and a rotoprinting factory and in children of women laboratory workers. Lancet 2:322-5.

Gehring PJ, Watanabe PG, Blau GE. (1979) Risk assessment of environmental carcinogens utilizing phamacokinetic parameters. Ann NY Acad Sci 329:137-52.

Gehring PJ, Watanabe PG, Park CN. (1978) Resolution of dose-response toxicity data for chemicals requiring metabolic activation: example-vinyl chloride. Toxicol Appl Pharmacol 44:581-91.

Gehring PJ, Watanabe PG, Young JD. (1977) The relevance of dose dependent pharmacokinetics in the assessment of carcinogenic hazard of chemicals. In: Hiatt HH, Watson JD, Winsten JA., eds. Origins of human cancer, book A, incidence of cancer in humans. Cold Spring Harbor, NE: Cold Spring Harbor Laboratory, Cold Spring Harbor Conferences on Cell Proliferation, Volume 4, pp 187-203.

Gill DP, Ahmed AE. (1981) Covalent binding of [C] benzene to cellular organelles and bone marrow nucleic acids. Biochem Pharmacol 30:1127-31.

Goldstein BD, Snyder CA. (1982) Benzene leukemogenesis. In: Tice RR, Costa DL, Schaich KM, eds. Genotoxic effects of airborne agents. New York, London: Plenum Press 1982, pp 277-89.

Goldstein BD. (1983) Benzene toxicity: review of recent literature. Prepared for the American Petroleum Institute. Piscataway, NJ: Department of Environmental and Community Medicine, UMDNJ-Rutgers Medical School.

Goldstein BD. (1977) Hematotoxicity in humans. In: Laskin S, Goldstein BD. Benzene toxicity. A critical evaluation. J Toxicol Environ Health (Suppl) 2:69-105.

Green JD, Leong BKJ, Laskin S. (1978) Inhaled benzene fetotoxicity in rats. Toxicol Appl Pharmacol 46:9-18.

Greene MH, Hoover RN, Eck RL, Fraumeni JF Jr. (1979) Cancer mortality among printing plant workers. Environ Res 20:66-73.

Griesemer RA, Cueto C Jr. (1980) Toward a classification scheme for degrees of experimental evidence for the carcinogenicity of chemicals for animals. In: Montesano R, Bartsch H, Tomatis L, eds. Molecular and cellular aspects of carcinogen screening tests. Lyon, France: International Agency for Research on Cancer.

Hanis NM, Holmes TM, Shallenberger LG, Jones KE. (1982) Epidemiology study of refinery and chemical plant workers. JOM 24:203-212.

Hartley HO, Sielken RL. (1977) Estimation of "safe doses" in carcinogenic experiments. Biometrics 33:1-30.

Haseman JK, Hoel DG, Jennrich RI. (1981) Some practical problems arising from the use of the gamma multihit model for risk estimation. J Toxicol Environ Health 8:379-86.

Hattis D. (1982) From presence to health impact: models for relating presence to exposure to damage. In; MIT, Center for Policy Alternative. Analyzing the benefits of health, safety, and environmental regulations. CPA 82-16. Cambridge, MA: Massachusetts Institute of Technology, Center for Policy alternatives, ch 5.

Hattis D, Mendez W. (1980) Discussion and critique of the Carcinogenicity Assessment Group's report on population risk due to atmospheric exposure to benzene. Cambridge, MA: Massachusetts Institute of Technology, Center for Policy Alternatives.

Hawley GG. (1981) The condensed chemical dictionary. New York: Van Nostrand Reinhold Company, p 116.

Heddle J. (1973) A rapid in vitro test for chromosomal damage. Mutat Res 18:187-90.

Holmberg PC. (1982) Central-nervous-system defects in children born to mothers exposed to organic solvents during pregnancy. Lancet 2:177-9.

Hook GJ, Benz RD, Carsten AL, Tice RR. (1984) The induction of sister chromatid exchanges (SCEs) in mouse bone marrow cells by benzene: strain and sex differences (Abstract Cb-18). Environ Mutagen 6:407.

Hudak A, Ungvary G. (1978) Embryotoxic effects of benzene and its methyl derivatives: toluene, xylene. Toxicology 11:55-63.

Hsu, TC, ed. (1982) Cytogenetic Assays of Environmental Mutagens. Totowa, NJ: Allanheld, Osman and Co. Publishers, Inc.,

Infante PF, Rinsky RA, Wagoner JK, Young RJ. (1977) Leukemia in benzene workers. Lancet 2:76-8.

International Agency for Research on Cancer (IARC). (1983) Approaches to classifying chemical carcinogens according to the mechanism of activity. Joint IARC/IPCS/CEC Working Group Report,
IARC Internal Technical Reports 83/001. Lyon:IARC.

International Agency for Research on Cancer (IARC). (1982) IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans: some industrial chemicals and dyestuffs, volume 29.
Lyon: IARC, pp 93-148.

International Agency for Research on Cancer. (1980a) IARC monographs on the Long-term and short-term screening assays for carcinogens: a critical appraisal. Supplement 2. Lyon, France.

International Agency for Research on Cancer (IARC). (1980b) IARC monographs on an evaluation of chemicals and industrial processes associated with cancer in humans based on human and animal data, volumes 1-20, report of an IARC working group Cancer Res 40:1-12.

International Agency for Research on Cancer (IARC). (1978) IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans: some N-nitroso compounds, volume 17.
Lyon: IARC, 1978, pp 193-206.

International Agency for Research on Cancer (IARC). (1974) IARC monographs on the evaluation of carcinogenic risk of chemicals to man: some anti-thyroid and related substances, nitrofurans and industrial chemials, volume 7.
Lyon: IARC, p 216.

International Agency for Research on Cancer (IARC). (1972-1983) IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans, volumes 1-30 and supplements 1 and 4. Lyon, France: IARC.

International Agency for Research on Cancer. (1980a) Long-term and short-term screening assays for carcinogens: a critical appraisal. IARC monographs suppl 2. Lyon, France: IARC.

Irons RD, Dent JG, Baker TS, Rickert DE. (1980) Benzene is metabolized and covalently bound in bone marrow in situ. Chem Biol Interact 30:241-5.

Jones HB, Grendon A. (1975) Environmental factors in the origin of cancer and estimation of the possible hazard to man.
Food Cosmet Toxicol 13:251-68.

Jung R, Beermann D, Glatt HR, Oesch F. (1981) Mutagenicity of structurally related oxiranes derivatives of benzene and its hydrogenated cogeners. Mutat Res 81:11-9.

Kale PG, Baum JW. (1983) Genetic effects of benzene in Drosophila meloanogaster males. Environ Mutagen 5:223-6.

Killian DJ, Daniel RC. (1978) A cytogenic study of workers exposed to benzene in the Texas Divisiion of Dow Chemical. Cited in US EPA, 1984,p 23481.

Kinoshita T, Santella R, Pulkrabek P, Jeffrey AM. (1981) Benzene oxide: genetic toxicity. Mutat Res 91, 99-102.

Krewski DR, Van Ryzin J. (1981) Dose response models for quantal toxicity data. In: Csorgo M, Dawson J, Rao JNK, Saleh E, eds. Statistics and related topics. Amsterdam: North Holland Press, pp201-231.

Kuna RA, Kapp RW Jr. (1981) The embryotoxic/teratogenic potential of benzene. Toxicol Appl Pharmacol 57:1-7.

Lebowitz H, Brusick D, Matheson D, et al. (1979) Commonly used fuels and solvents evaluated in a battery of short-term bioassays
(Abstract No. Eb-8). Environ Mutagen 1:172-3.

Leven DL, Devesa SS, Godwin JD II, Siverman DT. (1974) Cancer rates and risks, DHEW Publication No. (NIH) 75-691.
Washington, DC US Government Printing Office, p 12.

Linos A, Kyle RA, O'Fallon WM, Kurland LT. (1980) A case-control study of occupational exposures and leukaemia. Int J Epidemiol 9:131-135.

Lyon JP. (1976) Mutagenicity Studies with benzene (Abstract, Ph.D. Thesis, University of California, 1975) Dissert Abstracts B36:5537

Lutz WK, Schlatter C. (1977) Mechanism of the carcinogenic action of benzene: irreversible binding to rat liver DNA.
Chem Biol Interact 18:241-5.

Maltoni C, Conti B, Cotti G. (1983a) Benzene: a multipotertial carcinogen. Results of long-term bioassays performed at the Bologna Institute of oncology. Am J Ind Med 4:589-630.

Maltoni, C. (1983b) Myths and facts in the history of benzene carcinogenicity. In: Mehlman MA, ed. Carcinogenicity and toxicity of benzene. Princeton, NJ: Princeton Scientific Publishers, Inc., Advances in modern toxicology,
volume IV, pp 1-15.

Mantel N, Bryan W. (1961) "Safety" testing of carcinogenic agents. J National Cancer Inst: 27:455-470

Mantel N, Schneiderman MA. (1975) Estimatng "safe" levels, a hazardous undertaking. Cancer Res, 35:1379-1386

McMichael AJ, Spirtas R, Kupper LL, Gamble JF. (1975) Solvent exposure and leukemia among rubber workers: An epidemiologic study. J Occup Med 17:234-9.

McMichael AJ, Andjelkovich DA, Tyroler HA. (1976) Cancer mortality among rubber workers: An epidemiology study. Ann NY Acad Sci 271:125-137

Monson RR, Fine LJ. (1978) Ca. mortality and morbility among rubber workers. JNCI 61: 1047-1053.

Monson RR, Nakano KK. (1976) Mortality among rubber workers. 1. White male union employees in Akron, Ohio. Am J Epidemiol 103, 284-296. See also AJ Epi 103:297-303.

Morimoto K. (1983) Induction of sister chromatid exchanges and cell division delays in human lymphocytes by micorsomal activation of benzene. Cancer Res 43:1330-4.

Morimoto K, Wolff S, Koizumi A. (1983) Induction of sister chromatid exchanges in human lymphocytes by microsomal activatin of benzene metabolites. Mutat Res 119:355-60.

Morimoto K, Wolff S. (1980a) Increase of sister chromatid exchanges and perturbations of cell division kinetics in human lymphocytes by benzene metabolites. Cancer Res 40:1189-93.

Morimoto K, Wolff S. (1980b) Benzene metabolites increase sister chromatid exchanges and disturb cell division kinetics in human lymphocytes. Environ Mutagen 2:274-5.

Mukhametova IM, Vozovaya MA. (1972) Reproductive power and incidence of gynecological infections in female workers exposed to the combined effect of benzene and chlorinated hydrocarbons. Gig Tr Prof Zabol 16(11):6-9.

Mulvihill JJ. (1975) Congenital and genetic diseases. In: Fraument JF Jr, ed. Persons at high risk of cancer. An approach to cancer etiology and control. New York: Academic Press, Inc. pp 3-37.

Murray FJ, John JA, Rampy LW, et al. (1979) Embryotoxicity of inhaled benzene in mice and rabbits. Am Ind Hyg Assoc J 40:993-8.

National Institute for Occupational Safety and Health (NIOSH). (1974) Criteria Document: recommendations for an occupational standard for benzene. HEW Publication No. (NIOSH) 74-137. Washington, DC: US Government Printing Office.

National Research Council (NRC), Safe Drinking Water Committee. (1977) Drinking water and health, volume 1. Washington, DC: National Academy of Sciences.

National Toxicology Program (NTP). (1983) NTP technical report on the toxicology and carcinogenicity studies of benzene (CAS No. 71-43-2) in F344/N rats and B6C3F1 mice (gavage studies), NIH publication No. 84-2545. Research Triangle Park, NC: US Dept. of Health and Human Services, Board Draft, July 1984.

National Toxicology Program (NTP). (1984) Report of the Ad Hoc Panel on Chemical Carcinogenesis Testing and Evaluation of the National Toxicology Program Board of Scientific Counselors. Washington, DC: U S Government Printing Office.

Nawrot PS, Staples RE. (1979) Embryofetal toxicity and teratogenicity of benzene and toluene in the mouse (Abstract). Teratology 19:41A.

Nylander PO, Olofsson H, Rasmuson B, Svahlin H. (1978) Mutagenic effects of petrol in Drosophila melanogaster. 1) Effects of benzene and 1,2-dichloroethane. Mutat Res 57:163-167.

Office of Science and Technology Policy (OSTP). (1984) Chemical carcinogens; notice of review of the science and its associated principles. Federal Register 49(May 22):21594-21661.

Ott MG, Townsend JC, Fishbeck WA, Langner RA. (1978) Mortality among individuals occupationally exposed to benzene. Arch Environ Health 33:3-9.

Peto R. (1977) Epidemiology, multistage models, and short-term mutagenicity tests. In: Hiatt HH, Watson JD, Winsten JA, eds. Origins of human cancer, book C, human risk assessment. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory, Cold Spring Harbor Conferences on Cell Proliferation, vol 4, pp 1403-28.

Peto R, Lee P, Paige W. (1972) Statistical analysis of the bioassay of continuous carcinogens. Br J Cancer 26:258-261.

Peto R, Pike MC, Day NE, et al. (1980) Guidelines for simple sensitive significance tests for carcinogenic effects in long-term animal experiments.
In: IARC Monographs, supplement 2. Lyon: IARC, 1980, pp 311-423.

Picciano D. (1979) Cytogenetic study of workers exposed to benzene. Environ Res 19:33-38.

Picciano D. (1980) Monitoring industrial populations by cytogenic procedures. In: Infante PF, Legator MS, eds. Proceeding of a workshop on methodology for assessing reproductive hazards in the workplace April 19-22, 1978. Cincinnati OH: US National Institute for Occupational Safety and Health.

Pike MC. (1966) A method of analysis of a certain class of experiments in carcinogenesis. Biometrics 22:142-62.

Purchase IFH. (1980) Interspecies comparison of carcinogenicity Br J Cancer 41:453-68

Rai K, Van Ryzin J. (1981) A generalized multihit dose-response model for low-dose extrapolation. Biometrics 37:341-52.

Rall DP. (1979) Validity of extrapolation of results of animal studies to man. Ann NY Acad Sci 329:85-130.

Rappeport JM, Nathan DG. (1982) Acquired aplastic anemias: pathophysiology and treatment. Adv Intern Med 27:547-90.

Reitz RH, Quast JF, Schumann AM, et al. (1980) Non-linear pharmacokinetic parameters needed to be considered in high dose/low dose extrapolitation. Arch Toxicol (Suppl) 3:79-94.

Rinsky RA, Young RJ, Smith AB. (1981) Leukemia in benzene workers. Am J Ind Med 2:217-45.

Rosenkranz HS, Leifer Z. (1980) Determining the DNA-modifying activity of chemicals using DNA-polymerase-deficient Escherichia coli. In: de Serres FJ, Hollaender A, eds. Chemical Mutagens. Principles and Methods for their Detection, Vol 6. New York: Plenum, pp 109-147.

Rushton L, and Alderson MR. (1981) A case-control study to investigate the association between exposure to benzene and deaths from leukaemia in oil refinery workers. Br J Cancer 43:77-84.

Sarto F, Cominato I, Pinton AM, et al. (1984) A cytogenic study on workers exposed to low concentrations of benzene. Carcinogenesis 5:827-32.

Sato A, Nakajima T. (1979) Dose-dependent metabolic interaction between benzene and toluene in vivo and in vitro. Toxicol Appl Pharmacol 48:249-56.

Schmid W. (1975) The micronucleus test. Mutat Res 31:9-15.

Schottenfeld D, Warshauer ME, Zauber AG, et al. (1981) A prospective of morbidity and mortality in petroleum industry employees in the United States - a preliminary report. (1981) In: Peto R, Schneiderman MA, eds. Banbury report No. 9: quantification of occupational cancer. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, pp 247-265.

Schrier SL. (1984) The leukemias and myeloproliferative disorders. In: Rubenstein E, Federman DD, eds. Scientific American Medicine. New York: Scientific American, Inc, Ch 5, Sect VIII, p 1.

Shahin MM, Fournier F. (1978) Suppression of mutation induction and failure to detect mutagenic activity with Athabasca tar sand fractions. Mutat Res 58:29-34.

Sokal RR, Rohlf FJ. (1969) Biometry: principles and practice of statistics in biological research. San Francisco, CA: W.H. Freeman and Company.

Sontag JM, Page NP, Saffiotti U. (1976) Guidelines for carcinogenic bioassay in small rodents. Bethesda MD: US National Cancer Institute.

Stallones RA, Syblic D. (1977) Report on mortality from leukemia. Cited in EPA, 1984, p 23487.

Styles JA, Richardson CR. (1984) Cytogenetic effects of benzene: dosimetric studies on rats exposed to benzene vapor. Mutat Res 135:203-9.

Tabershaw Cooper Associates. (1974) A mortality study of petroleum workers. American Petroleum Institute Medical Research Report No. EA 7402. Washington, DC: American Petroleum Institute.

Tatrai E, Rodics K, Ungvary G. (1980) Embryotoxic effects of simultaneously applied exposure of benzene and toluene. Folia Morphol 28:286-9.

Thomas TI, Waxweiler RJ, Moure-Eraso R, et al. (1982) Mortality patterns among workers in three Texas oil refineries. JOM 24:135-141.

Thorpe JJ. (1974) Epidemiologic survey of leukemia in persons potentially exposed to benzene. J Occup Med 16:375-82.

Tice RR, Sawey MJ, Drew RT, Cronkite EP. (1984) Benzene-induced micronuclei (MN) in the peripheral blood (PB) of mice: a retrospective analysis (Abstract No. FA-1). Environ Mutagen 6:421

Tomatis L. (1979) The predictive value of rodent carcinogenicity tests in the evaluation of human risks. Ann Rev Pharmacol Toxicol 19:511-530.

Tomatis L, Agthe C, Bartsch H, et al. (1978) Evaluation of the carcinogenicity of chemicals: a review of the monograph program of the International Agency for Research on Cancer, 1971-1977. Cancer Res 38:877-85

Tomatis L, Partensky C, Montesano R. (1973) The predictive value of mouse liver tumor induction in carcinogenicity testing: a literature survey. Int J Cancer 12:1-20

Tough IM, Court Brown WM. (1965) Chromosome aberrations and exposure to ambient benzene. Lancet 1:684.

Tough IM, Smith PG, Court Brown WM, Harnden DG. (1970) Chromosome studies on workers exposed to atmospheric benzene. The possible influence of age. Eur J Cancer 6:49-55.

Tsai SP, Wen CP, Weiss NS, et al. (1983) Retrospective mortality and medical surveillance studies of workers in benzene areas of refineries. JOM 25:685-692.

US Congress: Office of Technology Assessment. (1981) Assessment of technologies for determining cancer risks fom the environment. Washington, DC: US Government Printing Office.

US Inter-Agency Regulatory Liaison Group. (1979) Scientific basis for identification of potential carcinogens and estimation of risk. JNCI 63:244-68.

Van Raalte HGS, Grasso P. (1982) Hematological, myelotoxic, clastogenic, carcinogenic, and leukemogenic effects of benzene. Regul Toxicol Pharmacol 2:153-76.

Vianna NJ, Polan A. (1979) Lymphomas and occupational benzene exposure. Lancet 1:1394-5.

Vigliani EC. (1976) Leukemia associated with benzene exposure. Ann NY Acad Sci 271:143-51.

Watanabe G-I, Yoshida S. (1970) The teratogenic effect of benzene in pregnant mice. Acta Med Biol 17:285-91.

Watanabe PG, Young JD, Gering PJ. (1977) The importance of non-linear (dose-dependent) pharmacokinetics in hazard assessment. J Environ Pathol Toxicol 1:147-59.

Weissburger JH, Willliams GM. (1983) The distinct health risk analyses required for genotoxic carcinogens and promoting agents. Environ Health Perspect; 50:233-45.

Worcester J, Wilson EB. (1943) The determinants of LD_{50} and its sampling error. Proc Natl Acad Sci USA 29:79-85.

APPENDIX A

Generic issues relating to Thresholds in the Action of "Epigenetic" or "Cytotoxic" carcinogens

1. "Epigenetic" vs "Genetic" Carcinogens

The fact that some carcinogens do not cause mutations or other evidence of DNA damage in short-term tests has prompted some authors (Weissburger and Williams, 1983) to suggest that they may be acting by an "epigenetic mechanism". Furthermore, it has been proposed that these substances would have "threshold" dose levels below which no carcinogenic effect would occur. As a consequence, safe levels could be derived for these substances by dividing the no-effect level observed in a cancer bioassay by some safety factor.

Epigenetic mechanisms are theoretically possible. An agent, for example, which suppresses or enhances the activity of an oncogene by interacting with DNA-methylating enzymes would not directly act on genetic material. Such an agent might, in theory, have a threshold. Alternatively, changing the tertiary (three dimensional) structure of the DNA may be another example of an epigenetic mechanism of carcinogenesis (IARC, 1983).

The methods that are used to detect "epigenetic" agents. "Epigenetic" agents are operationally defined as substances that fail to produce a

response in a DNA-binding assay or in other short-term tests. To place confidence in a method of identification that is based on negative evidence, we must know the frequency of false negatives, and this frequency must be quite low.

Unfortunately, negative results may be produced in many of these methods for reasons other than the assumption that an agent is operating by an "epigenetic" mechanism. For example, these methods gave false negative response for genotoxic carcinogens before metabolic activation was introduced. These carcinogens would have been incorrectly designated as "epigenetic" agents. Improvements in test systems have demonstrated that they are, in fact, mutagenic and are carcinogens which operate by genetic mechanisms. False negative results may arise in a DNA-binding assay even though thousands of molecules of a carcinogen are bound to the DNA because of the limits of sensitivity of this assay (i.e., limited specific activity of the radioactive carcinogenic species). Because of the difficulty of conclusively identifying epigenetic agents, IARC has stated "...at present, no classification of carcinogens according to mechanism could be exhaustive or definitive. On the other hand, classification of mechanisms has considerable value for particular scientific purposes" (IARC, 1983).

Some authors (Weissburger and Williams, 1983) have alluded to dose-response data for several "epigenetic" substances, including saccharin and phenobarbital, that they believe provide evidence for the presence of biological thresholds. Relevant experiments include those of Nakanishi, et al. (1980); Peraino, et al.(1977); Ito, et al. (1983); and Kunz, et al. (1983). On examining the original data for these chemicals, the DHS staff

concluded that the failure of these studies to produce a statistically significant increase in cancer incidence in the low-dose groups is a reflection of the limited sensitivity of the bioassay designs rather than an indication of a "threshold" dose-level. The lack of response is what would be expected for any study that used a small number of animals in the low-dose groups and in which the carcinogen acted by a nonthreshold mechanism. As a general principle, an apparent zero slope for the dose-response curve is not necessarily evidence for a biological threshold unless the size of the study affords sufficient power to rule out the nonthreshold model.

In summary, because short-term tests and/or the shapes of dose-response curves from animal bioassays cannot reliably distinguish between "genetic" and "epigenetic" carcinogens, we are in agreement with IARC that there is not, at present, sufficient scientific basis to warrant the separation of carcinogens into two distinct classes for which separate methods of risk assessment are used.

2. Cytotoxicity

The concept of cytotoxicity (chronic cellular damage and regeneration) as a mechanism of carcinogenesis is one that, if supported in fact, would have important implications for risk assessment. The theory particularly suggests that thresholds exist for agents that operate by a cytotoxic mechanism.

The theory states that high doses of a cytotoxic chemical can cause cell death and stimulate cellular regeneration at a rate that outstrips the

capacity of the cell to repair the DNA damage. The result is that errors in the DNA go uncorrected and are incorporated into the replicating DNA. In this fashion carcinogenesis is promoted. A cytotoxic agent thus acts as a promoter to enhance either its own intrinsic initiating activity or the initiating activity of background carcinogens. Tumors that arise from chronic exposure to a cytotoxic noncarcinogen would arise from the interaction between the cytotoxic agent and background carcinogens. In this case, the theory would predict the existence of a threshold for the cytotoxic agent below which neither cell death nor carcinogenic effect would be expected to occur. On the other hand, a cytotoxic agent which is also a carcinogen would promote its own initiating activity. As a consequence, its dose-response curve would be expected to be disproportionately steep at high doses but would not have a threshold.

Unfortunately, little evidence is available to support the notion that cells exposed to cytotoxic substances experience a decreased efficiency of the DNA repair. Thus, there is little reason to assume that rapid cell turnover would necessarily overload the cell's capacity to repair the DNA. Furthermore, acc rding to theory, low doses of a cytotoxic agent that produce no significant cellular damage should produce no tumors. However, data available to support this assumption are not convincing. Reitz et al. (1980) concluded that chloroform acts as a cytotoxic agent. This conclusion was based on studies that demonstrated that tumors were produced only at the high doses that produced cellular damage. However, this cancer bioassay had little statistical power to detect a carcinogenic effect, especially at low doses, so the significance of the negative result is open to question. In contrast, significant excesses of kidney and thyroid tumors were produced

both in rats and mice at dose levels that did not produce cellular damage in an NCI bioassay on chloroform (NCI, 1976, Hooper et al., 1979). It appears that if chloroform does act by some cytotoxic mechanism at high doses, it can also act as a carcinogen at lower doses. Therefore, if a carcinogen is cytotoxic, its dose-response curve may climb steeply upward at high doses but will not exhibit a threshold.

To demonstrate the lack of carcinogenic activity of cytotoxic agents, one must design a cancer bioassay such that the power of the test to detect positive effects is maintained at successively lower doses by the inclusion of larger numbers of animals as the dose decreases. If carcinogenic effects and cell death were found to occur in the high-dose groups but not in the low-dose groups, it would provide evidence in support of the cytotoxic argument. Such experimental data, is however, unfortunately absent, and the argument for a cytotoxic mechanism for carcinogensis remains hypothetical. At present there do not appear to be convincing scientific or public health grounds to justify incorporating the cytotoxic theory into the risk assessment process.

3. References

Hooper NK, Harris RH, Ames BN. (1979) Chemical carciogens. Science 203:602-3.

International Agency for Research on Cancer (IARC). (1983) Approaches to classifying chemical carcinogens according to mechanism of activity. Joint IARC/IPCS/CEC Working Group Report, IARC Internal Technical Reports 83/001. Lyon, France: IARC.

Ito N, Fukushima S, Shirai T, Nakanishi K, (1983) Effects of promotors on N-butyl-N-(4-hydroxylbutyl) nitrosamine-induced urinary bladder carcinogenesis in the rat. Environ Health Perspect 50:61-9.

Kunz HW, Tennekes HA, Port RE, et al. (1983) Quantitative aspects of chemical carcinogenesis and tumor promotion in liver. Environ Health Perspect 50:113-22.

Nakanishi K, Hagiwara A, Shibata M, et al. (1980) Dose-response of saccharin in urinary bladder hyperplasias in Fisher 344 rats pretreated with N-butyl-N-(4-hydroxybutyl)nitrosamine. JNCI 65:1005-9

National Cancer Institute (NCI). (1976) Report on carcinogenesis bioassay of chloroform. US DHEW publication no. NIH-76-1279. Bethesda, MD: National Cancer Institute.

Peraino C, Fry TJM, Satteldt E. (1977) Effects of varying the onset and duration of exposure to phenobarbital on its enhancement of 2-acetylamino-flourene-induced hepatic tumorigenesis. Cancer Res 34:3623-7.

Reitz RH, Quast JF, Stott WT, et al. (1980) Pharmacokinetics and macromolecular effects of chloroform in rats and mice: implications for carcinogenetic risk estimation. In: Jolley RL, Brungs WA, Cumming RB, eds. Water chlorination - environmental impact and health effects. Proceedings of the Third Colorado Springs Conference on Water chlorination: Environmental Impact and Health Effects. Ann Arbor, MI: Ann Arbor Sciences Publishers, Inc., vol 3, pp 983-93.

Weissburger JH, Williams GM. The distinct health risk analyses required for genotoxic carcinogens and promoting agents. Environ Health Perspect 1983;50:233-45.

WORLD HEALTH ORGANIZATION

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

IARC MONOGRAPHS

ON THE

EVALUATION OF THE CARCINOGENIC RISK OF CHEMICALS TO HUMANS

Some Industrial Chemicals and Dyestuffs

VOLUME 29

This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of the Carcinogenic Risk of Chemicals to Humans which met in Lyon,

13-20 October 1981

May 1982

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

BENZENE

This substance was considered by a previous Working Group, in June 1974 (IARC, 1974). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

1. Chemical and Physical Data

1.1 Synonyms and trade names

Chem. Abstr. Services Reg. No.: 71-43-2

Chem. Abstr. and IUPAC Systematic Names: Benzene

Synonyms: (6)-Annulene; benzin[1]; benzine[1]; benzol; benzole; benzolene; bicarburet of hydrogen; carbon oil; coal naphtha; cyclohexatriene; mineral naphtha; motor benzol; phene; phenyl hydride; pyrobenzol; pyrobenzole

Trade Name: Polystream

1.2 Structural and molecular formulae and molecular weight

C_6H_6  Mol. wt: 78.1

1.3 Chemical and physical properties of the pure substance

From Purcell (1978), unless otherwise specified

(*a*) *Description*: Colourless liquid

---

[1] These names are no longer used for benzene; they were used for many years to describe a low-boiling petroleum fraction predominantly containing aliphatic hydrocarbons (Purcell, 1978).

(*b*) *Boiling-point*: 80.1C

(*c*) *Melting-point*: 5.5°C

(*d*) *Density*: d_4^{20} 0.8787 (Weast, 1979)

(*e*) *Refractive index*: n_D^{20} 1.5011 (Weast, 1979)

(*f*) *Spectroscopy data*: λ_{max} 243, 249, 256 and 261 nm (in ethanol) (Weast, 1979); mass spectra and carbon-13 nuclear magnetic resonance spectra have been tabulated (NIH/EPA Chemical Information System, 1980).

(*g*) *Identity and purity test*: Conversion to *meta*-dinitrobenzene, which is recrystallized and found to be identical with a standard sample in melting-point tests

(*h*) *Solubility*: Slightly soluble in water (1.8 g/l at 25°C); miscible with acetic acid, acetone, chloroform, diethyl ether and ethanol (Weast, 1979)

(*i*) *Viscosity*: 0.6468 cP at 20°C

(*j*) *Volatility*: Vapour pressure, 100 mm at 26.1°C

(*k*) *Stability*: Stable; combustible (flash-point, -11.1°C)

(*l*) *Reactivity*: Undergoes substitution, addition and cleavage of the ring

(*m*) *Conversion factor*: ppm = 0.313 x mg/m³

1.4 Technical products and impurities

Benzene is available in the US in three grades: refined, nitration grade and industrial grade, all of which must be free of hydrogen sulphide and sulphur dioxide. Only the refined grade is required to contain no more than 0.15% non-aromatics and 1 mg/kg thiophene. The refined and nitration-grade products must have a distillation range of not more than 1°C including 80.1°C, a specific gravity of 0.8820-0.8860 (15.56/15.56°C), and contain no trace of acidity. The minimum solidification points are 5.35°C (dry basis) for the refined grade and 4.85°C (anhydrous basis) for the nitration grade. The industrial grade must have a distillation range of not more than 2°C including 80.1°C and a specific gravity of 0.875-0.886 (Purcell, 1978). One manufacturer lists the following typical composition for its nitration-grade benzene: 99.9+% purity, 0.03% nonaromatics, 0.02% water, 0.01% toluene, 0.1-0.2 mg/kg thiophene and no xylene (USS Chemicals, 1980).

Benzene available in Japan has the following specifications: boiling-range, 80.1 ± 1°C; freezing-point, a minimum of 5.2°C; specific gravity, 0.882-0.886 (15/4°C); a maximum of 0.001 g thiophene per 100 ml; and a maximum of 0.0005 g carbon disulphide per 100 ml.

2. Production, Use, Occurrence and Analysis

2.1 Production and use

Recent reviews on benzene include those by Hancock (1975) and Purcell (1978).

(a) Production

Benzene was first isolated by Faraday in 1825 from a liquid condensed by compressing oil gas; Mitscherlich first synthesized it in 1833 by distilling benzoic acid with lime. Benzene was first recovered commercially from light oil derived from coal-tar in 1849, and from petroleum in 1941 (Purcell, 1978).

It is recovered commercially from both petroleum and coal sources; those of petroleum were the basis for an estimated 92% of US production in 1978. Petroleum sources include refinery streams (primarily catalytic reformate), pyrolysis gasoline (a by-product of the manufacture of ethylene by cracking naphtha or gas oil), and toluene hydrodealkylation. Coal-derived benzene is recovered from light oil produced in coke manufacture.

Catalytic reforming (the source of approximately 44% of the benzene produced in the US in 1978) converts the naphthenes and paraffins in naphtha to a product rich in aromatic hydrocarbons. When a high yield of benzene is desired, a suitable naphtha fraction is reformed under severe conditions, and approximately half of the benzene contained in the reformate is recovered by solvent extraction (e.g., with sulpholane or tetraethylene glycol). The rest of the benzene is left in the reformate, which is used in the production of gasoline.

In western Europe, benzene is produced in almost equal quantities from catalytic reformate, pyrolysis gasoline and toluene hydrodealkylation; coke-oven operations provide less than 10% of total production.

Pyrolysis gasoline is believed to be the single largest source of benzene in Japan, and a significant percentage of the total capacity is based on the use of multiple feeds. Coalderived benzene is estimated to constitute less than 10% of the total.

Preliminary data indicate that US production of all grades of benzene by 33 companies in 1980 totalled 1563 million gallons (5217 thousand tonnes) (US International Trade Commission, 1981). In 1979, 31 US companies reported a total production of 1° and 2° benzene (which includes refined, nitration and industrial grades) of 5351 thousand tonnes (US International Trade Commission, 1980). It is estimated that almost as much additional benzene was produced in both years but was not isolated from the various streams (catalytic reformate, etc) and was used for fuel purposes.

US imports of benzene in 1980 totalled 94.7 million gallons (316 thousand tonnes) (US Department of Commerce, 1981a), and exports were 11.8 million gallons (39.4 thousand tonnes) (US Department of Commerce, 1981b).

An estimated 4800 thousand tonnes of benzene were produced in western Europe in 1979. Annual production capacity in 1980 is estimated to have been at least 6877 thousand tonnes. This compound is produced by 66 companies in 11 western European countries, the major producers being the UK (nine producers), the Federal Republic of Germany (16) and The Netherlands (four). Recent production of benzene in thousands of tonnes by COMECON countries is estimated to have been as follows: USSR, 1538 (1977); Czechoslovakia, 195 (1979); Romania, 162 (1979); Bulgaria, 61 (1979); Hungary, 34 (1979) and Poland, 15 (1979).

About 2170 thousand tonnes of benzene were produced in Japan in 1979, approximately 185 thousand tonnes of which were derived from coal. The combined annual production capacity of the 22 Japanese producers in 1980 is estimated to have been 2882 thousand tonnes. Japanese exports of benzene in 1979 were about 173 thousand tonnes.

World production of benzene in 1977 is estimated to have been over 12 million tonnes, making it the fourth or fifth largest volume organic chemical produced on a worldwide basis. The areas with the largest production, apart from the US, Europe and Japan, are Canada and South America.

(b) Use

The use pattern for recovered benzene in the US in 1978 was as follows: ethylbenzene/styrene, over 50%; cumene/phenol, close to 20%; cyclohexane, 15-16%; nitrobenzene/aniline, 4-5%; and maleic anhydride, chlorobenzenes, detergent alkylate and other uses, 2.5-3.0% each.

Over 97% of all US production of ethylbenzene is based on the alkylation of benzene with ethylene; all but minor amounts of the ethylbenzene produced are dehydrogenated to styrene. The latter, an important monomer for a variety of polymers (both plastics and elastomers), was the subject of an earlier IARC monograph (IARC, 1979a). Preliminary data indicate that US production of styrene in 1980 totalled 3135 million kg (US International Trade Commission, 1981).

Benzene is alkylated with propylene to produce cumene (isopropylbenzene), all but minor quantities of which are oxidized to cumene hydroperoxide, which is split into phenol and acetone. Phenol, essentially all of which is derived from cumene in the US, is an intermediate in the manufacture of phenol-formaldehyde resins (See monograph on formaldehyde in this volume for further information), bisphenol A (used in the manufacture of epoxy resins), and caprolactam [the subject of an earlier IARC monograph, IARC, 1979b]. Acetone (60% of which was derived from cumene in the US in 1979) is an important solvent and chemical intermediate. Its most important derivative in the US is methyl methacrylate, a monomer for acrylic resins, which was the subject of an earlier IARC monograph (IARC, 1979c).

Approximately 85% of all cyclohexane produced in the US is made by the catalytic hydrogenation of benzene. Cyclohexane is a chemical intermediate for three chemicals used in the manufacture of nylon fibres and resins: caprolactam [see IARC, 1979b], adipic acid and hexamethylenediamine.

Of the other chemicals derived from benzene, the following have been the subjects of IARC monographs: aniline (IARC, 1982a), *ortho*- and *para*-dichlorobenzenes (this volume), hexachlorobenzene (IARC, 1979d), hexachlorocyclohexane (IARC, 1979e), and the two dihydroxybenzenes, hydroquinone and resorcinol (IARC, 1977).

In the past, benzene was used widely as a solvent, but the amounts used now for this purpose are believed to be relatively small and decreasing.

Use of an estimated 4864 thousand tonnes of recovered benzene in western Europe in 1979 was as follows: ethylbenzene/styrene, 48%; cumene, 20%; cyclohexane, 14%; nitrobenzene/aniline, 7%; detergent alkylate, 4%; maleic anhydride, 3%; chlorobenzenes, 2%; and other uses, 2%.

The use pattern in Japan in 1980 for recovered benzene was as follows: ethylbenzene/styrene, 51%, cyclohexane, 24%; cumene/phenol, 13%; detergent alkylate, 4%; maleic anhydride, 2%; and other uses, 6%.

Fifteen countries have been reported to limit occupational exposure to benzene by regulation or recommended guideline. Their standards are listed in Table 1. Benzene is

Table 1. National occupational exposure limits for benzene[a]

Country	Year	Concentration mg/m³	Concentration ppm	*Interpretation*[b]	Status
Australia	1978	30	10	TWA[c]	Guideline
Belgium	1978	30	10	TWA[c]	Regulation
Czechoslovakia	1976	50	—	TWA	Regulation
		80	—	Ceiling (10 min)	
Finland	1975	32	10	TWA[c]	Regulation
Hungary	1974	20	—	TWA[d]	Regulation
Italy	1978	30	10	TWA[c]	Guideline
Japan	1978	80	25	Ceiling	Guideline
The Netherlands	1978	30	10	TWA[c]	Guideline
Poland	1976	30	—	Ceiling[c]	Regulation
Romania	1975	50	—	Maximum[c]	Regulation
Sweden	1978	15	5	TWA[c]	Guideline
		30	10	Maximum (15 min)	
Switzerland	1978	6.5	2	TWA[c]	Regulation
USA[a]					
OSHA	1980	—	10	TWA	Regulation
		—	25	Ceiling	
		—	50	Peak[e]	
ACGIH	1981	30	10	TWA	Guideline
		75	25	STEL	
NIOSH	1980	3.2	1	Ceiling (60 min)	Guideline
USSR	1980	5	—	Ceiling[c]	Regulation
Yugoslavia	1971	50	15	Ceiling[c]	Regulation

a From American Conference of Governmental Industrial Hygienists (ACGIH) (1981); International Labour Office (1980); National Institute for Occupational Safety and Health (NIOSH) (1980); US Occupational Safety and Health Administration (OSHA) (1980)

b TWA, time-weighted average; STEL, short-term exposure limit

c Skin irritant notation added

d May be exceeded 5 times per shift as long as average does not exceed value

e Peak limit above ceiling - 10 minutes

recognized as being carcinogenic by six countries (Finland, the Federal Republic of Germany, Italy, Japan, Sweden and Switzerland) and is designated as being a suspected carcinogen in two others (Australia and the USA) (International Labour Office, 1980).

A ban on all consumer products (except gasoline and solvents or reagents for laboratory use) containing benzene as an intentional ingredient or as a contaminant constituting 0.1% or more by volume was proposed by the US Consumer Products Safety Commission in 1978. In 1981, the Commission withdrew its proposed ban on the basis of information that benzene, as currently used in consumer products, did not present a significant risk to consumers. Data from contacts in industry and information obtained from manufacturers, importers and labellers of such products indicated that benzene is not currently used intentionally in consumer products (US Consumer Product Safety Commission, 1981).

The US Environmental Protection Agency (EPA) (1979) requires that notification be given whenever discharges containing 454 kg or more of benzene are made into waterways. In 1980, the EPA proposed a national standard for benzene emissions from maleic anhydride plants that would prohibit detectable emissions from new sources and limit emissions from existing sources to 0.3 kg per 100 kg of benzene fed to the reactor (US Environmental Protection Agency, 1980a).

The EPA has also identified benzene as a toxic waste and requires that persons who generate, transport, treat, store or dispose of it comply with the regulations of a Federal hazardous waste management programme. Bottom sediment sludge from the treatment of waste-waters from wood-preserving processes involving use of creosote and/or pentachlorophenol, water or caustic cleaning wastes from painting equipment, tank cleaning from paint manufacture, emission control dust or sludge from paint manufacture, and distillation or fractionating column bottoms from the production of chlorobenzenes are included in a list of hazardous wastes in which benzene was identified as one of the hazardous constituents (US Environmental Protection Agency, 1980b,c). However, in early 1981, the second and third of these four wastes were removed from the list, and the following wastes were added: the combined waste-water streams generated from nitrobenzene/aniline production and the separated aqueous stream from the reactor product washing step in the production of chlorobenzenes (US Environmental Protection Agency, 1981a).

The EPA proposed a national emission standard for 'fugitive emissions' (from supposedly sealed installations) of benzene in early 1981, which would prohibit detectable benzene emissions from processing equipment (e.g., pumps, valves) that contains materials which have a benzene concentration of 10% or more by weight (US Environmental Protection Agency, 1981b). The EPA has also proposed a regulation to limit effluent discharges into publicly owned treatment works of benzene from beehive cokemaking operations. The proposed limit is 63.8 mg benzene per thousand kg of product [0.0638 ppm] (US Environmental Protection Agency, 1981c).

The Bureau of Alcohol, Tobacco and Firearms of the US Department of the Treasury (1981) lists benzene among the approved denaturants for three of the prescribed formulae for denaturing alcohol.

As part of the US Department of Transportation (1980) Hazardous Materials Regulations, shipments of benzene are subject to a variety of labelling, packaging, quantity and shipping restrictions consistent with its designation as a hazardous material.

The Commission of the European Communities (1980) prohibits the use of benzene in products intended for use as toys (e.g., children's ballons).

2.2 Occurrence

(a) *Natural occurrence*

Benzene is a natural constituent of crude oil (Brief *et al.*, 1980).

(b) *Occupational exposure*

There is or has been occupational exposure to benzene in numerous industries because of its presence as a component of many fuels and as an impurity in organic chemicals made from it. For example, it is present in straight-run petroleum distillates and in coal-tar distillates (Ayers and Muder, 1964). It has been estimated that about two million workers in the US are potentially exposed to benzene (Brief *et al.*, 1980).

The early uses of benzene, particularly as a solvent, resulted regularly in concentrations in workplace air of about 1600 mg/m³ [500 ppm] and sometimes concentrations in excess of 3200 mg/m³ [1000 ppm]. Table 2 gives examples of concentrations of benzene reported in workplace air in various industries since 1935.

Concentrations of benzene found in the air around certain operations in various US rubber tyre factories are given in Table 3.

In four different central telephone offices in the US, benzene was present in the workplace air in concentrations ranging from 1.3 to 28.8 µg/m³ [0.41-9 ppb]. When levels at three of the offices were 58, 16 and 2.6 µg/m³ [18, 5 and 0.8 ppb], outside air contained 3, 9.6, and 0.6 µg/m³ [1, 3 and 0.2 ppb] of benzene, respectively. In one US telephone business office, a level of 16 µg/m³ [5 ppb] was found (Oblas *et al.*, 1980).

(c) *Air*

Rural background concentrations of benzene have been reported to range from 0.3-54 µg/m³ [0.1-17 ppb]. It has been suggested that these levels are related to biological sources; for example, ambient benzene concentrations increase after forest fires and oil seeps (Brief *et al.*, 1980). The general urban atmosphere reportedly contains 0.05 mg benzene/m³ [0.02 ppm] of air. Assuming a daily inhalation of 24 m³ of air and a benzene

Table 3. Concentrations of benzene in the US tyre industry, 1973-1977[a]

Operation	*Personal breathing sample*		*Area sample*	
	mg/m³	ppm	mg/m³	ppm
Cement mixing	0.6-15.4	0.2-4.8	0-52.8	0-16.5
Extrusion	< 0.3-16.3	< 0.1-5.1	1.3-14.7	0.4-4.6
Tyre building	0.3-7.7	0.1-2.4	0.3-6.4	0.1-2.0
Curing preparation	< 0.3-18.9	< 0.1-5.9	1.3-3.8	0.4-1.2
Inspection and repair	< 0.3-6.1	< 0.1-1.9	0.03-7.0	0.01-2.2
Maintenance	0.6-1.5	0.2-0.5	1.5-4.8	0.5-1.5
Warehouse	—		0.03-1.5	0.01-0.5

a From Van Ert *et al.* (1980)

Table 2. Occupational exposures to benzene in various industries

Industry	Concentration (mg/m³ air) [ppm] Range	Other	Year	Reference[a]
Rubber coating (churn room)	304-832 [95-260]		1935-1937	NIOSH (1974)
(spreader machine)	208-640 [65-200]		1935-1937	NIOSH (1974)
Rubber factory	320[b] [100]	1600 [500][c]	1942	EPA (1980d)
Rubber coating	83-416 [25-125]		1961	EPA (1980d)
	64-80 [20-25][b,d]	448 [140][c]	1960-1963	NIOSH (1974)
Rubber raincoat factory	438-698 [137-218]		1977	EPA (1980d)
Printing	32-3392 [10-1060]		1939	NIOSH (1974)
Artificial leather, rubber goods or shoe manufacture	320-1600 [100-500]		1936-1939	NIOSH (1974)
Leatherette factory	150-1000 [47-310]		1953-1957	NIOSH (1974)
	80-150 [25-47][e]		1953-1957	NIOSH (1974)
Shoe factory	1017-1504 [318-470]		1956	NIOSH (1974)
	100-500 [31-156]		1960-1963	NIOSH (1974)
	130-140 [41-44][f]		1964	NIOSH (1974)
	480-2080 [150-650]		1966-1978	EPA (1980d)
Chemical plant[g]	0.3-1.3 [0.1-0.4]		1979	Evans and Wilcox (1979)
Petroleum refinery (quality control lab)	2-103 [0.6-32][h]		1978	Markel and Elesh (1979)
Waste-water treatment plant at petroleum factory	0.06-1.5 [< 0.02-0.51] (personal samples)		1978	Gorman and Slovin (1980)
	0.06-21.7 [< 0.02-7.22] (area samples)		1978	Gorman and Slovin (1980)
Coal liquefaction plant	0.06 [0.02]		1980	Anon. (1980)
Australian Air Force workshop	33-116 [10-35][h]	4480 [1400][h]	1947	EPA (1980d)
Paint manufacture	trace-362 [trace-78.5]		1963	NIOSH (1974)
Painting of metal products	0.5-10 [0.17-3.1]		1978	McQuilkin (1980)

a NIOSH, National Institute for Occupational Safety and Health; EPA, US Environmental Protection Agency
b Average c Peak
d Following removal of benzene from use in the industry; naphtha solvents containing up to 7.5% benzene were being used
e Following improved control measures
f Following replacement of a technical benzene with toluene in shoe adhesives
g Producing hexabromocyclododecane and dialkylaminoethyl chloride hydrochlorides
h Maximum measured at benzene distillation operation

retention of 50%, human beings would inhale 0.6 mg of benzene per day (National Research Council, 1980). Table 4 is a summary of the results of several studies conducted since 1963 on concentrations of benzene in ambient air. Estimated annual emissions of benzene to the air in the US from various sources are summarized in Table 5.

Table 4. *Levels of benzene in ambient air*

Location (year)	Concentration µg/m³	Concentration ppb	Source
Los Angeles Basin, USA (1965)	16-70	5-22	Howard and Durkin (1974)
Central Los Angeles, USA (1963)	48-192	15-60	Howard and Durkin (1974)
Los Angeles Basin, USA (1968)	48 182	182 57[b]	Howard and Durkin (1974)
Los Angeles, USA (1977)	19	6[a]	Martin *et al.* (1980)
Riverside, California, USA (1973)	22-26	7-8	Howard and Durkin (1974)
Dallas, Texas, USA (1977)	5	1.6[a]	Martin *et al.* (1980)
Chicago, Illinois, USA (1977)	18	6[a]	Martin *et al.* (1980)
Toronto, Ontario, Canada (1973)	42 31	13[a] 98[b]	Howard and Durkin (1974)
Vancouver, B.C., Canada (1965)	3-32	1-10	Howard and Durkin (1974)
Delft, The Netherlands	3 26	0.93[a] 8[b]	Brief *et al.* (1980)
The Hague, The Netherlands	29 93	9[a] 29[b]	Brief *et al.* (1980)
Zurich, Switzerland	112 237	35[a] 74[b]	Brief *et al.* (1980)
Zurich, Switzerland (1971)	173	54	Howard and Durkin (1974)
Prague, Czechoslovakia	0.3	0.1[a]	Brief *et al.* (1980)
London, UK	573	179[b]	Brief *et al.* (1980)
London airport, UK	293	92	Thorburn and Colenutt (1979)
Uxbridge, Middlesex, UK	278-358	87-112	Colenutt and Thorburn (1980)
Rural location in UK	194	61	Thorburn and Colenutt (1979)

a Average
b Maximum

e Following improved control measures
f Following replacement of a technical benzene with toluene in sho[illegible]sives
g Producing hexabromocyclododecane and dialkylaminoethyl chlori[illegible]ydrochlorides
h Maximum measured at benzene distillation operation

Table 5. Annual emissions of benzene to air from various sources[a]

Source	Emission (thousand tonnes)
Component of gasoline[b]	40.0-80.0
Production of other chemicals	44.0-56.0
Indirect production of benzene[c]	23.0-79.0
Production of benzene from petroleum	1.8-7.3
Solvents and miscellaneous sources	1.5
Imports of benzene	0.013

a From JRB Associates, Inc. (1980)
b Production, storage, transort, vending and combustion
c Coke ovens, oil spills, nonferrous metals manufacturing, ore mining, wood processing, coal mining, and textile industry

Concentrations of benzene in air at various locations in Texas, USA, were found to be as follows: in an oil-field, 9.6-512 μg/m³ [3-160 ppb]; near a crude-oil tank farm, 12.8-41.6 μg/m³ [4-13 ppb]; near an oil refinery, 6.4-41.6 μg/m³ [2-13 ppb]; in a remote, non-industrial area, 9.6-12.8 μg/m³ [3-4 ppb] (Oldham *et al.*, 1979). In a survey of 17 US states, five million people were estimated to be exposed to 0.3-3.0 μg/m³ [0.1-1.0 ppb] benzene from petroleum refineries, and 3000 people to 3-13 μg/m³ [1.1-4.0 ppb] (Suta, 1980).

Average benzene concentrations in 24-hour air samples taken near coke-oven operations at a steel plant in Pennsylvania, USA, were in the range of 4-19 μg/m³ [1-6 ppb] (Fentiman *et al.*, 1979). In a survey of 12 US states, 300 000 people were estimated to be exposed to benzene in the air from coke-oven operations (Suta, 1980).

Table 6 is a summary of concentrations of benzene found in air samples taken near US chemical factories where benzene was used.

Table 6. Benzene concentrations in the air near US chemical manufacturing factories

Source	Concentration μg/m³	ppb	Reference
Nitrobenzene manufacture	3-11	1-4	Fentiman *et al.* (1979)
Cumene manufacture	25-51	9-19	Fentiman *et al.* (1979)
Maleic anhydride manufacture	2-32	1-10	Fentiman *et al.* (1979)
From pyrolysis gas	6-35	2-13	Fentiman *et al.* (1979)
Detergent alkylate manufacture	2.7-55.4	1-18	Fentiman *et al.* (1979)
Other factories using benzene	1.9-108.6	0.6-34	Suta (1980)

In a survey of 22 US states, it was estimated that about six million people were exposed to 0.3-3.0 μg/m³ [0.1-1.0 ppb] benzene from chemical factories, about one million to 3.0-13.0 μg/m³ [1.1-4.0 ppb], about 200 000 to 13.0-32.0 μg/m³ [4.1-10.0 ppb] and about 80 000 people to more than 32.0 μg/m³ [10.0 ppb] (Suta, 1980).

The benzene content of ambient air sampled in the vicinity of one solvent reclamation plant was found to be 74 mg/m³ [23 ppm] (Howard and Durkin, 1974).

US gasolines contain an average of 0.8% benzene and European gasolines contain an average of 5% (US Environmental Protection Agency, 1980d). Several studies that have been made of the levels of benzene in the air at gasoline service stations and loading facilities are summarized in Table 7. Suta (1980) has estimated that 37 million people in the US are exposed to benzene in the air from self-service gasoline stations.

Benzene comprises about 2.15% of total hydrocarbon emissions from a gasoline engine, or about 4% of automotive exhaust (US Environmental Protection Agency, 1980d). Data on benzene found in ambient air in areas associated with automobile use are summarized in Table 8. Brief *et al.* (1980) also reported that levels of benzene in the air near major roadways correlate with traffic levels. It has been reported that older automobiles (1975 and earlier models) emit larger quantities of benzene in exhaust than do newer, catalyst-equipped automobiles (Briggs *et al.*, 1977).

(d) Water and sediments

Benzene has been detected in lake, river and well water, raw and finished drinking-water, and in effluents from oil and coal processing, chemical factories, raw sewage and sewage treatment plants (Shackelford and Keith, 1976; Hushon *et al.*, 1980).

Assuming an intake of 2 l/day and a level of 1 μg benzene per l of US drinking-water, the dose of benzene to humans from water would be 2 μg/day (National Research Council, 1980).

Annual emissions of benzene to water in the US from various sources are summarized in Table 9.

Concentrations of benzene in waste-water from coal preparation plants have been reported to be in the range of 0.3-48 μg/l (Randolph *et al.*, 1979). Concentrations in effluents from plants which manufacture or use benzene have been found to be in the range of <1-179 μg/l; river and stream water near the plants contained <1-13 μg/l, while samples taken further downstream contained 2 μg/l or less (Fentiman *et al.*, 1979).

Benzene concentrations in water in several countries are summarized in Table 10.

(e) Soil and plants

Benzene has been found at concentrations of <2 to 191 μg/kg in soil samples taken near factories where benzene was used or produced (Fentiman *et al.*, 1979).

(f) Food, beverages, feed

Benzene has been reported in several foods: eggs, 500-1900 μg/kg; Jamaican rum, 120 μg/kg; irradiated beef, 19 μg/kg; heat-treated or canned beef, 2 μg/kg (US Environmental

Table 7. Benzene in the air near gasoline facilities and operations in the US and Europe

Sampling site	Average concentration mg/m³	ppm	Reference[a]
Service stations	0.4	0.123	NRC (1980)
	0.6-10.2	0.2-3.2	NIOSH (1980)
	0.08-10	0.02-3.2	Brief *et al.* (1980)
	0.001-0.007	0.0003-0.0023	Fentiman *et al.* (1979)
Residential neighbourhood upwind of service station	0.004-0.006	0.0013-0.002	Fentiman *et al.* (1979)
Downwind of service station during refuelling of underground tanks	0.032-0.069	0.01-0.024	Fentiman *et al.* (1979)
Bulk-loading facilities	0.3-24.6	0.1-7.7	NIOSH (1974)
	4.5-31.7	1.4-9.9	Brief *et al.* (1980)
Loading and discharging of road tankers	4.5-30.1	1.4-9.4	NIOSH (1974)
Loading of rail tankers	5-8	1.6-2.5	NIOSH (1974)
Distribution facility	0.003-0.032	0.001-0.01	Oldham *et al.* (1979)

[a] NCR, National Research Council; NIOSH, National Institute for Occupational Safety and Health

Table 8. Benzene in air associated with automobile use

Sampling site	Concentration µg/m³	ppb	Reference
Residential neig ibourhood	5	1.5	Fentiman *et al.* (1979)
Central business district	12	3.8	Fentiman *et al.* (1979)
Busy highway leading into business district	9-28	3-8.6	Fentiman *et al.* (1979)
Roadway intersection	16-150	5-47	Suyama *et al.* (1980)
Highway	490	153	Thorburn and Colenutt (1979)
Urban area with much traffic	393	123	Thorburn and Colenutt (1979)
Urban streets, parking garages, car repair shops, inside automobiles, and in a home above a parking garage	25-600	7.8-190	Jonsson and Berg (1980)

Table 9. Annual benzene emissions to water in US[a]

Source	Emissions (tonnes)
Indirect production of benzene[b]	200-11 000
Solvent and miscellaneous uses	1450
Production of chemicals other than benzene	1000
Production of benzene from petroleum	630
Imports of benzene	13

a From JRB Associates, Inc. (1980)
b Coke ovens, oil spills, nonferrous metal manufacture, ore mining, wood processing, coal mining and textile manufacture

Table 10. Benzene concentrations in water samples

Location	Concentration (µg/l)	Reference[a]
Lake (UK)	6.5-8.9	Colenutt and Thorburn (1980)
Stream (UK)	18.6	Colenutt and Thorburn (1980)
River (UK)	6.8	Colenutt and Thorburn (1980)
Rainwater (UK)	87.2	Colenutt and Thorburn (1980)
Drinking-water (Czechoslovakia)	0.1	EPA (1980d)
Drinking-water (US)	0.1-0.3	EPA (1980d); NCR (1977, 1980); Coleman *et al.* (1976)
Groundwater (US)	> 100	EPA (1980d)
Subsurface brine	10 000	Ochsner *et al.* (1979)
Subsurface water[b]	24 000	Ochsner *et al.* (1979)

a EPA, US Environmental Protection Agency; NCR, National Research Council
b Taken near extensive gas and oil deposits

Protection Agency, 1980d). In another study, levels in irradiated beef were <0.1 mg/kg (Federation of American Societies for Experimental Biology, 1979). Benzene has also been detected (no levels were reported) in the following foods: haddock, cod, red beans, roasted filberts and peanuts, potato tubers, blue and Cheddar cheese, cayenne pineapple, strawberries, black currants, hothouse tomatoes, soya bean milk, cooked chicken, boiled beef and canned beef stew (Chang and Peterson, 1977; US Environmental Protection Agency, 1980d).

Conventional cooking may produce an increase in the benzene content of food (Chang and Peterson, 1977; Federation of American Societies for Experimental Biology, 1979).

The National Research Council (1980) estimated that the average US urban dweller may receive about 650 µg of benzene daily from food and air and that the dietary intake of benzene may be as high as 250 µg/day. Levels of 24 to 60 µg/m³ benzene [8-20 ppb] have been found in the breath of individuals without specific benzene exposure, suggesting that the source may be the diet.

(g) Tobacco and tobacco smoke

Benzene has been identified in cigarette smoke at levels of 47-64 ppm [150-204 mg/m³] (Lauwerys, 1979).

(h) Pyrolysis products

Benzene is one of the volatile components resulting from the burning of various shipboard materials; the following concentrations have been reported: wall insulation material, 60 mg/m³ [18.8 ppm]; polyvinyl chloride cable jacket, 9 mg/m³ [2.8 ppm]; and hydraulic fluid, 800 mg/m³ [250 ppm] (Zinn *et al.*, 1980).

Benzene has been reported to be a thermal degradation product of polyvinyl chloride food-wrapping film when it is cut with a hot wire; concentrations measured during this operation ranged from 5 to 20 ng per cut (Boettner and Ball, 1980).

2.3 Analysis

Several methods for the analysis of benzene were described in the earlier monograph (IARC, 1974). Typical methods for the analysis of benzene in various matrices are summarized in Table 11.

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals[1]

(a) Oral administration

Rat: Three groups of 30 or 35 male and 30 or 35 female Sprague-Dawley rats, 13 weeks old, received 50 or 250 mg/kg bw benzene [purity unspecified] dissolved in pure olive oil by stomach tube once daily on 4 or 5 days each week during 52 weeks. Groups of 30 male and 30 female controls received olive oil only. The rats were allowed to live until spontaneous death or were killed at 144 weeks, the end of the experiment; average survival times were unspecified. Of females of the control, low- and high-dose groups,

[1] The Working Group was aware of a study in progress of oral administration of benzene to rats and mice (IARC, 1981).

Table 11. Methods for the analysis of benzene

Sample matrix	Sample preparation	Assay procedure[a]	Limits of detection	Reference
Aircraft fuels	Add to pentane; mix and centrifuge	GC/MS	not given	Warner and Kanan (1979)
Air	Trap onto adsorbent; desorb thermally	GC/MS	not given	Jonsson and Berg (1980)
	Trap onto adsorbent; desorb thermally	GC/FID	0.3 µg/m³ [0.09 ppb]	Baxter *et al.* (1980)
	Trap in charcoal tube; desorb with carbon disulphide	GC/FID	0.3 µg/m³ 0.09 ppb	Baxter *et al.* (1980)
	Inject air directly into chromatograph	GC/MS	not given	Fujii *et al.* (1979)
	Pass through silica gel treated with 5 % cerium sulphate in fuming sulphuric acid	Indicator tube (visual analysis)	5 µg/l (± 15 %)	Kolljkowsky (1969)
	Collect on silica gel; compare with absorbence in iso-octane	S	< 100 µg/l	Maffett et al. (1956)
Water	Sparge sample with helium; trap on porous polymeric adsorbent; desorb thermally	GC/MS	< 5 µg/l	Pereira and Hughes (1980)
	Pass nitrogen through sample and through a carbon adsorbent; desorb with carbon disulphide	GC/FID	not given	Colenutt and Thorburn (1980)
	Sparge with helium; trap in Tenax GC tube	GC/MS	0.1 µg/l	Fentiman *et al.* (1979)
Landfill vapours	Adsorb on carbon in glass tubes; desorb with carbon disulphide	GC/FID	not given	Colenutt and Davies (1980)
Soil	Sparge with nitrogen; trap in Tenax GC tube	GC/FID	0.1 µg/kg	Fentiman *et al.* (1979)
Blood	Dissolve in pentane	GC/FID	0.8-4.8 µg/l	Ghimenti *et al.* (1978)
	Mix with sodium citrate solution	GC/FID	20 µg/l	Pinigina and Mal'tseva (1978)
Adipose tissue, brain, kidney, liver, lung, muscle, pancreas, and spleen	Treat with chloro-benzene, ethanol and water at 60°C; inject vapour phase into gas chromatograph	GC/MS	not given	Nagata *et al.* (1978)

[a] Abbreviations: GC/MS, gas chromatography/mass spectrometry; GC/FID, gas chromatography/flame ionization detection; S, spectrophotometry

0/30, 2/30 and 8/32, respectively, developed Zymbal gland carcinomas [Cochran-Armitage test for positive trend: p = 0.001; Fisher exact test for control *versus* high-dose group: p = 0.003]; 3/30, 4/30 and 7/32 developed mammary gland carcinomas; and 1/30, 2/30 and 1/32 developed leukaemias [type unspecified]. No such tumours were found in males, except that leukaemias occurred in 4/33 high-dose males [Cochran-Armitage test for positive trend: p = 0.008; Fisher exact test: p < 0.069]. The background incidence of Zymbal gland carcinomas in several thousand male and female rats of the same strain was said by the authors to be about 0.7%. The average latent period of the mammary gland carcinomas was 88 weeks in each of the test groups *versus* 110 weeks in the control group. In the high-dose group, two females had a skin carcinoma, one male had a hepatoma, and one male had a subcutaneous angiosarcoma; no such tumour was seen in the control or low-dose group (Maltoni and Scarnato, 1979).

(b) Skin application

In many experiments in which a variety of chemicals were applied to the skin of mice as solutions in benzene, a large number of control animals were treated with benzene alone. In none has there been any indication that benzene has induced skin tumours; however, not all possible tumour sites were examined in all of the experiments. Some of the most pertinent studies were carried out by Burdette and Strong (1941), Kirschbaum and Strong (1942), Neukomm (1962), Coombs and Croft (1966), Laerum (1973) and Fukuda *et al.* (1981).

(c) Inhalation

Mouse: Anaemia, lymphocytopenia and bone-marrow hypoplasia were found in 50 male AKR/J mice, 6 weeks of age at the start of the study, which were exposed to 300 mg/m³ [100 ppm] benzene for 6 hours/day, 5 days/week, for life. The exposure ended at 505 days with the death of the last test animal. The incidence or induction time of the viral-induced lymphomas commonly seen in this strain of mice was not influenced by exposure to benzene, the incidences being 29/49 and 24/50 in the test and control group, respectively (Snyder *et al.*, 1980).

Two groups of 40 male C57BL/6J mice, 6 weeks old, were exposed to atmospheres containing 0 or 900 mg/m³ [300 ppm] benzene for 6 hours/day, 5 days/week, for life. The exposure ended after 488 days with the death of the last test mouse. In addition to anaemia, lymphocytopenia, neutrophilia and bone-marrow hyperplasia, 6 of 40 mice exposed to benzene developed lymphocytic lymphoma with thymic involvement (p < 0.01 for lymphomas, according to Peto's log-rank method), 1 plasmacytoma and 1 haematocytoblastic leukaemia. The average survival time of the 8 tumour-bearing mice was 262 days. Two of the 40 control animals died from lymphocytic lymphoma with no thymic involvement after 282 and 608 days, respectively. The differences in incidence and induction time of tumours between the groups were statistically significant (Snyder *et al.*, 1980). [The Working Group noted that the thymus was not examined routinely.]

Male Charles River CD-1 mice [number unspecified] were exposed for 6 hours/day, 5 days/week, for life to atmospheres containing benzene at levels of 0 (control), 100 ppm [320 mg/m³] or 300 ppm [958 mg/m³]. Two mice in the high-exposure group developed myelogenous (myeloid) leukaemia (Snyder *et al.*, 1978a).

Rat: There was no evidence of a leukaemic response in 45 male 6-week-old Sprague-Dawley rats exposed to an atmosphere containing 900 mg/m³ [300 ppm] benzene for 6 hours/day, 5 days/week, for life. Exposure was terminated at week 99 when the last test animal died. The controls were 27 males of the same strain and age (Snyder *et al.*, 1978b).

(d) Subcutaneous and/or intramuscular administration

Mouse: Lignac (1932) reported the occurrence of different types of leukaemia in 8/33 male and female albino mice injected subcutaneously with 0.001 ml benzene [0.88 mg/kg bw] (chemically pure, thiophene-free) in 0.1 ml olive oil weekly for 17-21 weeks (total dose, about 1 mg/kg bw). The time between first injection and death of the 8 tumour-bearing mice ranged from 4 to 11 months. [The Working Group noted that no controls were used and that the study could therefore not be evaluated.]

Of 20 mice [sex unspecified] of the high leukaemia F strain given weekly s.c. injections of 0.001 ml benzene [purity unspecified] in sesame oil [0.88 mg/kg bw], 6 (30%) developed leukaemia at 200-300 days of age. Of 212 untreated mice, 29 (14%) developed leukaemia before 300 days of age (Kirschbaum and Strong, 1942) [Fisher exact test: $p = 0.06$.]

Groups of 30 male AKR, DBA/$_2$, C3H or C57BL6 mice were given weekly s.c. injections of 0.001 ml benzene (purity unspecified) in 0.1 ml olive oil [0.88 mg/kg bw] for life. No tumours other than those that occur normally in such animals were found in mice of the DBA/$_2$, C3H or C57BL6 strains, the maximum lifespan being 730 days. Leukaemia was seen in both treated and control AKR mice (Amiel, 1960).

Weanling male C57BL/6N mice were given s.c. injections of corn oil (20 mice) or of a 30% solution of benzene (99% pure) in corn oil (80 mice), or were not treated (20 mice). The injections were given twice weekly for 44 weeks, and then once weekly until 54 weeks. Mice given the 30% benzene solution received 0.05, 0.1, 0.1 and 0.2 ml for the first 4 weeks, respectively, followed by 0.2 ml until the last injection (total dose of benzene, about 4.9 g/animal). Vehicle controls received 0.025, 0.05, 0.05 and 0.1 ml for the first 4 weeks, respectively, followed by 0.1 ml until the last injection. At 104 weeks after the first injection all surviving mice were killed. The numbers of tumour-bearing mice were 26/45, 10/16 and 12/20 in the benzene, vehicle control and untreated groups, respectively. The incidences of granulocytic leukaemia were 8/45, 1/16 and 2/20, respectively; and of lymphomas, 6/45, 4/16 and 3/20. Tumours also occurred in the liver, stomach and lungs, but there was no significant difference from controls in tumour types or multiplicity of tumours (Ward *et al.*, 1975).

3.2 Other relevant biological data

(a) Experimental systems

Toxic effects

The oral LD_{50} of reagent-grade benzene in male Sprague-Dawley rats was reported to be 0.93 (0.71-1.23) g/kg bw (Cornish and Ryan, 1965); however, Kimura *et al.* (1971) reported oral LD_{50}s in male Sprague-Dawley rats of 3.4 g/kg bw in young adults (80-160 g) and 4.9 g/kg bw in older animals (300-470 g). An oral LD_{50} of 5.6 g/kg bw was reported

in male Wistar rats (Wolf *et al.*, 1956). Withey and Hall (1975) reported an oral LD_{50} in male Sprague-Dawley rats of 5.96 g/kg bw (95% confidence limits, 5.08-7.00). The i.p. LD_{50} of benzene in mice was 0.34 ml/kg bw (0.299 g/kg bw) (95% confidence limits, 0.28-0.42 ml/kg bw [0.25-0.37 g/kg bw]) (Kocsis *et al.*, 1968); the i.p. LD_{50} in female Sprague-Dawley rats was 2.94 g/kg bw (95% confidence limits, 2.45-3.53) (Drew and Fouts, 1974).

In acute inhalation experiments, 3/8 male Long Evans rats died within 24 hours after exposure to 130 000 mg/m³ [40 000 ppm] benzene for five 20-35-min periods. Death also occurred in 2/10 rats exposed to 33 000 mg/m³ [10 000 ppm] benzene for 12.5-30 min daily for 1 or 12 days (Furnas and Hine, 1958). The LC_{50} in female Sprague-Dawley rats was 13 700 ppm (95% confidence limits, 13 050-14 380) [43 770; 41 690-45 940 mg/m³] following a single four-hour exposure (Drew and Fouts, 1974).

Decreases in circulating blood cells of animals treated with benzene have been reported frequently (Snyder and Kocsis, 1975): Decreased leucocyte levels have been seen in rabbits (Santesson, 1897; Weiskotten *et al.*, 1915; Selling, 1916; Kissling and Speck, 1972), rats (Latta and Davies, 1941; Nomiyama, 1962; Gerarde and Ahlstrom, 1966) and mice (Nomiyama and Minai, 1969) given benzene subcutaneously. Uptake of radioactive iron into red cells, as a measure of erythrocyte production, was also decreased following s.c. administration of benzene (Lee *et al.*, 1974).

The severity of myelotoxicity is related to the dose, duration of treatment and test species. Doses of 0.4-2.2 g/kg bw per day are effective from within a few days (higher doses) to weeks (repeated lower doses). Rats are the most resistant species; rabbits and mice are relatively more sensitive (Lee *et al.*, 1974; Andrews *et al.*, 1977; Snyder *et al.*, 1978b; Andrews *et al.*, 1979; Sammett *et al.*, 1979; Longacre *et al.*, 1980, 1981a). Longacre *et al.* (1980, 1981a,b) have shown that DBA/2 and CD-1 are more sensitive than C57BL/6 mice.

Selling (1916) produced marrow aplasia in rabbits by giving benzene subcutaneously, in studies that were instrumental in initiating the concept of chemically induced aplastic anaemia. Later, Kissling and Speck (1972) succeeded in reproducing these results.

Weiskotten *et al.* (1920) first demonstrated that inhalation of 240 ppm [767 mg/m³] benzene for 1[illegible] hours/day for 2 weeks could induce leucopenia in rabbits. Slight leucopenia was reported in rats, guinea-pigs and rabbits exposed to 280 mg/m³ [88 ppm] for 7 hours/day for up to 269 days. Leucopenia was also seen in rats given 132 daily oral doses of 10 mg/kg bw during 187 days (Wolf *et al.*, 1956). No effect on the blood picture was seen in rats, guinea-pigs and dogs exposed continuously to 56 mg/m³ [17.6 ppm] for up to 127 days (Jenkins *et al.*, 1970). Slight leucopenia has been reported in rats exposed to 140 mg/m³ [44 ppm] benzene for 5 hours/day on 4 days/week for 8 weeks (Deichmann *et al.*, 1963). Leucopenia has also been produced in rats exposed to 400 ppm [1278 mg/m³] for 7 hours/day for 14 weeks (Boje *et al.*, 1970) or to 1000 ppm [3195 mg/m³] for 2 weeks (Ikeda and Ohtsuji, 1971).

Sprague-Dawley rats and AKR mice exposed to benzene (300 ppm [958 mg/m³]) for 6 hours/day, 5 days/week for life had lymphocytopenia, with little evidence of anaemia. AKR mice were more sensitive to benzene-induced leucopenia than were rats (Snyder *et al.*, 1978b). Lifetime exposure of C57BL/6J mice to 100 or 300 ppm [320 or 958 mg/m³] benzene produces anaemia, lymphocytopenia and neutrophilia associated with a relative increase in the number of immature leucocytes and a decrease in mature leucocytes in circulation (Snyder *et al.*, 1980). Subcutaneously administered benzene led to a selective

depression in B-lymphocytes in rabbits, whereas T lymphocytes were more resistant (Irons and Moore, 1980).

Several groups are studying the effects of benzene on bone-marrow cell cultures. In marrow cells taken from BDF_1 mice exposed to 4680 ppm [14 950 mg/m³] for 8 hours there was a significant depletion of colony-forming cells of the CFU-C (colony-forming units, leucocyte percursors) type one day after exposure, but recovery was noted by seven days. The effect was enhanced by multiple exposures. There was also evidence of depression of CFU-S (erythroid precursors) in the spleen colony-forming assay (Uyeki *et al.*, 1977). In CD-1 mice exposed to 1.1-4862 ppm [3.3-15 534 mg/m³] benzene for 6 hours/day on 5 days a week, concentrations of 103 ppm [329 mg/m³] and higher produced a significant decrease in the cellularity of the marrow and the spleen; splenic but not marrow GM-CFU-C (granulocyte macrophage-colony-forming unit-committed macrophage precursors) were also depressed. When exposure was to 9.6 ppm [31 mg/m³] for 50 days, no change in marrow activity was seen; but splenic cellularity and CFU-S were elevated. When the dose was raised to 302 ppm [965 mg/m³] for 26 weeks, marrow and spleen cellularity and CFU-S and marrow GM-CFU-C were decreased (Green *et al.*, 1981a,b).

In rabbits (Moeschlin and Speck, 1967; Kissling and Speck, 1972) and rats (Boje *et al.*, 1970) treated with benzene, there was decreased uptake of tritiated thymidine into bone-marrow DNA.

After repeated s.c. administration of benzene to rats, there was an increase in the number of bone-marrow cells in the G_2 and M phases of the cell cycle. No inhibition of DNA synthesis was reported, but benzene treatment resulted in an increase in cell proliferative activity, as measured by cytofluorometry and ^{3}H-thymidine uptake (Irons *et al.*, 1979).

Effects on reproduction and prenatal toxicity

Rats, guinea-pigs and rabbits exposed to 80-88 ppm [256-281 mg/m³] for 7 hours per day for 30-40 weeks had increased testicular weight and degeneration of the seminiferous tubules, as well as other signs of toxicity (Wolf *et al.*, 1956). Alteration of oestrous cycles has been reported in rats exposed to 1.6 or 9.4 ppm [5 or 30 mg/m³] for 4 months (Avilova and Ulanova, 1975), but there was no effect on their subsequent fertility or litter size. Gofmekler (1968) showed that continuous exposure of female rats to 210 ppm [670 mg/m³] for 10-15 days completely prevented pregnancy; it was not stated if this was due to failure to mate or to other causes. Exposure to lower concentrations, 0.3-20 ppm (1-63.3 mg/m³) was without effect. In C3H(JAX) mice whose ovaries were painted directly with benzene and which were later mated, a high incidence of subcutaneous haemorrhages and tail defects was observed in the offspring, which persisted through four generations (Sridharan *et al.*, 1963).

A single s.c. injection of 3 ml/kg bw benzene on one of days 11-15 of gestation to CFI mice caused cleft palate, agnathia and micrognathia in the offspring (Watanabe and Yoshida, 1970). [No controls were used, and it is very likely that these effects were produced by the stress of the injection.] Several other studies in pregnant mice exposed to benzene - 2 and 4 ml/kg bw subcutaneously (Matsumoto *et al.*, 1975), 0.3-1.0 ml/kg bw orally (Nawrot and Staples, 1979) or 500 ppm [1597 mg/m³] by inhalation for 7 hours/day (Murray *et al.*, 1979) - all failed to show any teratogenic effect, although reduced fetal weight and occasional embryolethality were observed. Similarly, several

Inhalation studies in rats have shown embryolethality and reduced fetal weight but only occasional teratogenic effects: Sprague-Dawley rats exposed to 10, 50 or 500 ppm [32, 160 or 1600 mg/m^3] for 7 hours/day had a low incidence of brain and skeletal defects but no embryolethality at 50 or 500 ppm, and no abnormality or embryolethality at the lower levels (Kuna and Kapp, 1981). No teratogenic effect was seen in pregnant rats exposed to 10 or 40 ppm [32 or 128 mg/m^3] for 6 hours/day (Murray *et al.*, 1979), to 313 ppm [1000 mg/m^3] for 24 hours/day (Hudak and Ungvary, 1978) or for 6 hours/day (Green *et al.*, 1978) or to 400 mg/m^3 [125 ppm] for 24 hours/day (Tatrai *et al.*, 1980).

No teratogenic effect has been reported in rabbits injected subcutaneously with 0.25 ml/kg of a 40% benzene solution daily during pregnancy (Desoille *et al.*, 1963) or in rabbits exposed by inhalation to 500 ppm [1600 mg/m^3] for 7 hours/day on days 6-18 of pregnancy (Murray *et al.*, 1979).

Absorption, distribution, excretion and metabolism

Lazarew *et al.* (1931) claimed that rabbits absorbed benzene through the skin. Exhalation is a major route of excretion of unchanged benzene in dogs (Schrenk *et al.*, 1941), rabbits (Parke and Williams, 1953a), mice (Andrews *et al.*, 1977) and rats (Rickert *et al.*, 1979). In rats, excretion *via* the lung follows a biphasic pattern, suggesting a two-compartment model for distribution, with an initial t1/2 of 0.7 hour (Rickert *et al.*, 1979). Simultaneous administration of benzene with toluene (Andrews *et al.*, 1977; Sato and Nakajima, 1979) or with piperonyl butoxide (Timbrell and Mitchell, 1977) increases the excretion of unchanged benzene in the breath, presumably because of interference with benzene metabolism.

Benzene metabolism was recently reviewed by Snyder *et al.* (1981)

Metabolism occurs most rapidly in the liver, where benzene is converted to benzene oxide (Jerina and Daly, 1974) by mixed-function oxidases (Gonasun *et al.*, 1973). Benzene oxide then rearranges spontaneously to form phenol, reacts enzymatically with glutathione to yield a premercapturic acid (Jerina *et al.*, 1968), or is hydrated *via* epoxide hydratase (Oesch *et al.*, 1977) to the dihydrodiol (Sato *et al.*, 1963), which is then oxidized to catechol (Ayengar *et al.*, 1959; Jerina *et al.*, 1968; Vogel *et al.*, 1980). Another major metabolite, hydroquinone [see IARC, 1977], may be formed by further reaction of phenol with the mixed-function oxidase; but that pathway has yet to be clarified. Other reported metabolites, such as trihydroxylated benzene (Parke and Williams, 1953a; Greenlee *et al.*, 1981) and muconic acid (Parke and Williams, 1953a), appear to be formed by as yet unresolved pathways.

Conjugated phenolic metabolites of benzene appear in the urine mainly as ethereal sulphates and glucuronides (Parke and Williams, 1953b; Williams, 1959). In fasted rats, formation of ethereal sulphate conjugates is decreased (Cornish and Ryan, 1965). Less than 5% of metabolites are recovered in the urine as phenylmercapturic acid (Parke and Williams, 1953a; Longacre *et al.*, 1981b).

The metabolism of benzene in liver homogenates can be stimulated by treating animals with enzyme-inducing agents. Benzene, phenobarbital, 3-methylcholanthrene, dimethyl sulphoxide, chlordiazepam, diazepam and oxazepam all induce benzene hydroxylase activity (Snyder and Remmer, 1979). Carbon monoxide, aniline, metyrapone, SKF-525A, aminopyrine, cytochrome *c* (Gonasun *et al.*, 1973), aminotriazole (Hirokawa and Nomiyama, 1962) and toluene (Andrews *et al.*, 1977) inhibit benzene metabolism *in vitro*.

Myelotoxic effects of benzene were alleviated by pretreating rats with phenobarbital (Ikeda and Ohtsuji, 1971; Gill *et al.*, 1979) or with either of two polychlorinated biphenyls: 2,4,5,2',4',5'-hexachlorobiphenyl or 3,4,3',4'-tetrachlorobiphenyl (Greenlee and Irons, 1981). The available evidence supports the concept that benzene toxicity is caused by one or more metabolites of benzene (Snyder *et al.*, 1981,1982). Parmentier and Dustin (1948) demonstrated that benzene metabolites containing two or three hydroxyl groups inhibited mitosis. Toluene, which inhibits benzene metabolism (Ikeda *et al.*, 1972; Sato and Nakajima, 1979), protected animals against benzene-induced myelotoxicity (Andrews *et al.*, 1977). Benzene toxicity could be correlated with the appearance of benzene metabolites in bone marrow (Andrews *et al.*, 1977; Rickert *et al.*, 1979; Greenlee *et al.*, 1981; Longacre *et al.*, 1981a,b). Although it is clear that benzene can be metabolized in bone marrow (Andrews *et al.*, 1979; Irons *et al.*, 1980), the observation (Sammett *et al.*, 1979) that partial hepatectomy protects against benzene toxicity suggests that a metabolite formed in liver is essential for benzene toxicity.

Irons and coworkers in a series of papers (see below) have stressed the importance of polyhydroxylated derivatives of benzene and their semiquinones (Irons *et al.*, 1982). They have shown that hydroquinone inhibits rat brain microtubule polymerization (Irons and Neptun, 1980); that hydroquinone and *para*-benzoquinone are the most potent inhibitors of T- and B-lymphocyte function, as measured in mouse spleen cells in culture (Wierda *et al.*, 1981); that hydroquinone inhibits lectin-stimulated lymphocyte agglutination in rat spleen preparations *in vitro* (Pfeifer and Irons, 1981); and that *para*-benzoquinone is the metabolite most likely to be responsible for suppression of lymphocyte transformation and microtubule assembly in rat spleen cells in culture (Irons *et al.*, 1982). However, administration of these compounds to animals does not produce the typical picture of benzene toxicity, i.e., leucopenia, anaemia, thrombocytopenia and, eventually, aplastic anaemia. Engelsberg and Snyder (1982) have administered the major metabolites of benzene to mice and failed to observe decreases in red cell production, using the ^{59}Fe uptake technique of Lee *et al.* (1974,1981). Goldstein *et al.* (1982) have suggested that ring-opening products may play a role in benzene toxicity. Tunek *et al.* (1981) reported that in mice benzene treatment suppressed subsequent CFU-C formation from bone-marrow cells *in vitro*. Treating the animals with phenol, hydroquinone or benzene dihydrodiol failed to suppress CFU-C. Thus, the toxic metabolites of benzene have yet to be identified.

Lutz and Schlatter (1977) demonstrated radioactivity in a nucleic acid fraction from rat liver following administration of either ^{3}H- or ^{14}C-labelled benzene. It has been shown that benzene binds covalently to protein in liver, bone marrow, kidney, lung, spleen, blood and muscle (Snyder *et al.*, 1978c; Longacre *et al.*, 1981a,b). Less covalent binding was observed to the protein of bone marrow, blood, and spleen of C57BL/6 mice, which are more resistant to the benzene-induced effects on red cell production, than to that of sensitive DBA/2 mice (Longacre *et al.*, 1981b). Irons *et al.* (1980) demonstrated covalent binding of benzene to protein in perfused bone-marrow preparations. Tunek *et al.* (1978) have argued that a metabolite of phenol binds to liver protein more efficiently than does benzene oxide, and they have electrophoretically separated hepatic proteins to which benzene preferentially binds (Tunek *et al.*, 1979). Gill and Ahmed (1981) argue that covalent binding to mitochondria is a prominent feature of benzene metabolism. They reported further that there is relatively more radioactivity in a nucleic acid-rich fraction of a benzene metabolite isolated from mouse bone-marrow cells than in a similar fraction from liver.

Mutagenicity and other short-term tests

Benzene is not mutagenic in bacterial systems. In a detailed study with *Salmonella typhimurium* strains TA98 and TA100, benzene was tested at concentrations of 0.1 to 1.0 µl per plate, with and without microsomal fractions obtained from liver homogenates of 3-methylcholanthrene or phenobarbital-treated rats. In some experiments, 1,1,1-trichloro-2,3-epoxypropane was added to the liver homogenate mixture in the activated bacterial plate assay in an attempt to block the possible biodegradation of an epoxide metabolite. Bone-marrow homogenates from 3-methylcholanthrene-treated rats were also tested in place of liver homogenates. Finally, a host-mediated assay was performed with *Salmonella* strain TA1950, in which mice were given two s.c. injections of 0.1 ml/kg benzene. In none of these studies was there an increase in the reversion rates (Lyon, 1976).

The lack of a mutagenic effect of benzene has since been confirmed with a large range of tester strains. Benzene showed no mutagenic activity in assays with *Salmonella typhimurium* (Dean, 1978; Shahin and Fournier, 1978; Lebowitz *et al.*, 1979) or *Bacillus subtilis* (Tanooka, 1977), no resistance to 8-azaguanine as a marker in *S. typhimurium* (Kaden *et al.*, 1979), and no mutagenicity to *Saccharomyces cerevisiae* (Cotruvo *et al.*, 1977). It was also negative in the *Escherichia coli* pol A test (Rosenkranz and Leifer, 1980). A preliminary study suggests, however, that benzene oxide, a postulated intermediary metabolite of benzene, may be mutagenic in *S. typhimurium* (Kinoshita *et al.*, 1981). [The Working Group considered that the data of the last study are equivocal and must be confirmed.]

No mutagenic effect of benzene was seen in *Drosophila melanogaster* in a sex-linked genetically unstable test system involving a transposable genetic element, in which mutagenicity was measured by the frequency of somatic mutations for eye pigmentation (red sectors). Newly hatched larvae were placed in a medium containing 1.0% or 2.0% benzene (Nylander *et al.*, 1978).

Benzene was not mutagenic in the mouse lymphoma forward mutation assay in L5178Y ($TK^{+/-}$) cells (Lebowitz *et al.*, 1979). Clastogenic effects of benzene on mammalian chromosomes have been observed *in vitro*. A statistically significant increase in chromosomal aberrations, mainly chromatid-type deletions and gaps, has been reported in human lymphocytes (Koizumi *et al.*, 1974; Morimoto, 1976) and in HeLa cells (Koizumi *et al.*, 1974) exposed *in vitro* to 0.2-3.0 mM benzene. Gerner-Smidt and Friedrich (1978), who analysed only 60 cells, could detect no increase in chromosomal aberrations in human lymphocytes treated *in vitro* with similar concentrations of benzene.

In experiments parallel to those described above, Gerner-Smidt and Friedrich (1978) found no enhancement in the frequency of sister chromatid exchange in benzene-treated cultures. Diaz *et al.* (1979) reported that when benzene was present during the first 24 hours of culture, but not later, the frequency of sister chromatid exchange was enhanced; when benzene treatment was carried out in the presence of metabolic activation by rat liver microsomes, a further increase in sister chromatid exchange/cell was observed. Morimoto and Wolff (1980) found that a 72-hour incubation with up to 5 mM benzene did not increase the frequency of sister chromatid exchange or affect cell cycle kinetics, whereas its principal metabolites, catechol and hydroquinone, induced high levels of sister chromatid exchange at much lower concentrations. Phenol produced only a weak effect. The sum of these results suggests that the apparent clastogenic effects of benzene *in vitro* may result from its biologically active metabolites rather than from benzene itself.

Treatment with 0.2-1.0 mM benzene significantly enhanced the number of rings and dicentrics induced in chromosomes of human lymphocytes by administration of 100 rads (1 Gy) of γ radiation (Morimoto, 1976).

In male rats, no dominant lethality was induced by a single i.p. injection of 0.5 ml/kg bw benzene (Lyon, 1976); however, a positive effect was observed in mice given 3000 mg/kg bw (Pavlenko *et al.*, 1979). [Due to the experimental design, the Working Group found it difficult to interpret the latter result.]

An increase in polychromatic erythrocytes with micronuclei (micronucleus test) has been observed in benzene-treated animals in four separate investigations (Lyon, 1976; Diaz *et al.*, 1980; Hite *et al.*, 1980; Meyne and Legator, 1980). Hite *et al.* (1980) gave 0.0625 to 2.0 ml/kg bw per day of benzene in two daily oral doses to male and female Charles River (CD-1) mice. The mice were sacrificed at from 6 hours to 16 days after the second dose, and bone marrow was obtained. Animals sacrificed 6 hours to 5 days after treatment showed significant increases in micronuclei with doses of 0.25 ml/kg per day, and in some cases with 0.125 ml/kg per day. A similar result was reported by Lyon (1976) in rats sacrificed 6 hours after a second dose of 0.05-0.5 ml/kg bw per day of benzene administered intraperitoneally; and by Diaz *et al.* (1980) in male mice (F_1 hybrids from the cross CS:V × CS No. 1) sacrificed 6 or 30 hours after s.c. administration of 0.2-2 ml/kg per day. A significant dose-effect correlation was found in both of these studies (Lyon, 1976; Diaz *et al.*, 1980). In another study (Meyne and Legator, 1980), oral administration of benzene induced a higher frequency of micronuclei in female and male Swiss (CD-1) mice than did intraperitoneal injection of the same doses.

Male mice are more sensitive than females to the induction of micronuclei by benzene administered either orally or intraperitoneally (Meyne and Legator, 1980; Siou *et al.*, 1980). Castration of males reduces their sensitivity to that of females (Siou *et al.*, 1980).

Numerous studies have demonstrated the induction of chromosomal aberrations in bone-marrow cells from mice (Meyne and Legator, 1978, 1980), rats (Dean, 1969; Philip and Krogh Jensen, 1970; Lyapkalo, 1973; Lyon, 1976; Dobrokhotov and Enikeev, 1977; Anderson and Richardson, 1979) and rabbits (Kissling and Speck, 1971) treated with single or multiple daily doses of benzene ranging from about 0.2 to 2.0 ml/kg per day and given either subcutaneously or intraperitoneally. Most of the induced aberrations were chromatid breaks or deletions; but chromosome-type aberrations also occurred (Kissling and Speck, 1971; Lyon, 1976), particularly after prolonged exposure, when toxicity, manifested by a drop in the peripheral blood leucocyte count, appeared (Kissling and Speck, 1971). The persistence of these changes has varied: A significantly elevated level of aberrations was seen up to 8 days after a single i.p. injection of 0.5 ml/kg bw in rats (Lyon, 1976), whereas aberrations were significantly increased in mice 24 hours but not 7 days after receiving a similar dose (0.5 ml/kg bw) (Meyne and Legator, 1978). In the former study (Lyon, 1976), double minutes (small supernumerary chromosomal fragments) were seen up to 70 days after benzene treatment.

The route of administration may influence some of the mutagenic effects of benzene. Experiments done in parallel to the micronucleus test described above revealed similar frequencies of chromosomal aberrations after oral and i.p. treatment with benzene (Meyne and Legator, 1980). Exposure of adult male and female DBA/2 mice to 3100 ppm [10 000 mg/m³] benzene by inhalation for 4 hours significantly increased the frequency of sister chromatid exchange but not of chromosomal aberrations in bone-marrow cells. This treatment also inhibited marrow cellular proliferation, but only in male mice.

Treatment with sodium phenobarbital prior to benzene exposure enhanced the frequency of sister chromatid exchange in female mice, and led to a significant yield of chromatid-type aberrations in animals of both sexes. The authors suggested that different metabolites of benzene might be involved in different biologic endpoints (Tice *et al.*, 1980).

(*b*) *Humans*

Toxic effects

Single exposures to concentrations of 66 000 mg/m³ [20 000 ppm] commercial benzene have been reported to be fatal in man within 5-10 minutes (Flury, 1928). At lower levels, loss of consciousness, irregular heart-beat, dizziness, headache and nausea are observed (Deutsche Forschungsgemeinschaft, 1974). In cases of acute poisoning, inflammation of the respiratory tract, haemorrhages of the lungs, congestion of the kidneys and cerebral oedema have been observed at autopsy; but even with levels in blood of up to 2 mg/100 ml, no changes were observed in the blood picture (Winek and Collom, 1971).

It has been known since the earliest reports, of Santesson (1897) and Selling (1916), that benzene can cause aplastic anaemia; and the recent reports of Aksoy *et al.* (1972) further support these observations. Early stages in the progression to pancytopenia have been stated to be anaemia (Hunter, 1939; Goldwater, 1941; Helmer, 1944), leucopenia in which lymphocytopenia predominated (Greenburg *et al.*, 1939; Goldwater, 1941), leucopenia in which neutropenia predominated (Hamilton-Paterson and Browning, 1944) and thrombocytopenia (Savilahti, 1956).

Of 60 individuals (58 women and 2 men) who had displayed signs of benzene toxicity and were reevaluated 16 months after use of benzene in the factory where they worked had been terminated, 46 had recovered, 12 still exhibited signs of benzene toxicity and 2 had died (Helmer, 1944). In workers exposed to about 25 ppm [80 mg/m³] benzene over several years, increases in mean corpuscular volume and minimal decreases in mean haemoglobin and haematocrit levels were seen. These values returned to normal after cessation of exposure (Fishbeck *et al.*, 1978). However, it has not been possible to establish with certainty the degree of exposure below which no adverse haematological effects of benzene in humans would occur.

Smolik and coworkers (Lange *et al.*, 1973a; Smolik *et al.*, 1973) studied a large number of workers exposed to but not seriously intoxicated by benzene and found that serum complement levels, IgG and IgA, were decreased but that IgM levels did not drop and were in fact slightly higher. Immunotoxicological studies of benzene had not previously concerned workers, since early reports on rabbits had described increased susceptibility to tuberculosis (White and Gammon, 1914) and pneumonia (Hirschfelder and Winternitz, 1913; Winternitz and Hirschfelder, 1913), decreased production of red cell lysins, agglutinins for killed typhoid bacilli and opsonins (Simonds and Jones, 1915) and reduction or absence of antibacterial antibodies (Camp and Baumgartner, 1915; Hektoen, 1916). Recently, Wierda *et al.* (1982) have demonstrated that administration of benzene to C57BL/6 mice *in vivo* inhibits the function of B- and T-lymphocytes studied *in vitro*. These observations, taken together with the well-known ability of benzene to depress leucocytes, which themselves play a significant role in protection against infectious agents, may explain why benzene-intoxicated individuals readily succumb to infection and why the terminal event in severe benzene toxicity is often an acute, overwhelming infection.

Lange *et al.* (1973b) also found that levels of leucocyte agglutinins were elevated in selected individuals exposed to benzene. They suggested that in some people benzene toxicity may be accounted for in part by an allergic blood dyscrasia.

Increases in red-cell δ-aminolaevulinic acid were found in 16 of 27 workers exposed to benzene (Kahn and Muzyka, 1973). Similar results were observed in animals (Kahn and Muzyka, 1973). Inhibition of reticulocyte haem was observed *in vitro* (Wildman *et al.*, 1976; Greenblatt *et al.*, 1977).

Effects on reproduction and prenatal toxicity

Benzene crosses the human placenta, and levels in cord blood are similar to those in maternal blood (Dowty *et al.*, 1976). Menstrual disturbances have been reported more frequently in women exposed industrially to benzene and other solvents than in unexposed controls (Michon, 1965; Mikhailova *et al.*, 1971).

Severe anaemia caused by solvents such as benzene or by other factors, such as chloramphenicol, may lead to death of mothers at parturition (Messerschmitt, 1972).

Absorption, distribution, excretion and metabolism

The most frequent route by which humans are exposed to benzene is *via* inhalation. Toxic effects in humans have often been attributed to combined exposure by both respiration and through the skin: e.g., rotogravure workers wash ink from their hands in open vats of benzene (Hunter, 1978).

When benzene was placed on the skin under a closed cup it was absorbed at a rate of 0.4 mg/cm² per hour (Hanke *et al.*, 1961), a rate equal to 2% of that of ethylbenzene (Dutkiewicz and Tyras, 1967) and 2-3% of that of toluene (Dutkiewicz and Tyras, 1968).

Following exposure to benzene, it is eliminated unchanged in expired air (Srbova *et al.*, 1950; Hunter, 1968; Sherwood and Carter, 1970; Nomiyama and Nomiyama, 1974a,b; Sato and Nakajima, 1979). In men and women exposed to 52-62 ppm [166-198 mg/m³] benzene for 4 hours, a mean of 46.9% was taken up, 30.2% was retained and the remaining 16.8% excreted as unchanged benzene in expired air. Pharmacokinetic plots of respiratory elimination were interpreted as indicating that there are three phases to the excretion, described by three rate constants, with no significant differences between men and women (Nomiyama and Nomiyama, 1974,a,b). When humans were exposed to 100 ppm [300 mg/m³] benzene, it was detected in expired air 24 hours later, suggesting that it is possible to back-extrapolate to the benzene concentration in the inspired air (Hunter, 1968). This interpretation has since been supported (Berlin *et al.*, 1980).

Subjects who inhaled concentrations of 340 mg/m³ [106 ppm] benzene in air for 5 hours excreted 29% as phenol, 3% as catechol and 1% as hydroquinone in the urine, mostly as ethereal sulphates. Most of the phenol and catechol was excreted within 24 hours, and the hydroquinone within 48 hours (Teisinger *et al.*, 1952). A correlation has been shown in a small number of workers between the concentration of benzene in air during 8-hour exposures and the excretion of phenol in urine (Rainsford and Lloyd Davies, 1965).

The percentage of inorganic sulphate in urine can also be used as an index of benzene exposure (Hammond and Hermann, 1960).

Mutagenicity and chromosomal effects

Numerous studies have been carried out on the chromosomes of bone-marrow cells and peripheral lymphocytes from people known to have been exposed to benzene (Dean, 1978). The populations included in these studies fall into two general categories: (1) patients with either a current or a past history of benzene-induced blood dyscrasias ('benzene haemopathies'), often associated with extensive exposure to benzene; and (2) workers with known current or past exposure to benzene but with no obvious clinical effects. In many of these studies, significant increases in chromosomal aberrations have been seen, which in some cases have persisted for years after cessation of exposure.

Pollini *et al.* (Pollini and Colombi, 1964a,b; Pollini *et al.*, 1964, 1969; Pollini and Biscaldi, 1976, 1977) examined bone-marrow cells and peripheral lymphocytes from workers with current severe blood dyscrasias, and followed several workers by repeated cytogenetic studies up to 12 years after recovery from benzene-induced pancytopenia. Gross chromosomal abnormalities were characteristic of these cells; 70% of the bone-marrow cells and lymphocytes in patients with acute poisoning showed karyotypic abnormalities (Pollini and Colombi, 1964a,b). The authors could not relate the frequency or type of chromosomal alterations to the severity of the blood dyscrasia (Pollini *et al.*, 1964). Five years after poisoning, all of five patients studied still showed stable (C_s) and unstable (C_u) chromosomal aberrations in their lymphocytes, although only 40% of the cells were now abnormal (Pollini *et al.*, 1969). By 12 years (Pollini and Biscaldi, 1977), no cytogenetic abnormalities remained in the four patients studied.

Forni and collaborators (Forni *et al.*, 1971a,b) examined two groups of workers with chronic benzene poisoning; one group included 25 subjects who had recovered from benzene haemopathy one to 18 years previously (plus four others showing acute toxicity at the time of first chromosome examination); and the other group comprised 34 workers in a rotogravure plant who had been exposed in 1952-1953 to concentrations of 125-532 ppm [400-1700 mg/m^3] benzene in air, which had led to toxic effects. Lymphocytes from both groups showed significantly higher levels of C_u and C_s than those from age-matched controls. Although in the first group C_u and C_s were present several years after cessation of benzene exposure, follow-up cytogenetic studies indicated a tendency toward a decrease in C_u and a persistence or increase in C_s (Forni *et al.*, 1971a).

The finding of significant increases in chromosomal aberrations in blood and bone marrow (Forni and Moreo, 1967, 1969) and in lymphocytes from clinically symptomatic subjects exposed to benzene has been confirmed in several other investigations (Hartwich *et al.*, 1969; Sellyei and Kelemen, 1971; Erdogan and Aksoy, 1973; Hudak and Gombosi, 1977; Van den Berghe *et al.*, 1979). Forni and Moreo (1967, 1969) hypothesized that such aberrations are involved in the eventual development of leukaemia in benzene-exposed individuals.

Tough and others (Tough and Court Brown, 1965; Tough *et al.*, 1970) studied workers in three different factories who had been exposed to benzene in the atmosphere for approximately one to 25 years. The workers were apparently asymptomatic as far as evidence of acute benzene toxicity was concerned. The first two groups consisted of a total of 38 workers who had been exposed to 25-150 ppm [80-480 mg/m^3] benzene until two to four years prior to sampling; the incidence of cells with unstable chromosomal aberrations (C_u) in these groups was higher than was expected from the general population. Workers in the third factory had been exposed intermittently to approximately 12 ppm [38 mg/m^3] benzene for 2-26 years; their lymphocytes showed no increase in

chromosomal abnormalities. The authors hesitated, however, to relate these effects to benzene exposure alone, since there was evidence that age and other environmental factors may also have been contributory.

Funes-Cravioto *et al.* (1977) studied lymphocytes from 73 workers in several chemical laboratories and in the printing industry, and found a significantly increased frequency of chromosomal breaks, as compared with cells from 49 control subjects. In 12 subjects studied, the frequency of sister chromatid exchange was also enhanced. Exposure to organic solvents, including benzene, was common to all work environments studied; and, in 29 workers specifically, exposure to benzene had been heavy during the 1960s. However, no particular solvent could be singled out as the direct clastogenic agent. Picciano (1979) examined lymphocytes from 52 workers who had been exposed to less than 10 ppm [<32 mg/m³] benzene for periods of one month to 26 years. A statistically significant increase in the rate of chromosomal aberrations was found, as compared with 44 people in a control group. Although these workers were also exposed to other aromatic hydrocarbon solvents, the degree of contact with those solvents was less than with benzene.

Three other studies also report increased levels of chromosomal aberrations in asymptomatic workers who had been exposed to benzene (Hartwich and Schwanitz, 1972; Khan and Khan, 1973; Fredga *et al.*, 1979). In one of these studies (Hartwich and Schwanitz, 1972), the mean aberration rate of cells from nine refinery workers exposed to 'relatively low' levels of benzene was significantly elevated when compared with that in controls, but the rate in individual workers was at the upper limit of normal. In a study of lymphocytes from 65 people (Fredga *et al.*, 1979), a statistically significant increase in chromosomal aberrations was found in both petrol- and milk-tanker drivers and in 12 industrial gasworks workers exposed to benzene, but not in petrol tanker crews or petrol station staff. As no other etiological factor could be identified, the increase in the 12 industrial workers studied was regarded as being due to benzene exposure (5-10 ppm; 16-32 mg/m³). However, Watanabe *et al.* (1980) found no evidence of an increased frequency of chromosomal aberrations or of sister chromatid exchange among nine female workers engaged in painting ceramics who had been exposed to <1-9 ppm [<3-29 mg/m³] for 1-20 years, or in seven female workers who had been exposed to 3-50 ppm [10-160 mg/m³] benzene for 2-12 years.

3.3 Case reports and epidemiological studies of carcinogenicity in humans

(a) Case reports

An association between long-term exposure to benzene and the occurrence of leukaemia was suggested as early as 1928 by Delore and Borgomano (1928), who described acute lymphoblastic leukaemia in a worker who had been exposed to benzene for five years.

The industrial exposure to commercial benzene (benzol) of 89 workers involved in the manufacture either of artificial leather or of shoes (which involves the use of rubber cements containing benzene) and their short- and long-term health records were investigated in a series of studies (Bowditch and Elkins, 1939; Hunter, 1939; Mallory *et al.*, 1939). One of the workers, a 28-year old male who had been exposed to commercial

benzene for 10 years, died from acute myeloblastic leukaemia (Hunter, 1939). Mallory *et al.* (1939) reported on 19 cases of prolonged exposure to commercial benzene. Two cases of leukaemia were described: that reported by Hunter (1939) and a lymphoblastic leukaemia in a 12-year old boy who had 'frequently' used a paint remover known to contain commercial benzene.

DeGowin (1963) reported that an indoor house painter who had thinned his paints with benzene for 13 years developed aplastic anaemia; although he was not subsequently exposed to benzene, he developed acute myeloid leukaemia 15 years later.

Tareeff *et al.* (1963) described 16 cases of leukaemia (6 acute and 10 chronic) in workers in the USSR occupationally exposed to benzene for 4-27 years (average, 15 years). In 3/6 acute cases, a latent period of 2-5 years between the cessation of exposure and the development of leukaemia was noted.

Vigliani and Saita (1964) reported 6 cases of leukaemia seen at the Clinica de Lavoro, Milan, between 1942 and 1963. The patients included a spreader and calender operator at a leathercloth factory (9 years' exposure), an assistant operator in a rotogravure firm (5 years' exposure), a spray varnisher (8 years' exposure), a rotogravure operator (11 years' exposure) and two workers using glues containing benzene (19 and 3 years' exposure). At the Institute of Occupational Health in Pavia, 5 cases of leukaemia were seen between 1961 and 1963 in shoemakers exposed to glues containing benzene.

During the period 1950-1965, 50 cases of leukaemia were observed in workers with confirmed exposure to benzene in industries in the Paris region (Goguel *et al.*, 1967). Clinical data were given for 44 previously unpublished cases (37 men and 7 women), which comprised 13 cases of chronic myeloid leukaemia, 8 cases of chronic lymphoid leukaemia and 23 cases of acute leukaemia, of which 2 were erythroleukaemias. Measurements were made of benzene in the blood for 19 of the cases; in 7 of these, the level was high.

Four cases of acute leukaemia were reported in shoemakers in Istanbul exposed to benzene for 6-14 years. Three of the cases were of the myeloblastic type and the fourth a monocytic type (Aksoy *et al.*, 1972). The concentration of benzene in the working environment was reported to range between 15-30 ppm [48-96 mg/m^3] outside working hours and rose to a maximum of 210 ppm [670 mg/m^3] when adhesives containing benzene were being used (Aksoy *et al.*, 1971).

Ludwig and Werthemann (1962) described one case of myeloid leukaemia and one of a tumour-like reticulosis among 44 workers in two chemical factories exposed to benzene and toluene between 1940 and 1961.

Cases of erythromyelosis have also been described in workers with short- and long-term exposure to benzene (Galavotti and Troisi, 1950; Nissen and Soeborg Ohlsen, 1953; Di Guglielmo and Iannaccone, 1958; Rozman *et al.*, 1968; Bryon *et al.*, 1969; Forni and Moreo, 1969).

In a study carried out during 1966-1969 in two Lyon hospitals, 17/140 (12%) patients with acute leukaemia, 9/61 (15%) with chronic lymphoid leukaemia and 4/56 (7%) with myeloid leukaemia were found to have been exposed to benzene or toluene. Five cases of exposure to benzene were found among 124 (4%) control patients without blood disorders (Girard and Revol, 1970).

A number of additional case reports have been the subject of a review (Goldstein, 1977). Most cases of malignancy in which an association with exposure to benzene was reported have been leukaemias, particularly of the acute myelogenous type. There have, however, been a number of discrepant reports: in some, all of the cases were nonmyelogenous leukaemia; in others, cases of acute and chronic lymphatic leukaemia and of monocytic leukaemia were seen. There have been scattered reports of an excess of lymphomas both of the Hodgkin's and non-Hodgkin's variety. Aksoy (1980) summarized 83 cases - 42 leukaemias, 9 malignant lymphomas, 5 lung cancers, 2 myeloid metaplasias, 2 paroxysmal nocturnal haemoglobinurias and 3 multiple myelomas - all of which involved chronic exposure to benzene in Turkey. The author suggested that '... the frequent finding of this association suggests that benzene may not only cause leukaemia but also be involved in other types of malignancy'. [It is impossible to ascribe risk estimates to reports of this type, since most do not describe appropriate control groups, i.e., they are based on reports of case series. Most of the reports do not include information on environmental measurements; i.e., putative exposure to benzene is based on historical information.]

(b) Epidemiological studies

A case-control study of leukaemia was reported from Japan (Ishimaru *et al.*, 1971). All cases diagnosed as definite or probable leukaemia, resident in Hiroshima or Nagasaki City at the time of the onset of the disease between 1945 and 1967 were included. One control per case was chosen from the Atomic Bomb Casualty Commission Leukemia Registry sampling frame and matched on five characteristics: city, sex, date of birth $\pm$ 30 months, distance from the atomic bomb explosion, and alive and resident in either Hiroshima or Nagasaki at the time of disease onset in the patient. There were 492 leukaemia cases identified; information could be obtained for only 413 matched case-control pairs. In analysing 303 adult pairs with respect to occupations in which there was potential exposure to benzene, the authors arrived at a risk ratio of 2.5 ($p < 0.01$), on the basis of 42 exposure-discordant pairs. Of these, 41 pairs could be used to investigate the degree to which the benzene-associated relative risk was modified by exposure to the atomic bomb radiation. The relative risk was highest in those exposed to 1-99 rads [0.01-0.99 Gy] (5 exposure-discordant pairs). [The Working Group noted a discrepancy between the figures in table 2 and those in table 4 of that paper; they also noted the small numbers involved and the considerable uncertainty involved in using occupation as an index of exposure to benzene. The increased risk may also have been influenced by exposures to substances other than benzene, since in none of the occupations considered would benzene have been the only chemical encountered.]

Aksoy *et al.* (1974; Aksoy, 1977) estimated the incidence of acute leukaemia or 'preleukaemia' among 28 500 shoe-workers in Turkey on the basis of case ascertainment by contact with medical care. Thirty-four cases were identified. They estimated that the incidence of acute leukaemia was significantly greater among workers chronically exposed to benzene, which was used as a solvent by these workers, than in the general population. Occupational exposures were determined by work histories and by environmental measurements. There was said to be exposure only to benzene in small, poorly ventilated work areas; peak exposures to benzene were reported to be 210-650 ppm [670-2075 mg/m^3]. Duration of exposure was estimated to have been 1-15 years (mean, 9.7 years). The annual incidence was estimated to be 13/100 000, giving an approximate relative risk of 2 when compared with the annual estimate for the general population, 6/100 000. [These estimates are limited by the study design characteristics and by uncertainty about the way in which cases were ascertained and how many of the study population were exposed and how many unexposed.]

The distribution of cell types among the leukaemia cases with a history of exposure to benzene was compared with that of patients with no such history (Table 12) (Aksoy, 1977).

Table 12. Comparison of types of leukaemia found in 40 individuals with chronic benzene poisoning and in 50 nonexposed patients

Type of leukaemia	Exposed Number	%	Nonexposed Number	%
Acute myeloblastic leukaemia	15	37.5	8	16.0
Acute lymphoblastic leukaemia	4	10.0	13	20.0
Preleukaemia[a]	7	17.5	1	2.0
Acute erythroleukaemia	7	17.5	2	4.0
Acute monocytic or myelonomonocytic leukaemia	3	7.5	3	6.0
Acute unidentified leukaemia	1	2.5	0	0.0
Acute promyelocytic leukaemia	1	2.5	0	0.0
Chronic myeloid leukaemia	2	5.0	10	20.0
Chronic lymphoid leukaemia	0	0.0	13	25.0

The ratio of acute non-lymphocytic leukaemia to chronic leukaemia was 27:2 in the exposed group and 13:23 in the non-exposed, suggesting a relative risk of about 24 for acute nonlymphocytic leukaemia - an order of magnitude higher than the value of 2, which was based on the hypothetical incidence rates. Indirect support for the higher figure can be derived from a follow-up of patients with ankylosing spondylitis (Court Brown and Doll, 1957, 1965), in whom the distribution of leukaemia cell types is similar to that reported by Aksoy, which gave a relative risk of about 12 of dying 3 to 9 years after first coming under observation.

[Aksoy (1978) also followed 44 benzene-exposed patients with pancytopenia. Six cases of leukaemia developed within 6 years of follow-up, giving a proportion of 14%.]

Vigliani (1976) summarized the experience at the Institute of Occupational Health of Milan from 1942 to 1975 with 66 cases of benzene haemopathy, 11 of which were leukaemia, and the experience at the Institute of Occupational Health of Pavia from 1959 to 1974, in which 13 cases of leukaemia were ascertained among 135 instances of benzene haemopathy. [The cumulative incidence of leukaemia among individuals with clinically ascertained benzene haemopathy was about 17% in Milan and about 10% in Pavia.] Occupational exposures were identified in rotogravure plants and shoe factories. Benzene concentrations near rotogravure machines were 200-400 ppm [640-1280 mg/m^3], with peaks up to 1500 ppm [4800 mg/m^3]; benzene concentrations in air near workers handling glue in shoe factories were 25-600 ppm [80-1920 mg/m^3], but were 'mostly around 200-500 ppm' [640-1600 mg/m^3]. Estimated latency (years from start of exposure to clinical diagnosis of leukaemia) ranged from 3-24 years (median, 9 years). Vigliani estimated that the relative risk of acute leukaemia was at least 20:1 for workers heavily exposed to benzene in the rotogravure and shoe industries in the provinces studied, when compared with the general population. [The relative risk is based on a non-validated estimate.]

Fishbeck *et al.* (1978) reported on a small series of 10 chemical workers exposed to benzene (at the same company reported on by Ott *et al.*, see below). Data on exposure to benzene and on periodic health surveillance were available for the period 1953-1963. Benzene levels exceeded a time-weighted average of 25 ppm [80 mg/m^3]; in the job with the highest exposure ('operator'), the eight-hour time-weighted average exposure was 30-35 ppm [96-112 mg/m^3], with peaks of 937 ppm [2994 mg/m^3]. The total period of exposure ranged from 3 years 7 months to 29 years 9 months, and averaged 14 years 6 months. All 10 workers had first been exposed more than 16 years before the study. In 1963, all of these workers showed laboratory evidence of changes in peripheral blood, with slight reduction in haemoglobin and increased mean corpuscular volume. The investigators reported that '... monitoring of their health status has shown no persisting, significant adverse health effects'. [These data provide limited information. Given the presence of benzene-induced pancytopenia, the subsequent risk of leukaemia may be in the range of 10-15% in 10 years. The limited power of this study is such that subsequent cumulative risks of up to 25% could not be excluded.]

Thorpe (1974) reported on mortality from leukaemia among workers in eight European affiliates of a large US oil company. Eighteen cases (14 deaths and 4 incident cases assumed to be dead) were reported for the period 1962 to 1971. Estimates of the number of deaths expected in a combined population of 38 000 workers were based on rates for the general populations in the countries represented by the affiliates. The overall SMR (O/E) of 77 was calculated on the basis of a total of 383 276 person-years. No association was found between an index of potential exposure to benzene (based on estimates by the affiliates) and leukaemia mortality rate. Although no statistically significant excess was observed within any geographic affiliate (the highest SMR was 108 in one affiliate), SMRs of 121 and 60 were observed in all exposed and in all nonexposed workers, respectively. The 95% confidence interval was 37-205 for the exposed. [The study suffers from problems of ascertainment, specificity and validity of diagnoses and the 'healthy worker effect' in the calculation of SMRs. Further, it was impossible to identify the leukaemia type in the majority of instances (12 of the 18 cases). The population at risk was constructed from personnel files, the completeness of which, particularly for work processes and their potential benzene exposure, was not validated. Cases were ascertained from periodic health examinations, absenteeism records and insurance claims by methods idiosyncratic to each affiliate, not designed for research purposes, and of indeterminate completeness and accuracy. Limited data were reported on potential exposure to benzene, and no information was given on the induction-latent period. The power of this study is limited, since only eight of the cases were observed among exposed workers.]

(c) Epidemiological studies of mixed exposures

A number of epidemiological studies of workers potentially exposed to benzene among other agents have shown statistically significant excesses of leukaemia. As the actual exposure to benzene in these studies is often not documented, the relevance of the studies to the issue of benzene carcinogenicity remains undecided. The Working Group chose to include them not only because of their possible relevance in respect to benzene but also because they draw attention to the more general problem of the relationship between exposure to 'solvents' and cancer.

McMichael *et al.* (1975), Monson and Nakano (1976) and Tyroler *et al.* (1976) have summarized studies on the US rubber industry which disclosed excesses of mortality from chronic lymphatic leukaemia, myelogenous leukaemia and lymphosarcoma [see IARC,

1982b]. [There was historical evidence that exposure was to a mixture of agents, including benzene.]

Brandt *et al.* (1978) performed a case-control study of 50 male Swedish patients aged 20-65 seen at the University Hospital, Lund, in 1969-1977 with acute nonlymphocytic leukaemia and reported an excess history of exposure to petroleum products among cases. [No further details were provided regarding potential exposure to benzene or other environmental factors.]

Another Swedish case-control study (Flodin *et al.*, 1981), based on 42 cases of acute myeloid leukaemia from the University Hospital of Linkoping and 244 other deaths from the same area as controls, indicated an approximately six-fold, statistically significantly increased risk from exposure to solvents of various types, including petroleum products. [This study seems to confirm the results obtained in the study by Brandt *et al.*] Estimated background radiation from building materials (stone, concrete, wood, etc) in homes and workplace buildings significantly increased the risk of acute myeloid leukaemia; in particular, there seemed to be a strong effect of the combination of solvent exposure and high background radiation.

In a case-control study at the University Hospital of Umea, comprising 169 cases of malignant lymphoma (60 of which were of the Hodgkin's type) and 338 controls, frequencies of exposure to a number of agents were investigated, including phenoxy acids and organic solvents (Hardell *et al.*, 1981). A risk ratio of 4.6 (95% confidence limits, 1.9-11.4) was reported with regard to combined, 'high-grade' exposure to styrene, trichloroethylene, perchlorethylene and benzene; only one of the cases was explicitly reported to have been exposed to benzene. High-grade exposure to unspecified solvents resulted in a risk ratio of 2.8 (95% confidence limits, 1.6-4.8). [The Working Group noted that the latter exposure might also have involved exposure to benzene in combination with other agents; and it is unclear whether such exposure was associated with development of lymphoma.]

Greene *et al.* (1979) reported that a proportionate cancer mortality study among employees of the US Government Printing Office showed a 'significantly higher proportion of deaths from multiple myeloma, leukemia, Hodgkin's disease....' The excess deaths 'from leukemia occurred primarily in bindery workers who may have had exposure to benzene'. The authors noted the limitations in the methods used, including small numbers, use of the proportionate mortality ratio technique and limitations of ascertainment and diagnosis, but indicated the consistency of their study with the findings of others.

Rushton and Alderson (1981) reported the results of a case-control study nested in a cohort study. All death certificates with mention of leukaemia in men employed over a period of 25 years at eight petroleum refineries in the UK were studied. Potential occupational exposure to benzene among these cases was contrasted with that of two sets of controls selected from the total refinery population - one control was matched for refinery and year of birth, the other for refinery, year of birth and length of service. Job history was used to classify potential benzene exposure into low, medium and high categories. Although there was no overall excess of deaths from leukaemia in the refinery workers, compared with expectations based on national rates (see Rushton and Alderson, 1980), and there was no excess of 'cytological types of leukaemia which have been shown to be particularly associated with benzene exposure', the risk for men with medium or high exposure relative to the risk for those with low benzene exposure did approach statistical significance when length of service was taken into account.

Ott *et al.* (1978) reported the mortality experience of 594 individuals occupationally exposed to benzene in chemical manufacture, using a retrospective cohort analysis of the period 1940-1973. Three deaths due to leukaemia (one myelogenous and one myeloblastic) and one due to aplastic anaemia were noted; 0.8 death from leukaemia, excluding lymphocytic or monocytic cell types, was expected on the basis of incidence data from the Third National Cancer Survey [SMR = 375] ($p < 0.05$). Data derived from work histories and industrial hygiene records were used to estimate cumulative exposure doses. [The authors could find no association between the cases and work areas and estimated exposure levels; however, the number of workers in any particular work area was limited, and the power of the study to detect any association between exposure levels and cases was correspondingly low.]

Infante et al. (1977a) made a retrospective cohort analysis of 748 workers occupationally exposed to benzene between 1940 and 1949 in two factories engaged in the manufacture of rubber hydrochloride. They achieved 75% vital status ascertainment of the cohort up to 1975. Rinsky *et al.* (1981) continued to follow up the same cohort, also to 1975, and ascertained vital status for 98%. Cases were ascertained from diagnoses as given on death certificates, coded according to the ICD in effect at time of death and converted to those of the 7th revision. Person-years of observation and deaths from 1 January 1950 to 30 June 1975 were used. Expected rates were derived from US white male mortality statistics. The SMR for all causes of death in the more recent analysis (Rinsky *et al.*, 1981) was 111 (180 observed; 161.3 expected). A statistically significant excess mortality from all leukaemias was seen (O/E - 7.0/1.25; SMR = 560). All of the leukaemias were myelogenous or monocytic, constituting a 10-fold excess over expected of deaths from myeloid and monocytic leukaemias combined (O/E = 7/0.7). (Estimates of cell-type distribution were derived from the Connecticut Tumor Registry.) (Infante *et al.*, 1977a). Four additional cases of leukaemia, three of them myelogenous, occurred in workers employed in the two plants, but were not included in the statistical analysis because death occurred after 1975, because the workers were salaried rather than hourly and thus fell outside the cohort definition, or because of inaccuracies in death certificate coding. The median duration of employment for the entire cohort in the two plants was less than one year.

Exposures to airborne benzene vapour at the two plants were evaluated by Infante *et al.* (1977a). They concluded on the basis of monitoring data that worker exposures were generally within the recommended limits in effect at the time of their employment. [The methods employed in the 1940s for measuring benzene concentrations in air, while reasonably accurate, were relatively less sensitive than those available today.] Recommended levels were as follows:

1941 100 ppm [320 mg/m³] maximum allowable concentration
1947 50 ppm [160 mg/m³] 8-hour time-weighted average
1948 35 ppm [112 mg/m³] 8-hour time-weighted average

Tabershaw and Lamm (1977) argued that the exposures were not as low as Infante *et al.* (1977a) had stated. At plant B, workers manufactured rubber hydrochloride only; at plant A they also manufactured tyres, hose, foams, rubber chemicals and metal products. Tabershaw and Lamm argued that environmental exposure was therefore probably mixed and potentially higher at plant A than at plant B.

Infante *et al.* (1977b) and Rinsky *et al.* (1981), in reply to the critique by Tabershaw and Lamm, evaluated past exposures in both plants in further detail, and reported that,

although other solvents were used in various areas of both plants, benzene was found to be the only solvent used in the manufacture of rubber hydrochloride, except for chloroform, which was used between 1936 and 1949 in one plant. Rinsky *et al.* agreed with Tabershaw and Lamm that occasional high excursions occurred in airborne benzene levels (up to several hundred ppm). They found, however, that most such excursions occurred in areas entered only infrequently by workers, and they estimated that workers' actual eight-hour time-weighted average breathing-zone exposure fell generally within accepted limits. To evaluate possible differences between the two plants, Rinsky *et al.* specifically analysed leukaemia mortality in each. They found excess mortality in both plants: in one, 2 cases were observed *versus* 0.58 expected (SMR = 345); and in the other, 5 cases were observed *versus* 0.67 expected (SMR = 746). [The Working Group accepted the central conclusion of Infante *et al.* and of Rinsky *et al.* that excessive mortality from myelogenous and monocytic leukaemia had occurred among workers with occupational exposure to benzene that was generally within accepted limits. However, the possible contribution of the occasional excursions in exposure and of the employment of some workers in other areas of the plant must be noted; and in the opinion of the Working Group those factors may have made some contribution to the observed excess in mortality from leukaemia.]

4. Summary of Data Reported and Evaluation

4.1 Experimental data

Benzene has been tested in rats by intragastric administration and inhalation exposure, and in mice by skin application, inhalation exposure and subcutaneous injection. Oral administration to rats resulted in an increase in the incidence of Zymbal-gland carcinomas. Anaemia, lymphocytopenia and bone-marrow hyperplasia and an increased incidence of lymphoid tumours occurred in male mice exposed by inhalation to benzene; in similar inhalation studies with another strain of mice and with rats there was no evidence of a leukaemic response. Experiments involving skin application or subcutaneous injection of benzene did not produce evidence of carcinogenicity, but most of these experiments were inadequate.

Benzene does not induce specific gene mutations in bacterial systems or in *Drosophila melanogaster*. A single report showed no evidence for the induction of point mutation in mammalian cells; however, benzene induced cytogenetic abnormalities (chromosomal aberrations and sister chromatid exchanges) in mammalian cells *in vitro*.

The micronucleus test in mice and rats has been consistently positive. Numerous studies have shown that benzene exposure of experimental animals *in vivo* leads to the induction of chromosomal aberrations in the bone-marrow cells.

Exposure to benzene may damage the testis. Evidence from most studies in mice, rats, guinea-pigs and rabbits suggests that benzene is not teratogenic at doses that are fetotoxic and embryolethal.

4.2 Human data

Workers and the general public are exposed to benzene as a result of a variety of activities in which it is processed, generated or used. Major contributors to benzene

emissions into air include: (1) gasoline production, storage, transport, vending and combustion; (2) production of other chemicals from benzene; and (3) indirect production of benzene (e.g., in coke ovens). The last is the major source of benzene emissions into water.

Chronic human exposure to benzene results in leucopenia, thrombocytopenia, anaemia or combinations of these. At early stages of such blood dyscrasias, these effects appear to be reversible. Exposure to high doses for longer periods of time may lead to pancytopenia, which results from aplasia of the bone marrow and is considered to be an irreversible stage of the disease.

Benzene crosses the human placenta. There is a clear correlation between exposure to benzene and the appearance of chromosomal aberrations in the bone marrow and peripheral lymphocytes of individuals exposed to high levels of benzene (>100 ppm). Such levels of exposure usually lead to clinical symptoms of benzene-induced blood dyscrasias. These aberrations may persist for many years after exposure and after manifestations of haematotoxicity. The results are not so clear with lower levels (<100 ppm). Although aberrations have been reported following chronic exposures to as little as 10 ppm, this has not been a consistent finding. Environmental factors and exposure to other agents may have interacted with benzene in these studies of low exposure.

Many case reports and case series have described the association of leukaemia with exposure to benzene, either alone or in combination with other chemicals. Most cases were acute myelogenous leukaemia, although some were monocytic, erythroblastic or lymphocytic; and some lymphomas have been noted.

Two follow-up studies showed high incidences of leukaemia among individuals ascertained as cases of benzene haemopathy.

A series of epidemiological studies, both cohort and case-control, showed statistically significant associations between leukaemia (predominantly myelogenous) and occupational exposure to benzene and benzene-containing solvents. These results were replicated in a number of countries and different industries. In the epidemiological studies of people exposed primarily to benzene, statistically significant excesses of leukaemia were observed. [See also Annex. Some Aspects of Quantitative Cancer Risk Estimation.]

4.3 Evaluation[1]

There is *limited evidence* that benzene is carcinogenic in experimental animals.

It is established that human exposure to commercial benzene or benzene-containing mixtures can cause damage to the haematopoietic system, including pancytopenia. The relationship between benzene exposure and the development of acute myelogenous leukaemia has been established in epidemiological studies.

Reports linking exposure to benzene with other malignancies were considered to be inadequate for evaluation.

There is *sufficient evidence* that benzene is carcinogenic to man.

---

[1] This evaluation should be read in conjunction with section 7(*b*)(*c*) of the preamble.

5. References

Aksoy, M. (1977) Leukemia in workers due to occupational exposure to benzene. *New Istanbul Contrib. clin. Sci.*, *12*, 3-14

Aksoy, M. (1978) Benzene and leukaemia. *Lancet*, *I*, 441

Aksoy, M. (1980) Different types of malignancies due to occupational exposure to benzene: A review of recent observations in Turkey. *Environ. Res.*, *23*, 181-190

Askoy, M., DinCol, K., Akgun, T., Erdem, S. and DinCol, G. (1971) Haematological effects of chronic benzene poisoning in 217 workers. *Br. J. ind. Med.*, *28*, 296-302

Aksoy, M., DinCol, K., Erdem, S., Akgun, T. and DinCol, G. (1972) Details of blood changes in 32 patients with pancytopenia associated with long-term exposure to benzene. *Br. J. ind. Med.*, *29*, 56-64

Aksoy, M., Erdem, S. and DinCol, G. (1974) Leukemia in shoe-workers exposed chronically to benzene. *Blood*, *44*, 837-841

American Conference of Government Industrial Hygienists (1981) *Threshold Limit Values for Chemical Substances and Physical Agents in the Workroom Environment with Intended Changes for 1981*, Cincinnati, OH, p. 10

Amiel, J.L. (1960) Negative test for the induction of leukaemia in mice by benzene (Fr.). *Rev. franc. Etudes clin. biol.*, *5*, 198-199

Anderson, D. and Richardson, C.R. (1979) Chromosome gaps are associated with chemical mutagenesis (Abstract No. Ec-9). *Environ. Mutagenesis*, *1*, 179

Andrews, L.S., Lee, E.W., Witmer, C.M., Kocsis, J.J. and Snyder, R. (1977) Effects of toluene on the metabolism, disposition and hemopoietic toxicity of [^{3}H]-benzene. *Biochem. Pharmacol.*, *26*, 293-300

Andrews, L.S., Sasame, H.A. and Gillette, J.R. (1979) ^{3}H-Benzene metabolism in rabbit bone marrow. *Life Sci.*, *25*, 567-572

Anon. (1980) Low levels of suspect carcinogens found in coal liquefaction facilities. *Occup. Saf. Health Rep.*, *9*, 1163-1164

Avilova, G.G. and Ulanova, I.P. (1975) Comparative characteristics of the effect of benzene on the reproductive function of adult and young animals (Russ.). *Gig. Tr. prof. Zabol.*, *2*, 55-57

Ayengar, P.K., Hayaishi, O., Nakajima, M. and Tomida, I. (1959) Enzymic aromatization of 3,5-cyclohexadiene-1,2-diol. *Biochem. biophys. Acta*, *33*, 111-119

Ayers, G.W. and Muder, R.E. (1964) *Benzene*. In: Kirk, R.E. and Othmer, D.F., eds, *Encyclopedia of Chemical Technology*, 2nd ed., Vol. 3, New York, John Wiley and Sons, pp. 367-401

Baxter, H.G., Blakemore, R., Moore, J.P. and Coker, D.T. (1980) The measurement of airborne benzene vapour. *Ann. occup. Hyg.*, *23*, 117-132

Berlin, M., Gage, J.C., Gullberg, B., Holm, S., Knudsen, P., Eng, C. and Tunek, A. (1980) Breath concentration as an index of the health risk of inhaled benzene. *Scand. J. Work environ. Health*, *6*, 104-111

Boettner, E.A. and Ball, G.L. (1980) Thermal degradation products from PVC film in food wrapping operations. *Am. ind. Hyg. Assoc. J.*, *41*, 513-522

Boje, H., Benkel, W. and Heiniger, H.J. (1970) Study of leucopenia in bone marrow of rats after chronic benzene inhalation (Ger.). *Blut*, *21*, 250-257

Bowditch, M. and Elkins, H.B. (1939) Chronic exposure to benzene (benzol). I. The industrial aspects. *J. ind. Hyg. Toxicol.*, *21*, 321-330

Brandt, L., Nilsson, P.G. and Mitelman, F. (1978) Occupational exposure to petroleum products in men with acute non-lymphocytic leukaemia. *Br. med. J.*, *I*, 553-554

Brief, R.S., Lynch, J., Bernath, T. and Scala, R.A. (1980) Benzene in the workplace. *Am. ind. Hyg. Assoc. J.*, *41*, 616-623

Briggs, T., Bertke, J.T. and Augenstein, D. (1977) *Atmospheric Benzene Emissions* (*EPA 450/3-77-029*). Prepared for US Environmental Protection Agency, Cincinnati, OH, PEDCo Environmental, Inc., p. 1-2

Bryon, P.-A., Coeur, P., Girard, R., Gentilhomme, O. and Revol, L. (1969) Acute erythromyelosis with benzene etiology (Fr.) *J. Med. Lyon*, *50*, 757-759

Burdette, W.J. and Strong, L.C. (1941) Comparison of methyl salicylate and benzene as solvents for methylcholanthrene. *Cancer Res.*, *1*, 939-941

Camp, W.E. and Baumgartner, E.A. (1915) Inflammatory reactions in rabbits with a severe leukopenia. *J. exp. Med.*, *22*, 174-193

Chang, S.S. and Peterson, R.J. (1977) Symposium: The basis of quality in muscle foods. Recent developments in the flavor of meat. *J. Food Sci.*, *42*, 298-305

Coleman, W.E., Lingg, R.D., Melton, R.G. and Kopfler, F.C. (1976) *The occurrence of volatile organics in five drinking water supplies using gas chromatography/mass spectrometry*. In: *Identification and Analysis of Organic Pollutants in Water*, Ann Arbor, MI, Ann Arbor Science, pp. 305-327

Colenutt, B.A. and Davies, D.N. (1980) The sampling and gas chromatographic analysis of organic vapours in landfill sites. *Int. J. environ. anal. Chem.*, *7*, 223-229

Colenutt, B.A. and Thorburn, S. (1980) Gas chromatographic analysis of trace hydrocarbon pollutants in water samples. *Int. J. environ. Stud.*, *15*, 25-32

Commission of the European Communities (1980) Proposal for a Council Directive amending for the sixth time Council Directive 76/769/EEC on the approximation of the laws, regulations and administrative provisions of the Member States relating to restrictions on the marketing and use of certain dangerous substances and preparations (benzene). *Off. J. Eur. Communities, C 285*, 2

Coombs, M.M. and Croft, C.J. (1966) Carcinogenic derivatives of cyclopenta(*a*)phenanthrene. *Nature, 210*, 1281-1282

Cornish, H.H. and Ryan, R.C. (1965) Metabolism of benzene in nonfasted, fasted, and aryl-hydroxylase inhibited rats. *Toxicol. appl. Pharmacol., 7*, 767-771

Cotruvo, J.A., Simmon, V.F. and Spanggord, R.J. (1977) Investigation of mutagenic effects of products of ozonation reactions in water. *Ann. N.Y. Acad. Sci., 298*, 124-140

Court Brown, W.M. and Doll, R. (1957) *Leukaemia and Aplastic Anaemia in Patients Irradiated for Ankylosing Spondylitis* (*Medical Research Council Special Report Series No. 295*), London, Her Majesty's Stationery Office

Court Brown, W.M. and Doll, R. (1965) Mortality from cancer and other causes after radiotherapy for ankylosing spondylitis. *Br. med. J., ii*, 1327-1332

Dean, B.J. (1969) Chemical-induced chromosome damage. *Lab. Anim., 3*, 157-174

Dean, B.J. (1978) Genetic toxicology of benzene, toluene, xylenes and phenols. *Mutat. Res., 47*, 75-97

DeGowin, R.L. (1963) Benzene exposure and aplastic anemia followed by leukemia 15 years later. *J. Am. med. Assoc., 185*, 748-751

Deichmann, W.B., MacDonald, W.E. and Bernal, E. (1963) The hemopoietic tissue toxicity of benzene vapors. *Toxicol. appl. Pharmacol., 5*, 201-224

Delore, P. and Borgomano, C. (1928) Acute leukaemia following benzene poisoning. On the toxic origin of certain acute leukaemias and their relation to serious anaemias (Fr.). *J. Med. Lyon, 9*, 227-233

Desolle, H., Albahary, C. and Philbert, M. (1963) Hormonal effects on chronic benzene toxicity in rabbits. IV. Influence of gestation on levels of red and white blood cells in female rabbits submitted or not to moderate benzene poisoning throughout pregnancy (Fr.). *Arch. Mal. prof. Med. Trav. Secur. soc., 24*, 867-879

Deutsche Forschungsgemeinschaft (1974) *Benzol am Arbeitsplatz* [*Benzene in the Work Place*], Weinheim, Verlag Chemie Gmbh

Diaz, M., Fijtman, N., Carricarte, V., Braier, L. and Diez, J. (1979) Effect of benzene and its metabolites on SCE in human lymphocytes cultures (Abstract No. 23). *In Vitro, 15*, 172

Diaz, M., Reiser, A., Braier, L. and Diez, J. (1980) Studies on benzene mutagenesis. I. The micronucleus test. *Experientia, 36*, 297-299

Di Guglielmo, G. and Iannaccone, A. (1958) Inhibition of mitosis and regressive changes of erythroblasts in acute erythropathy caused by occupational benzene poisoning. *Acta haematol., 19*, 144-147

Dobrokhotov, V.B. and Enikeev, M.I. (1977) Mutagenic effect of benzene, toluene and a mixture of these hydrocarbons in a chronic experiment (Russ.). *Gig. Sanit., 1*, 32-34

Dowty, B.J., Laseter, J.L. and Storer, J. (1976) The transplacental migration and accumulation in blood of volatile organic constituents. *Pediatr. Res., 10*, 696-701

Drew, R.T. and Fouts, J.R. (1974) The lack of effects of pretreatment with phenobarbital and chlorpromazine on the acute toxicity of benzene in rats. *Toxicol. appl. Pharmacol., 27*, 183-193

Dutkiewicz, T. and Tyras, H. (1967) A study of the skin absorption of ethylbenzene in man. *Br. J. ind. Med., 24*, 330-332

Dutkiewicz, T. and Tyras, H. (1968) The quantitative estimation of toluene skin absorption in man. *Int. Arch. Gewerbepathol. Gewerbehyg., 24*, 253-257

Engelsberg, B. and Snyder, R. (1982) Effects of benzene and other chemicals on 72 hr ^{59}Fe utilization in Swiss albino mice (Abstract). *Toxicologist, 2*, 121

Erdogan, G. and Aksoy, M. (1973) Cytogenetic studies in thirteen patients with pancytopenia and leukaemia associated with long-term exposure to benzene. *New Istanbul Contrib. clin. Sci., 10*, 230-247

Evans, W.A. and Wilcox, T. (1979) *Health Hazard Evaluation Determination Report HE 77-73-610, Velsicol Chemical Corporation, St Louis MI*, Cincinnati OH, National Institute for Occupational Safety and Health

Federation of American Societies for Experimental Biology (1979) *Evaluation of the Health Aspects of Certain Compounds Found in Irradiated Beef. Suppl. I. Further Toxicological Considerations of Volatile Compounds (ADA075399)*. Prepared for US Army Medical Research and Development Command, Bethesda, MD, Life Sciences Research Office, pp. v, vii, 1, 6-8

Fentiman, A.F., Neher, M.B., Kinzer, G.W., Sticksel, P.R., Coutant, R.W., Jungclaus, G.A., Edie, N.A., McNulty, J. and Townley, C.W. (1979) *Environmental Monitoring Benzene (PB-295 641)*. Prepared for US Environmental Protection Agency by Battelle Columbus Laboratories, Springfield, VA, National Technical Information Service, pp. 9-15, 26-110

Fishbeck, W.A., Townsend, J.C. and Swank, M.G. (1978) Effects of chronic occupational exposure to measured concentrations of benzene. *J. occup. Med., 20*, 539-542

Flodin, U., Andersson, L., Anjou, C.-G., Palm, U.-B., Vikrot, O. and Axelson, O. (1981) A case-referent study on acute myeloid leukemia, background radiation and exposures to solvents and other agents. *Scand. J. Work environ. Health, 7*, 169-178

Flury, F. (1928) Modern industrial poisoning in respect to pharmacology and toxicology (Ger.). *Naunyn-Schmiedeberg's Arch. exp. Pathol. Pharmakol., 138*, 65-82

Forni, A. and Moreo, L. (1967) Cytogenetic studies in a case of benzene leukaemia. *Eur. J. Cancer*, *3*, 251-255

Forni, A. and Moreo, L. (1969) Chromosome studies in a case of benzene-induced erythroleukaemia. *Eur. J. Cancer*, *5*, 459-463

Forni, A.M., Cappellini, A., Pacifico, E. and Vigliani, E.C. (1971a) Chromosome changes and their evolution in subjects with past exposure to benzene. *Arch. environ. Health*, *23*, 385-391

Forni, A., Pacifico, E. and Limonta, A. (1971b) Chromosome studies in workers exposed to benzene or toluene or both. *Arch. environ. Health*, *22*, 373-378

Fredga, K., Reitalu, J. and Berlin, M. (1979) *Chromosome studies in workers exposed to benzene*. In: *Genetic Damage in Man Caused by Environmental Agents*, New York, Academic Press, pp. 187-203

Fujii, T., Yokouchi, Y. and Ambe, Y. (1979) Survey and determination of trace components in air by serial mass-fragmentographic runs over the entire mass range. *J. Chromatogr.*, *176*, 165-170

Fukuda, K., Matsushita, H., Sakabe, H. and Takemoto, K. (1981) Carcinogenicity of benzyl chloride, benzal chloride, benzotrichloride and benzoylchloride in mice by skin application. *Gann*, *72*, 655-664

Funes-Cravioto, F., Kolmodin-Hedman, B., Lindsten, J., Nordenskjold, M., Zapata-Gayon, C., Lambert, B., Norberg, E., Olin, R. and Swensson, A. (1977) Chromosome aberrations and sister-chromatid exchange in workers in chemical laboratories and a rotoprinting factory and in children of women laboratory workers. *Lancet*, *ii*, 322-325

Furnas, D.W. and Hine, C.H. (1958) Neurotoxicity of some selected hydrocarbons. *Arch. ind. Health*, *18*, 9-15

Galavotti, B. and Troisi, F.M. (1950) Erythroleukaemia myelosis in benzene poisoning. *Br. J. ind. Med.*, *7*, 79-81

Gerarde, H.W. and Ahlstrom, D.B. (1966) Toxicologic studies on hydrocarbons. XI. Influence of dose on the metabolism of mono-*n*-alkyl derivatives of benzene. *Toxicol. appl. Pharmacol.*, *9*, 185-190

Gerner-Smidt, P. and Friedrich, U. (1978) The mutagenic effect of benzene, toluene and xylene studied by the SCE technique. *Mutat. Res.*, *58*, 313-316

Ghimenti, G., Galli, M. and Galli, E. (1978) Extraction and determination of solvents in biological samples. Part I. (Ital.) o *Ann. Ist Super. Sanita*, *14*, 583-587 [*Chem. Abstr.*, *92*, 175235g]

Gill, D.P. and Ahmed, A.E. (1981) Covalent binding of [^{14}C]benzene to cellular organelles and bone marrow nucleic acids. *Biochem. Pharmacol.*, *30*, 1127-1131

Gill, D.P., Kempen, R.R., Nash, J.B. and Ellis, S. (1979) Modifications of benzene myelotoxicity and metabolism by phenobarbital, SKF-525A and 3-methylcholanthrene. *Life Sci.*, *25*, 1633-1640

Girard, R. and Revol, L. (1970) The incidence of exposure to benzene in severe haemopathies. *Nouv. Rev. fr. Hematol.*, *10*, 477-484

Gofmekler, V.A. (1968) The embryotropic action of benzene and formaldehyde in experimental administration by inhalation (Russ.). *Gig. Sanit.*, *33*, 12-16

Goguel, A., Cavigneaux, A. and Bernard, J. (1967) Benzene leukaemias (Fr.). *Bull. Inst. natl Sante Rech. med.*, *22*, 421-441

Goldstein, B.D. (1977) Hematotoxicity in humans. *J. Toxicol. environ. Health, Suppl. 2*, 69-105

Goldstein, B.D., Witz, G., Javid, J., Amoruso, M.A., Rossman, T. and Wolder, B. (1982) *Muconaldehyde, a potential toxic intermediate of benzene metabolism.* In: Parke, D.V., Kocsis, J.J., Jollow, D.J., Gibson, G.G. and Witmer, C.M., eds, *Biological Reactive Intermediates. II. Chemical Mechanisms and Biological Effects*, New York, Plenum, pp. 331-340

Goldwater, L.J. (1941) Disturbances in the blood following exposure to benzol. *J. lab. clin. Med.*, *26*, 957-973

Gonasun, L.M., Witmer, C., Kocsis, J.J. and Snyder, R. (1973) Benzene metabolism in mouse liver microsomes. *Toxicol. appl. Pharmacol.*, *26*, 398-406

Gorman, R.W. and Slovin, D. (1980) *Health Hazard Evaluation Determination Report HE 79-26-674, Getty Refining and Marketing Company, Waste Water Treatment Plant, Delaware City DE*, Cincinnati, OH, National Institute for Occupational Safety and Health

Green, J.D., Leong, B.K.J. and Laskin, S. (1978) Inhaled benzene fetotoxicity in rats. *Toxicol. appl. Pharmacol*, *46*, 9-18

Green, J.D., Snyder, C.A., LoBue, J., Goldstein, B.D. and Albert, R.E. (1981a) Acute and chronic dose/response effect of benzene inhalation on the peripheral blood, bone marrow, and spleen cells of CD-1 male mice. *Toxicol. appl. Pharmacol.*, *59*, 204-214

Green, J.D., Snyder, C.A., LoBue, J., Goldstein, B.D. and Albert, R.E. (1981b) Acute and chronic dose/response effects of inhaled benzene on multipotential hematopoietic stem (CFU-S) and granulocyte/macrophage progenitor (GM-CFU-C) cells in CD-1 mice. *Toxicol. appl. Pharmacol.*, *58*, 492-503

Greenblatt, D.R., Rosman, J. and Freedman, M.L. (1977) Benzene and ethanol additive inhibition of rabbit reticulocyte heme and protein synthesis. *Environ. Res.*, *13*, 425-431

Greenburg, L., Mayers, M.R., Goldwater, L. and Smith, A.R. (1939) Benzene (benzol) poisoning in the rotogravure printing industry in New York City. *J. ind. Hyg.*, *21*, 395-420

Greene, M.H., Hoover, R.N., Eck, R.L. and Fraumeni, J.F., Jr (1979) Cancer mortality among printing plant workers. *Environ. Res.*, *20*, 66-73

Greenlee, W.F. and Irons, R.D. (1981) Modulation of benzene-induced lymphocytopenia in the rat by 2,4,5,2',4',5'-hexachlorobiphenyl and 3,4,3',4'-tetrachlorobiphenyl. *Chem.-biol. Interactions*, *33*, 345-360

Greenlee, W.F., Gross, E.A. and Irons, R.D. (1981) Relationship between benzene toxicity and the disposition of ^{14}C-labeled benzene metabolites in the rat. *Chem.-biol. Interactions*, *33*, 285-299

Hamilton-Paterson, J.L. and Browning, E. (1944) Toxic effects in women exposed to industrial rubber solutions. *Br. med. J.*, *I*, 349-352

Hammond, J.W. and Hermann, E.R. (1960) Industrial hygiene features of a petro-chemical benzene plant design and operation. *Am. Ind. Hyg. Assoc. J.*, *21*, 173-177

Hancock, E.G., ed. (1975) *Benzene and its Industrial Derivatives*, New York, John Wiley and Sons

Hanke, J., Dutkiewicz, T. and Piotrowski, J. (1961) The absorption of benzene through the skin in men (Pol.). *Med. Pracy*, *12*, 413-426

Hardell, L., Eriksson, M., Lenner, P. and Lundgren, E. (1981) Malignant lymphoma and exposure to chemicals, especially organic solvents, chlorophenols and phenoxy acids: A case-control study. *Br. J. Cancer*, *43*, 169-176

Hartwich, G. and Schwanitz, G. (1972) Chromosome studies after chronic benzene exposure (Ger.). *Dtsch. med. Wochenschr.*, *97*, 45-49

Hartwich, G., Schwanitz, G. and Becker, J. (1969) Chromosomal aberrations in a leukaemia due to benzene (Ger.). *Dtsch. med. Wochenschr.*, *94*, 1228-1229

Hektoen, L. (1916) The effect of benzene on the production of antibodies. *J. Infect. Dis.*, *19*, 69-84

Helmer, K.J. (1944) Accumulated cases of chronic benzene poisoning in the rubber industry. *Acta med. scand.*, *118*, 254-375

Hirokawa, T. and Nomiyama, K. (1962) Studies on the poisoning by benzene and its homologues. 5. Oxidation rate of benzene in the rat liver homogenate. *Med. J. Shinshu Univ.*, *7*, 29-39

Hirschfelder, A.D. and Wintemitz, M.C. (1913) Studies upon experimental pneumonia in rabbits. IV. Is there a parallelism between trypanocidal and pneumococcicidal action of drugs? *J. exp. Med.*, *17*, 666-678

Hite, M., Pecharo, M., Smith, I. and Thornton, S. (1980) Effect of benzene in the micronucleus test. *Mutat. Res.*, *77*, 149-155

Howard, P.H. and Durkin, P.R. (1974) *Sources of Contamination, Ambient Levels, and Fate of Benzene in the Environment* (*EPA 560/5-75-005*). Prepared for US Environmental Protection Agency, Syracuse, NY, Syracuse University Research Corp., pp. 10-29, 57-65

Hudak, A. and Gombosi, K. (1977) Chromosome impairment of workers in research laboratories under uncontrolled benzene exposure (Hung.). *Munkavedelem, 23*, 50-51

Hudak, A. and Ungvary, G. (1978) Embryotoxic effects of benzene and its methyl derivatives: toluene, xylene. *Toxicology, 11*, 55-63

Hunter, C.G. (1968) Solvents with reference to studies on the pharmacodynamics of benzene. *Proc. R. Soc. Med., 61*, 913-915

Hunter, D. (1978) *The Diseases of Occupations*, 6th ed., London, Hodder and Stoughton, p. 492

Hunter, F.T. (1939) Chronic exposure to benzene (benzol). II. The clinical effects. *J. ind. Hyg., 21*, 331-354

Hushon, J., Clerman, R., Small, R., Sood, S., Taylor, A. and Thoman, D. (1980) *An Assessment of Potentially Carcinogenic, Energy-Related Contaminants in Water.* Prepared for US Department of Energy and National Cancer Institute, McLean, VA, The Mitre Corporation, p. 68

IARC (1974) *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man*, Vol. 7, *Some anti-thyroid and related substances, nitrofurans and industrial chemicals*, Lyon, pp. 203-221

IARC (1977) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man*, Vol. 15, *Some Fumigants, the Herbicides 2,4-D and 2,4,5-T, Chlorinated Dibenzodioxins and Miscellaneous Industrial Chemicals*, Lyon, pp. 155-175

IARC (1979a) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 19, *Some Monomers, Plastics and Synthetic Elastomers and Acrolein*, Lyon, pp. 231-274

IARC (1979b) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 19, *Some Monomers, Plastics and Synthetic Elastomers and Acrolein*, Lyon, pp. 115-130

IARC (1979c) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 19, *Some Monomers, Plastics and Synthetic Elastomers and Acrolein*, Lyon, pp. 187-211

IARC (1979d) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 20, *Some Halogenated Hydrocarbons*, Lyon, pp. 155-178

IARC (1979e) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 20, *Some Halogenated Hydrocarbons*, Lyon, pp. 195-239

IARC (1981) *Information Bulletin on the Survey of Chemicals Being Tested for Carcinogenicity*, Lyon, No. 9, p. 130

IARC (1982a) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 27, *Some Aromatic Amines, Anthraquinones, Nitroso Compounds and Inorganic Fluorides Used in Drinking-Water and Dental Preparations*, Lyon, pp. 39-61

IARC (1982b) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 28, *The Rubber Industry*, Lyon

Ikeda, M. and Ohtsuji, H. (1971) Phenobarbital-induced protection against toxicity of toluene and benzene in rats. *Toxicol. appl. Pharmacol.*, *20*, 30-43

Ikeda, M., Ohtsuji, H. and Imamura, I. (1972) *In vivo* suppression of benzene and styrene oxidation by co-administered toluene in rats and effects of phenobarbital. *Xenobiotics*, *2*, 101-106

Infante, P.F., Rinsky, R.A., Wagoner, J.K. and Young, R.J. (1977a) Leukaemia in benzene workers. *Lancet*, *ii*, 76-78

Infante, P.F., Rinsky, R.A., Wagoner, J.K. and Young, R.J. (1977b) Benzene and leukaemia. *Lancet*, *ii*, 868-869

International Labour Office (1980) *Occupational Exposure Limits for Airborne Toxic Substances*, 2nd (rev.) ed. (*Occupational Safety and Health Series No. 37*), Geneva, pp. 48-49, 271-290

Irons, R.D. and Moore, B.J. (1980) Effect of short term benzene administration on circulating lymphocyte subpopulations in the rabbit: Evidence of a selective B-lymphocyte sensitivity. *Res. Commun. chem. Pathol. Pharmacol.*, *27*, 147-155

Irons, R.D. and Neptun, D.A. (1980) Effects of the principal of hydroxy-metabolites of benzene on microtubule polymerization. *Arch. Toxicol.*, *45*, 297-305

Irons, R.D., Heck, H.D.A., Moore, B.J. and Muirhead, K.A. (1979) Effects of short-term benzene administration on bone marrow cell cycle kinetics in the rat. *Toxicol. appl. Pharmacol.*, *51*, 399-409

Irons, R.D., Dent, J.G., Baker, T.S. and Rickert, D.E. (1980) Benzene is metabolized and covalently bound in bone marrow *in situ*. *Chem.-biol. Interactions*, *30*, 241-245

Irons, R.D., Neptun, D.A. and Pfeifer, R.W. (1982) Inhibition of lymphocyte transformation and microtubule assembly by quinone metabolites of benzene: Evidence for a common mechanism. *J. reticuloendothel. Soc.* (in press)

Ishimaru, T., Okada, H., Tomiyasu, T., Tsuchimoto, T., Hoshino, T. and Ichimaru, M. (1971) Occupational factors in the epidemiology of leukemia in Hiroshima and Nagasaki. *Am. J. Epidemiol.*, *93*, 157-165

Jenkins, L.J., Jr, Jones, R.A. and Siegel, J. (1970) Long-term inhalation screening studies of benzene, toluene, *o*-xylene, and cumene on experimental animals. *Toxicol. appl. Pharmacol.*, *16*, 818-823

Jerina, D.M. and Daly, J.W. (1974) Arene oxides: A new aspect of drug metabolism. *Science*, *185*, 573-582

Jerina, D., Daly, J., Witkop, B., Zaltzman-Nirenberg, P. and Udenfriend, S. (1968) Role of the arene oxide-oxepin system in the metabolism of aromatic substrates. I. *In vitro* conversion of benzene oxide to a premercapturic acid and a dihydrodiol. *Arch. Biochem. Biophys.*, *128*, 176-183

Jonsson, A. and Berg, S. (1980) Determination of 1,2-dibromomethane, 1,2-dichloroethane and benzene in ambient air using porous polymer traps and gas chromatographic-mass spectrometric analysis with selected ion monitoring. *J. Chromatogr.*; *190*, 97-106

JRB Associates, Inc. (1980) *Materials Balance for Benzene, Level I - Preliminary (EPA-560/13-80-014)*. Prepared for US Environmental Protection Agency, McLean, VA, p. xiii

Kaden, D.A., Hites, R.A. and Thilly, W.G. (1979) Mutagenicity of soot and associated polycyclic aromatic hydrocarbons to *Salmonella typhimurium*. *Cancer Res.*, *39*, 4152-4159

Kahn, H. and Muzyka, V. (1973) The chronic effect of benzene on porphyrin metabolism. *Work-environ. Health*, *10*, 140-143

Khan, H. and Khan, M.H. (1973) Cytogenetic studies following chronic exposure to benzene (Ger.). *Arch. Toxikol.*, *31*, 39-49

Kimura, E.T., Ebert, D.M. and Dodge, P.W. (1971) Acute toxicity and limits of solvent residue for sixteen organic solvents. *Toxicol. appl. Pharmacol.*, *19*, 699-704

Kinoshita, T., Santella, R., Pulkrabek, P. and Jeffrey, A.M. (1981) Benzene oxide: genetic toxicity. *Mutat. Res.*, *91*, 99-102

Kirschbaum, A. and Strong, L.C. (1942) Influence of carcinogens on the age incidence of leukemia in the high leukemia F strain of mice. *Cancer Res.*, *2*, 841-845

Kissling, M. and Speck, B. (1971) Chromosome aberrations in experimental benzene intoxication. *Helv. med. Acta*, *36*, 59-66

Kissling, M. and Speck, B. (1972) Further studies on experimental benzene induced aplastic anemia. *Blut*, *25*, 97-103

Kocsis, J.J., Harkaway, S., Santoyo, M.C. and Snyder, R. (1968) Dimethyl sulfoxide: interactions with aromatic hydrocarbons. *Science*, *160*, 427-428

Koizumi, A., Dobashi, Y., Tachibana, Y., Tsuda, K. and Katsunuma, H. (1974) Cytokinetic and cytogenetic changes in cultured human leukocytes and HeLa cells induced by benzene. *Ind. Health*, *12*, 23-29

Koljkowsky, P. (1969) Indicator-tube method for the determination of benzene in air. *Analyst*, *94*, 918-920

Kuna, R.A. and Kapp, R.W., Jr (1981) The embryotoxic/teratogenic potential of benzene vapor in rats. *Toxicol. appl. Pharmacol.*, *57*, 1-7

Laerum, O.D. (1973) Reticulum cell neoplasms in normal and benzene treated hairless mice. *Acta pathol. microbiol. scand., Sect. A.*, *81*, 57-63

Lange, A., Smolik, R., Zatonski, W. and Szymanska, J. (1973a) Serum immunoglobulin levels in workers exposed to benzene, toluene and xylene. *Int. Arch. Arbeitsmed.*, *31*, 37-44

Lange, A., Smolik, R., Zatonski, W. and Glazman, H. (1973b) Leukocyte agglutinins in workers exposed to benzene, toluene and xylene. *Int. Arch. Arbeitsmed.*, *31*, 45-50

Latta, J.S. and Davies, L.T. (1941) Effects on the blood and hemopoietic organs of the albino rat of repeated administration of benzene. *Arch. Pathol.*, *31*, 56-67

Lauwerys, R. (1979) *Industrial Health and Safety. Human Biological Monitoring of Industrial Chemicals. 1. Benzene*, Luxembourg, Commission of the European Communities

Lazarew, N.W., Brussilowskaja, A.J., Lawrow, J.N. and Lifschitz, F.B. (1931) Cutaneous permeability for petroleum ether and benzene (Ger.). *Arch. Hyg. Bakteriol.*, *106*, 112-122

Lebowitz, H., Brusick, D., Matheson, D., Jagannath, D.R., Reed, M., Goode, S. and Roy, G. (1979) Commonly used fuels and solvents evaluated in a battery of short-term bioassays (Abstract No. Eb-8). *Environ. Mutagens*, 1, 172-173

Lee, E.W., Kocsis, J.J. and Snyder, R. (1974) Acute effect of benzene on ^{59}Fe incorporation into circulating erythrocytes. *Toxicol. appl. Pharmacol.*, *27*, 431-436

Lee, E.W., Kocsis, J.J. and Snyder, R. (1981) The use of ferrokinetics in the study of experimental anemia. *Environ. Health Perspect.*, *39*, 29-37

Lignac, G.O.E. (1932) Benzene leukaemia in humans and albino mice (Ger.). *Krankheitsforsch.*, *9*, 403-453

Longacre, S.L., Kocsis, J.J. and Snyder, R. (1980) *Benzene metabolism and toxicity in CD-1, C57/B6 and DBA/2N mice.* In: Coon, M.J., Conney, A.H., Estabrook, R.W., Gelboin, H.V., Gillette, J.R. and O'Brien, P.J., eds, *Microsomes, Drug Oxidations and Chemical Carcinogenesis*, New York, Academic Press, pp. 897-902

Longacre, S.L., Kocsis, J.J., Witmer, C.M., Lee, E.W., Sammett, D. and Snyder, R. (1981a) Toxicological and biochemical effects of repeated administration of benzene in mice. *Toxicol. environ. Health*, *7*, 223-237

Longacre, S.L., Kocsis, J.J. and Snyder, R. (1981b) Influence of strain differences in mice on the metabolism and toxicity of benzene. *Toxicol. appl. Pharmacol.*, *60*, 398-409

Ludwig, H. and Werthemann, A. (1962) Benzene myelopathy (Ger.). *Schweiz. med. Wochenschr.*, *13*, 378-384

Lutz, W.K. and Schlatter, C. (1977) Mechanism of the carcinogenic action of benzene: Irreversible binding to rat liver DNA. *Chem.-biol. Interactions*, *18*, 241-245

Lyapkalo, A.A. (1973) Genetic activity of benzene and toluene (Russ.). *Gig. Tr. prof. Zabol.*, *17*, 24-28

Lyon, J.P. (1976) Mutagenicity studies with benzene (Abstract). *Diss. Abstr. Int. B.*, *36*, 5537

Maffett, P.A., Doherty, T.F. and Monkman, J.L. (1956) A direct method for the collection and determination of micro amounts of benzene or toluene in air. *Am. ind. Hyg. Assoc. J.*, *17*, 186-188

Mallory, T.B., Gall, E.A. and Brickley, W.J. (1939) Chronic exposure to benzene (benzol). III. The pathologic results. *J. ind. Hyg. Toxicol.*, *21*, 355-377

Maltoni, C. and Scarnato, C. (1979) First experimental demonstration of the carcinogenic effects of benzene. Long-term bioassays on Sprague-Dawley rats by oral administration. *Med. Lav.*, *70*, 352-357

Markel, H.L., Jr and Elesh, W.E. (1979) *Health Hazard Evaluation Determination Report No. 78-57-579, Amoco Texas Refining Company Texas City TX*, Cincinnati, OH, National Institute for Occupational Safety and Health

Martin, B.E., Clark, T., Bumgarner, J. and Evans, G.F. (1980) *Ambient Air Monitoring for Benzene. 24-Hour Integrated Sampling in Six Cities (EPA 600/4-80-027)*, Research Triangle Park, NC, US Environmental Protection Agency

Matsumoto, N., Iijima, S. and Katsunuma, H. (1975) Effect of benzene on fetal growth with special reference to the different stages of development in mice. *Congenital Anomalies*, *15*, 47-58

McMichael, A.J., Spirtas, R., Kupper, L.L. and Gamble, J.F. (1975) Solvent exposure and leukemia among rubber workers: An epidemiologic study. *J. occup. Med.*, *17*, 234-239

McQuilkin, S.D. (1980) *Health Hazard Evaluation Determination Report No. HE78-102-677, Continental Columbus Corporation, Columbus, WI*, Cincinnati, OH, National Institute for Occupational Safety and Health

Messerschmitt, J. (1972) Bone marrow aplasias during pregnancy (Fr.). *Nouv. Rev. fr. Hematol.*, *12*, 15-26

Meyne, J. and Legator, M.S. (1978) Cytogenetic analysis after an acute intraperitoneal exposure of mice to benzene. *Mamm. Chromosomes Newsl.*, *19*, 38

Meyne, J. and Legator, M.S. (1980) Sex-related differences in cytogenetic effects of benzene in the bone marrow of Swiss mice. *Environ. Mutagens*, *2*, 43-50

Michon, S. (1965) Disturbances of menstruation in women working in an atmosphere polluted with aromatic hydrocarbons (Pol.). *Pol. Tyg. Lek.*, *20*, 1648-1649

Mikhailova, I.M., Kobyets, G.P., Lyubomudrov, V.E. and Braga, G.F. (1971) The influence of occupational factors on diseases of the female reproductive organs (Russ.). *Pediatr. Akush. Ginekol.*, *33*, 56-58

Moeschlin, S. and Speck, B. (1967) Experimental studies on the mechanism of action of benzene on the bone marrow (radioautographic studies using ^{3}H-thymidine). *Acta haematol.*, *38*, 104-111

Monson, R.R. and Nakano, K.K. (1976) Mortality among rubber workers. I. White male union employees in Akron, Ohio. *Am. J. Epidemiol.*, *103*, 284-296

Morimoto, K. (1976) Analysis of combined effects of benzene with radiation on chromosomes in cultured human leukocytes. *Jpn. J. ind. Health (Sangyo Igaku), 18*, 23-34

Morimoto, K. and Wolff, S. (1980) Increase of sister chromatid exchanges and perturbations of cell division kinetics in human lymphocytes by benzene metabolites. *Cancer Res., 40*, 1189-1193

Murray, F.J., John, J.A., Rampy, L.W., Kuna, R.A. and Schwetz, B.A. (1979) Embryotoxicity of inhaled benzene in mice and rabbits. *Am. ind. Hyg. Assoc. J., 40*, 993-998

Nagata, T., Kageura, M. and Totoki, K. (1978) Mass fragmentographic determination of the kerosene components in biological materials (Jpn.). *Koenshu-Iyo Masu Kenkyukai, 3*, 77-82 [*Chem. Abstr., 92*, 192082X]

National Institute for Occupational Safety and Health (1974) *Criteria for a Recommended Standard ... Occupational Exposure to Benzene (HEW Publication No. (NIOSH) 74-137)*, Washington DC, US Department of Health, Education and Welfare, pp. 29-46, 57-64, 83-93

National Institute for Occupational Safety and Health (1980) *Summary of NIOSH Recommendations for Occupational Health Standards*, Rockville, MD

National Research Council (1977) *Drinking Water and Health*, Washington DC, National Academy of Sciences, pp. 688-691, 830

National Research Council (1980) *Drinking Water and Health*, Vol. 3, Washington DC, National Academy Press, pp. 80-86, 261-262

Nawrot, P.S. and Staples, R.E. (1979) Embryofetal toxicity and teratogenicity of benzene and toluene in the mouse (Abstract). *Teratology, 19*, 41A

Neukomm, S. (1962) Co-carcinogenic action of various fractions of tobacco smoke. *Acta unio int. cancrum, 18*, 33-36

NIH/EPA Chemical Information System (1980) *C-13 NMR Spectral Search System* and *Mass Spectral Search System*, Washington DC, CIS Project, Information Sciences Corporation

Nissen, N.I. and Soeborg Ohlsen, A. (1953) Erythromyelosis (morbus di Guglielmo). Review and report of a case in a benzene (benzol) worker. *Acta med. scand., 145*, 56-71

Nomiyama, K. (1962) Studies on the poisoning by benzene and its homologues. (6) Oxidation rate of benzene and benzene poisoning. *Med. J. Shinshu Univ., 7*, 41-48

Nomiyama, K. and Minai, M. (1969) Studies on poisoning by benzene and its homologues. (11) Strain difference in susceptibility to benzene in mice. *Ind. Health, 7*, 54-65

Nomiyama, K. and Nomiyama, H. (1974a) Respiratory retention, uptake and excretion of organic solvents in man. Benzene, toluene, *n*-hexane, trichloroethylene, acetone, ethyl acetate and ethyl alcohol. *Int. Arch. Arbeitsmed., 32*, 75-83

Nomiyama, K. and Nomiyama, H. (1974b) Respiratory elimination of organic solvents in man. Benzene, toluene, *n*-hexane, trichloroethylene, acetone, ethyl acetate and ethyl alcohol. *Int. Arch. Arbeitsmed.*, *32*, 85-91

Nylander, P.-O., Olofsson, H., Rasmuson, B. and Svahlin, H. (1978) Mutagenic effects of petrol in *Drosophila melanogaster*. I. Effects of benzene and 1,2-dichloroethane. *Mutat. Res.*, *57*, 163-167

Oblas, D.W., Dugger, D.L. and Lieberman, S.I. (1980) The determination of organic species in the telephone central office ambient. *Institute of Electrical and Electronics Engineers Transactions on Components, Hybrids, and Manufacturing Technology*, *3*, 17-20

Ochsner, J.C., Blackwood, T.R. and Zeagler, L.D. (1979) *Status Assessment of Toxic Chemicals: Benzene* (*EPA600/2-79-210d*). Prepared for US Environmental Protection Agency, Dayton, OH, Monsanto Research Corp., Austin, TX, Radian Corp.

Oesch, F., Bentley, P. and Glatt, H.R. (1977) *Epoxide hydratase: Purification to apparent homogeneity as a specific probe for the relative importance of epoxides among other reactive metabolites*. In: Jollow, D.J., Kocsis, J.J., Snyder, R. and Vainio, H., eds, *Biological Reactive Intermediates: Formation, Toxicity and Inactivation*, New York, Plenum, pp. 180-206

Oldham, R.G., Spraggins, R.L., Parr, J.L. and Lee, K.W. (1979) *Analysis of organics in ambient air*. In: Frederick, E.R., ed., *Proceedings of a Special Conference on Control Specific (Toxic) Pollution*, Pittsburgh, PA, Air Pollution Control Association, pp. 130-149

Ott, M.G., Townsend, J.C., Fishbeck, W.A. and Langner, R.A. (1978) Mortality among individuals occupationally exposed to benzene. *Arch. environ. Health*, *33*, 3-9

Parke, D.V. and Williams, R.T. (1953a) The metabolism of benzene containing [$^{14}C_1$]benzene. *Biochem. J.*, *54*, 231-238

Parke, D.V. and Williams, R.T. (1953b) The metabolism of benzene. (a) The formation of phenylglucuronide and phenylsulphuric acid from [^{14}C]benzene. (b) The metabolism of [^{14}C]phenol. *Biochem. J.*, *55*, 337-340

Parmentier, R. and Dustin, P., Jr (1948) Early effects of hydroquinone on mitosis. *Nature*, *161*, 527-528

Pavlenko, G.I., Domshlak, M.G., Chirkova, E.M. and Katoeova, L.D. (1979) Results of studying the mutagenic activity of benzene (Russ.). *Toksikol. Nov. Prom. Khim. Veshchestv*, *15*, 30-33, 145-150

Pereira, W.E. and Hughes, B.A. (1980) Determination of selected volatile organic priority pollutants in water by computerized gas chromatography-quadrupole mass spectrometry. *J. Am. Water Works Assoc.*, *72*, 220-230

Pfeifer, R.W. and Irons, R.D. (1981) Inhibition of lectin-stimulated lymphocyte agglutination and mitogenesis by hydroquinone: Reactivity with intracellular sulfhydryl groups. *Exp. mol. Pathol.* *35*, 189-198

Philip, P. and Krogh Jensen, M. (1970) Benzene induced chromosome abnormalities in rat bone marrow cells. *Acta pathol. microbiol. scand. Sect. A, 78*, 489-490

Picciano, D. (1979) Cytogenetic study of workers exposed to benzene. *Environ. Res., 19*, 33-38

Pinigina, I.A. and Mal'tseva, N.M. (1978) Determination of benzene in the blood (Russ.). *Gig. Sanit., 12*, 67-71 [*Chem. Abstr., 90*, 97238c]

Pollini, G. and Biscaldi, G.P. (1976) Persistence of karyotype alterations in lymphocytes 10 years after benzene poisoning (Ital.). *Med. Lav., 67* (Suppl. 5), 465-472

Pollini, G. and Bicaldi, G.P. (1977) Investigation of karyotype in the lymphocytes of subjects with benzene haemopathy twelve years after poisoning (Ital.). *Med. Lav., 68*, 308-312

Pollini, G. and Colombi, R. (1964a) Damage to bone-marrow chromosomes in benzolic aplastic anaemia (Ital.). *Med. Lav., 55*, 241-255

Pollini, G. and Colombi, R. (1964b) Chromosomal damage in lymphocytes during benzene haemopathy (Ital.). *Med. Lav., 55*, 641-655

Pollini, G., Strosselli, E. and Colombi, R. (1964) The relationship between chromosomal alterations in haemopoietic cells and the severity of benzene haemopathy (Ital.) *Med. Lav., 55*, 735-751

Pollini, G., Biscaldi, G.P. and Robustelli della Cuna, G. (1969) Chromosome changes in lymphocytes five years after benzene haemopathy (Ital.). *Med. Lav., 60*, 743-758

Purcell, W.P. (1978) *Benzene*. In: Kirk, R.E. and Othmer, D.F., eds, *Encyclopedia of Chemical Technology*, 3rd ed., Vol. 3, New York, John Wiley and Sons, pp. 744-771

Rainaford, S.G. and Lloyd Davies, T.A. (1965) Urinary excretion of phenol by men exposed to vapour of benzene: A screening test. *Br. J. ind. Med., 22*, 21-26

Randolph, K.B., Kay, L.B. and Smith, R.C., Jr (1979) *Characterization of preparation plant wastewaters.*. In: Rogers, S.E. and Lemmon, A.W., Jr, eds, *Proceedings: Symposium on Coal Cleaning to Achieve Energy and Environmental Goals (EPA 600/7-79-098b)*. Prepared for US Environmental Protection Agency, Columbus, OH, Battelle Columbus Laboratories, pp. 824-855

Rickert, D.E., Baker, T.S., Bus, J.S., Barrow, C.S. and Irons, R.D. (1979) Benzene disposition in the rat after exposure by inhalation. *Toxicol. appl. Pharmacol., 49*, 417-423

Rinsky, R.A., Young, R.J. and Smith, A.B. (1981) Leukemia in benzene workers. *Am. J. ind. Med., 2*, 217-245

Rosenkranz, H.S. and Leifer, Z. (1980) *Determining the DNA-modifying activity of chemicals using DNA-polymerase-deficient* Escherichia coli. In: de Serres, F.J. and Hollaender, A., eds, *Chemical Mutagens. Principles and Methods for their Detection*, Vol. 6, New York, Plenum, pp. 109-147

Rozman, C., Woessner, S. and Saez-Serrania, J. (1968) Acute erythromyelosis after benzene poisoning. *Acta haematol., 40*, 234-237

Rushton, L. and Alderson, M. (1980) the influence of occupation on health - some results from a study in the UK oil industry. *Carcinogenesis, 1*, 739-743

Rushton, L. and Alderson, M.R. (1981) A case-control study to investigate the association between exposure to benzene and deaths from leukaemia in oil refinery workers. *Br. J. Cancer, 43*, 77-84

Sammett, D., Lee, E.W., Kocsis, J.J. and Snyder, R. (1979) Partial hepatectomy reduces both metabolism and toxicity of benzene. *J. Toxicol. environ. Health, 5*, 785-792

Santesson, C.G. (1897) Chronic poisoning with coal tar benzene; four deaths. Clinical and pathological-anatomical observations of several colleges and illustrating animal experiments (Ger.). *Arch. Hyg. (Munchen), 31*, 336-376

Sato, A. and Nakajima, T. (1979) Dose-dependent metabolic interaction between benzene and toluene *in vivo* and *in vitro*. *Toxicol. appl. Pharmacol., 48*, 249-256

Sato, T., Fukuyama, T., Suzuki, T. and Yoshikawa, H. (1963) 1,2-Dihydro-1,2-dihydroxybenzene and several other substances in the metabolism of benzene. *J. Biochem. (Tokyo), 53*, 23-27

Savilahti, M. (1956) More than 100 cases of benzene intoxication in a shoe factory: Observations on hematological symptoms and course of the process in one year (Ger.). *Arch. Gewerbepathol. Gewerbehyg., 15*, 147-157

Schrenk, H.H., Yant, W.P., Pearce, S.J., Patty, F.A. and Sayers, R.R. (1941) Absorption, distribution and elimination of benzene by body tissues and fluids of dogs exposed to benzene vapor. *J. ind. Hyg. Toxicol., 23*, 20-34

Selling, L. (1916) Benzol as a leucotoxin. Studies on the degeneration and regeneration of the blood and hematopoietic organs. *Johns Hopkins Hosp. Rep., 17*, 83-142

Sellyei, M. and Kelemen, E. (1971) Chromosome study in a case of granulocytic leukemia with 'pelgerisation' 7 years after benzene pancytopenia. *Eur. J. Cancer, 7*, 83-85

Shackelford, W.M. and Keith, L.H. (1976) *Frequency of Organic Compounds Identified in Water (EPA-600/4-76-062)*, Athens, GA, US Environmental Protection Agency, pp. 37, 63-65

Shahin, M.M. and Fournier, F. (1978) Suppression of mutation induction and failure to detect mutagenic activity with Athabasca tar sand fractions. *Mutat. Res., 58*, 29-34

Sherwood, R.J. and Carter, F.W.G. (1970) The measurement of occupational exposure to benzene vapour. *Ann. occup. Hyg., 13*, 125-146

Simonds, J.P. and Jones, H.M. (1915) The effect of injections of benzol upon the production of antibodies. *J. med. Res., 33*, 197-209

Siou, G., Sourdeix, D. and Doinel, A. (1980) Mutagenic effects of benzene. 2. Influence of castration on male mouse susceptibility to the mutagenic effects of benzene (Fr.). *Cah. Notes doc.*, *101*, 531-534

Smolik, R., Grzybek-Hryncewicz, K., Lange, A. and Zatonski, W. (1973) Serum complement level in workers exposed to benzene, toluene and xylene. *Int. Arch. Arbeitsmed.*, *31*, 243-247

Snyder, C.A., Goldstein, B.D., Sellakumar, A., Albert, R.E. and Laskin, S. (1978a) The toxicity of inhaled benzene (Abstract No. 106). *Toxicol. appl. Pharmacol.*, *45*, 265

Snyder, C.A., Goldstein, B.D., Sellakumar, A., Wolman, S.R., Bromberg, I., Erlichman, M.N. and Laskin, S. (1978b) Hematotoxicity of inhaled benzene to Sprague-Dawley rats and AKR mice at 300 ppm. *J. Toxicol. environ. Health*, *4*, 605-618

Snyder, C.A., Goldstein, B.D., Sellakumar, A., Bromberg, I., Laskin, S. and Albert, R.E. (1980) The inhalation toxicology of benzene: incidence of hematopoietic neoplasms and hematotoxicity in AKR/J and C57BL/6J mice. *Toxicol. appl. Pharmacol.*, *54*, 323-331

Snyder, R. and Kocsis, J.J. (1975) Current concepts of chronic benzene toxicity. *Crit. Rev. Toxicol.*, *3*, 265-288

Snyder, R. and Remmer, H. (1979) Classes of hepatic microsomal mixed function oxidase inducers. *Pharmacol. Ther.*, *7*, 203-244

Snyder, R., Lee, E.W. and Kocsis, J.J. (1978c) Binding of labeled benzene metabolites to mouse liver and bone marrow. *Res. Commun. chem. Pathol. Pharmacol.*, *20*, 191-194

Snyder, R., Longacre, S.L., Witmer, C.M., Kocsis, J.J., Andrews, L.S. and Lee, E.W. (1981) *Biochemical toxicology of benzene.* In: Hodgson, E., Bend, J.R. and Philpot, R.M., eds, *Reviews in Biochemical Toxicology*, Vol. 3, New York, Elsevier/North-Holland Publishing Co., pp. 123-153

Snyder, R., Sammett, D., Witmer, C. and Kocsis, J.J. (1982) *An overview of the problem of benzene toxicity and some recent data on the relationship of benzene metabolism to benzene toxicity.* In: Tice, R., Costa, D. and Schaich, K., eds, *The Genotoxic Effects of Airborne Agents*, New York, Plenum, pp. 225-240

Srbova, J., Teisinger, J. and Skramovsky, S. (1950) Absorption and elimination of inhaled benzene in man. *Arch. ind. Hyg.*, *2*, 1-8

Sridharan, B.N., Batra, B.K. and Savkur, L.D. (1963) The effect of a carcinogen on the progeny of treated mice. *Indian J. Pathol. Bacteriol.*, *6*, 26-33

Suta, B.E. (1980) *Definition of Population-at-Risk to Environmental Toxic Pollutant Exposures*, Vol. II, Appendix C, *Human Population Exposures to Atmospheric Benzene*. Prepared for US Environmental Protection Agency, Washington DC, pp. 22, 34, 55, 63

Suyama, Y., Katagiri, Y. and Saiki, Y. (1980) Photochemical pollution (2-2). Measurements of aromatic hydrocarbons near intersections and nonmethane hydrocarbons on highways (Jpn.). *Kanogawa-ken Kogai Senta Nempa*, *11*, 25 [*Chem. Abstr.*, *93*, 172772q]

Tabershaw, I.R. and Lamm, S.H. (1977) Benzene and leukaemia. *Lancet*, *ii*, 867-868

Tanooka, H. (1977) Development and applications of *Bacillus subtilis* test systems for mutagens, involving DNA-repair deficiency and suppressible auxotrophic mutations. *Mutat. Res.*, *42*, 19-32

Tareeff, E.M., Kontchalovskaya, N.M. and Zorina, L.A. (1963) Benzene leukemias. *Acta unio int. Cancrum*, *19*, 751-755

Tatrai, E., Rodics, K. and Ungvary, G. (1980) Embryotoxic effects of simultaneously applied exposure of benzene and toluene. *Folia morphol.*, *28*, 286-289

Teisinger, J., Bergerova-Fiserova, V. and Kudrna, J. (1952) The metabolism of benzene in man (Pol.). *Pracov. Lek.*, *4*, 175-188

Thorburn, S. and Colenutt, B.A. (1979) A gas chromatographic comparison of volatile organic compounds in urban and rural atmospheres. *Int. J. environ. Stud.*, *13*, 265-271

Thorpe, J.J. (1974) Epidemiologic survey of leukemia in persons potentially exposed to benzene. *J. occup. Med.*, *16*, 375-382

Thorpe, J.J. (1975) Letters to the editor. Leukemia and potential benzene exposure. *J. occup. Med.*, *17*, 5-6

Tice, R.R., Costa, D.L. and Drew, R.T. (1980) Cytogenetic effects of inhaled benzene in murine bone marrow: induction of sister chromatid exchanges, chromosomal aberrations, and cellular proliferation inhibition in DBA/2 mice. *Proc. natl Acad. Sci. USA*, *77*, 2148-2152

Timbrell, J.A. and Mitchell, J.R. (1977) Toxicity-related changes in benzene metabolism *in vivo*. *Xenobiotica*, *7*, 415-423

Tough, I.M. and Court Brown, W.M. (1965) Chromosome aberrations and exposure to ambient benzene. *Lancet*, *I*, 684

Tough, I.M., Smith, P.G., Court Brown, W.M. and Hamden, D.G. (1970) Chromosome studies on workers exposed to atmospheric benzene. The possible influence of age. *Eur. J. Cancer*, *6*, 49-55

Tunek, A., Platt, K.L., Bentley, P. and Oesch, F. (1978) Microsomal metabolism of benzene to species irreversibly binding to microsomal protein and effects of modification of this metabolism. *Mol. Pharmacol.*, *14*, 920-929

Tunek, A., Schelin, C. and Jergil, B. (1979) Microsomal target proteins of metabolically activated aromatic hydrocarbons. *Chem.-biol. Interactions*, *27*, 133-144

Tunek, A., Olofsson, T. and Berlin, M. (1981) Toxic effects of benzene and benzene metabolites on granulopoietic stem cells and bone marrow cellularity in mice. *Toxicol. appl. Pharmacol., 59*, 149-156

Tyroler, H.A., Andjelkovic, D., Harris, R., Lednar, W., McMichael, A. and Symons, M. (1976) Chronic diseases in the rubber industry. *Environ. Health Perspect., 17*, 13-20

US Consumer Product Safety Commission (1981) Benzene-containing consumer products; withdrawal of proposed rule. *US Code Fed. Regul., Title 16*, Parts 1145, 1307, 1500, 1700; *Fed. Regist., 46*, (*No. 99*), pp. 27910-27911

US Department of Commerce (1981a) *US General Imports, Schedule A Commodity by Country*, FT135/December 1980, Bureau of the Census, Washington DC, US Government Printing Office, p. 2-49

US Department of Commerce (1981b) *US Exports, Schedule E Commodity by Country*, FT410/December 1980, Bureau of the Census, Washington DC, US Government Printing Office, p. 2-73

US Department of the Treasury (1981) Formulas for denatured alcohol and rum. *US Code Fed. Regul., Title 27*, Parts 21, 212; *Fed. Regist., 46* (*No. 19*), pp. 9644-9658; Bureau of Alcohol, Tobacco and Firearms

US Department of Transportation (1980) Identification numbers, hazardous wastes, hazardous substances, international descriptions, improved descriptions, forbidden materials, and organic peroxides. *US Code Fed. Regul., Title 49*, Parts 171-174, 176, 177; *Fed. Regist., 45* (*No. 101*), pp. 34560, 34596

US Environmental Protection Agency (1979) Water programs; determination of reportable quantities for hazardous substances. *US Code Fed. Regul., Title 40*, Part 117; *Fed. Regist., 44*, (*No. 169*), pp. 50766-50779

US Environmental Protection Agency (1980a) National emission standard for hazardous air pollutants; benzene emissions from maleic anhydride plants. *US Code Fed. Regul., Title 4* , Part 61; *Fed. Regist., 45* (*No. 77*), pp. 26660-26664

US Environmental Protection Agency (1980b) Hazardous waste management system: identification and listing of hazardous waste. *US Code Fed. Regul., Title 40*, Part 261; *Fed. Regist., 45* (*No. 98*), pp. 33084, 33122-33127, 33131-33133

US Environmental Protection Agency (1980c) Hazardous waste management system: identification and listing of hazardous waste. *US Code Fed. Regul., Title 40*, Part 261; *Fed. Regist., 45* (*No. 135*), pp. 47832-47834

US Environmental Protection Agency (1980d) *Ambient Water Quality Criteria for Benzene* (*EPA-440/5-80-018*), Washington DC, pp. C1-C8, C16-C35, C67-C100

US Environmental Protection Agency (1981a) Hazardous waste management system: identification and listing of hazardous waste. *US Code Fed. Regul., Title 40*, Part 261; *Fed. Regist., 46* (*No. 11*), pp. 4614-4620

US Environmental Protection Agency (1981b) National emission standard for hazardous air pollutants; benzene fugitive emissions. *US Code Fed. Regul., Title 40*, Part 61; *Fed. Regist., 46 (No. 2)*, pp. 1165-1193

US Environmental Protection Agency (1981c) Iron and steel manufacturing point source category effluent limitations guidelines, pretreatment standards and new source performance studies. *US Code Fed. Regul., Title 40*, Part 420; *Fed. Regist., 46 (No. 4)*, pp. 1858-1907

US International Trade Commission (1980) *Synthetic Organic Chemicals, US Production and Sales, 1979 (USITC Publication 1099)*, Washington DC, US Government Printing Office, pp. 15, 17

US International Trade Commission (1981) *Preliminary Report on US Production of Selected Synthetic Organic Chemicals (including Synthetic Plastics and Resin Materials), Preliminary Totals, 1980 (SOC Series C/P 81-1)*, Washington DC, pp. 1, 4, 5

US Occupational Safety and Health Administration (1980) Benzene. *US Code Fed. Regul., Title 29*, Parts 1910.19, 1910.1000, 1910.1028

USS Chemicals (1980) *Clairton Benzene - Typical Analysis*, Pittsburgh, PA, United States Steel Corporation

Uyeki, E.M., Ashkar, A.E., Shoeman, D.W. and Bisel, T.U. (1977) Acute toxicity of benzene inhalation to hemopoietic precursor cells. *Toxicol. appl. Pharmacol., 40*, 49-57

Van den Berghe, H., Louwagie, A., Broeckaert-Van Orshoven, A., David, G. and Verwilghen, R. (1979) Chromosome analysis in two unusual malignant blood disorders presumably induced by benzene. *Blood, 53*, 558-566

Van Ert, M.D., Arp, E.W., Harris, R.L., Symons, M.J. and Williams, T.M. (1980) Worker exposures to chemical agents in the manufacture of rubber tires: Solvent vapor studies. *Am. Ind. Hyg. Assoc. J., 41*, 212-219

Vigliani, E.C. (1976) Leukemia associated with benzene exposure. *Ann. N.Y. Acad. Sci., 271*, 143-151

Vigliani, E.C. and Saita, G. (1964) Benzene and leukemia. *New Engl. J. Med., 271*, 872-876

Vogel, K., Bentley, P., Platt, K.-L. and Oesch, F. (1980) Rat liver cytoplasmic dihydrodiol dehydrogenase. Purification to apparent homogeneity and properties. *J. biol. Chem., 255*, 9621-9625

Ward, J.M., Weisburger, J.H., Yamamoto, R.S., Benjamin, T., Brown, C.A. and Weisburger, E.K. (1975) Long-term effect of benzene in C57BL/6N mice. *Arch. environ. Health, 30*, 22-25

Warner, J.S. and Kenan, R.P. (1979) *Analytical Techniques for Aromatic Components in Aircraft Fuels (Report No. AFAPL-TR-79-2093)*. Prepared for US Air Force Aero Propulsion Laboratory by Battelle Columbus Laboratories, Columbus, OH, pp. 5-6, 22-24

Watanabe, G.-I. and Yoshida, S. (1970) The teratogenic effect of benzene in pregnant mice. *Acta med. biol.*, *17*, 285-291

Watanabe, T., Endo, A., Kato, Y., Shima, S., Watanabe, T. and Ikeda, M. (1980) Cytogenetics and cytokinetics of cultured lymphocytes from benzene-exposed workers. *Int. Arch. occup. environ. Health*, *46*, 31-41

Weast, A.C., ed. (1979) *CRC Handbook of Chemistry and Physics*, 60th ed., Cleveland, OH, Chemical Rubber Co., p. C-146

Weiskotten, H.G., Schwartz, S.C. and Steensland, H.S. (1915) The action of benzol. I. On the significance of myeloid metaplasia of the spleen. *J. med. Res.*, *33*, 127-140

Weiskotten, H.G., Gibbs, C.B.F., Boggs, E.O. and Templeton, E.R. (1920) The action of benzol. VI. Benzol vapor leucopenia (rabbit). *J. med. Res.*, *41*, 425-438

White, W.C. and Gammon, A.M. (1914) The influence of benzol inhalations on experimental pulmonary tuberculosis in rabbits. *Trans. Assoc. Am. Phys.*, *29*, 332-337

Wierda, D., Irons, R.D. and Greenlee, W.F. (1981) Immunotoxicity in C57BL/6 mice exposed to benzene and Aroclor 1254. *Toxicol. appl. Pharmacol.*, *60*, 410-417

Wildman, J.M., Freedman, M.L., Rosman, J. and Goldstein, B. (1976) Benzene and lead inhibition of rabbit reticulocyte heme and protein synthesis: Evidence for additive toxicity of these two components of commercial gasoline. *Res. Commun. chem. Pathol. Pharmacol.*, *13*, 473-488

Williams, R.T. (1959) *Detoxication Mechanisms*, 2nd ed., London, Chapman and Hall, pp. 188-236

Winek, C.L. and Collom, W.D. (1971) Benzene and toluene fatalities. *J. occup. Med.*, *13*, 259-261

Winternitz, M.C. and Hirschfelder, A.D. (1913) Studies upon experimental pneumonia in rabbits: Parts I-III. *J. exp. Med.*, *17*, 657-665

Withey, R.J. and Hall, J.W. (1975) The joint action of perchloroethylene with benzene or toluene in rats. *Toxicology*, *4*, 5-15

Wolf, M.A., Rowe, V.K., McCollister, D.D., Hollingsworth, R.L. and Oyen, F. (1956) Toxicological studies of certain alkylated benzenes and benzene. *Arch. ind. Health*, *14*, 387-398

Zinn, B.T., Browner, R.F., Powell, E.A., Pasternak, M. and Gardner, R.O. (1980) *The Smoke Hazards Resulting From the Burning of Shipboard Materials Used by the US Navy (NRL Report 8414)*. Prepared for the Naval Research Laboratory, Atlanta, GA, Georgia Institute of Technology

United States
Environmental Protection
Agency

Office of Air Quality
Planning and Standards
Research Triangle Park NC 27711

EPA-450/5-80-004
January 10, 1979

Air

EPA

Carcinogen Assessment Group's Final Report on Population Risk to Ambient Benzene Exposures

EPA-450/5-80-004

Carcinogen Assessment Group's Final Report on Population Risk to Ambient Benzene Exposures

by

Dr. Roy E. Albert, Chairman
Carcinogen Assessment Group

U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Air, Noise, and Radiation
Office of Air Quality Planning and Standards
Research Triangle Park, North Carolina 27711

January 10, 1979

DISCLAIMER

This report has been reviewed by the Strategies and Air Standards Division of the Office of Air Quality Planning and Standards, EPA, and approved for publication. Mention of trade names or commercial products is not intended to constitute endorsement or recommendation for use. Copies of this report are available through the Library Services Office (MD-35), U.S. Environmental Protection Agency, Research Triangle Park, N.C. 27711, or from National Technical Information Services, 5285 Port Royal Road, Springfield, Virginia 22161.

CONTENTS

TABLES

CARCINOGEN ASSESSMENT GROUP'S FINAL REPORT ON POPULATION RISK TO AMBIENT BENZENE EXPOSURES

I. Summary

There is substantial epidemiological evidence that benzene is a human leukemogen. However, no validated animal model has yet been developed for benzene as a carcinogen. There are several large series of case reports indicating a high risk of leukemia in individuals who developed aplastic anemia consequent to benzene exposure. In addition there are a number of epidemiological studies in the rubber, chemical and shoe industries that demonstrate an excess risk of leukemia associated with benzene exposure.

Three of these epidemiological studies provide enough information about exposure to benzene and the occurrence of leukemia to allow us to make crude quantitative estimates of the leukemia risk associated with current general population exposures to benzene in the United States. These studies were conducted by Infante et al, (1977), Ott, et al., (1977) and Askoy et al., (1977, 1976, 1974).

The Infante study, which showed an excess incidence of leukemia, is not yet completely analyzed by the authors. Hence, some assumptions made about the average duration and magnitude of exposures are necessary. The Ott study indicated a marginal excess myelogenous leukemia risk with

relatively well-documented exposures. The Askoy studies indicated a marked increase in non-lymphatic leukemia to individuals using benzene based adhesives in small shoe making shops, however, the exposure data in this situation was difficult to evaluate.

A linear non-threshold model was used to estimate the leukemia risk to the low average levels of about one part per billion to which the general population is exposed. The slope parameter of this model was taken as the geometric mean of the slope parameter estimates obtained from the three epidemiological studies. Using this extrapolation model, we estimated that the number of cases of leukemia per year in the general population due to ambient atmospheric benzene is about 90 with a 95% confidence interval from 34 to 235 assuming a precision of within two fold in the exposure estimate. This is from .23% to 1.62% of the total leukemia deaths in the United States based upon 1973 vital statistics.

The purpose of this calculation is to obtain a rough estimate of the carcinogenic hazard to benzene in the entire United States population. To do this lifetime averages of benzene exposure were estimated and these were combined with the non-threshold linear model of risk as a function of lifetime average exposure. In this report no attempt has been made to estimate the risks to selected sub-populations who may have greater or less than average exposure or sensitivity to benzene although it is certain that such groups exist.

II. Introduction

The Carcinogen Assessment Group (CAG) has been asked by the Office of Air Quality Planning and Standards (OAQPS) to estimate the carcinogenic risk to the United States population of ambient benzene concentrations. This type of information is useful in judging the overall contribution of benzene emissions to the national rates of cancer mortality, and will be used by OAQPS in the decision whether to regulate benzene.

As the basis for this estimation, the CAG is using three epidemiological studies that show a relationship between excess mortality due to leukemia and benzene exposure. Each of these studies have strengths and weaknesses that will be discussed, but taken together they represent convincing evidence that benzene is a human carcinogen.

To date, no clear evidence exists implicating benzene as a carcinogen from animal experiments. A study is in progress at New York University that appears to suggest that inhaled benzene is causing leukemia in rats. At the present time it is felt that it would be premature to base a risk extrapolation on this preliminary data. However, at the completion of this study the CAG will update the present risk analysis to take account of this new information.

III. General Approach to Utilizing Epidemiological Studies to Predict Lifetime Probability of Cancer Deaths Due to Benzene

As was noted in the benzene health document (Goldstein, et al., 1977), very little information exists that can be utilized to obtain a dose response relationship between benzene and leukemia in humans or animals.

However, if a number of simplifying assumptions are made, it is possible to construct crude dose response models whose parameters can be estimated using vital statistics, epidemiological studies, historical workplace benzene standards and monitoring data, and a recent environmental benzene exposure study (Mara and Lee, 1978).

A. Mathematical Model Employed

We assume that for low exposures the lifetime probability of death from leukemia may be represented by the linear equation

$$P = A+Bx$$

where A is the rate in the absence of benzene exposure and x is the average lifetime exposure to atmospheric benzene expressed in ppm. The term B is the change in the leukemia rate for each increase of one ppm of benzene in the air.

If we make the assumption that, "R", the relative risk of leukemia for benzene exposed workers compared to the general population is independent of the length or age of exposure but depends only upon the total exposure, it follows that

$$R = \frac{P_2}{P_1} \frac{A + B(x_1 + x_2)}{A + Bx_1}$$

or $RP_1 = A + B(x_1 + x_2)$

$P_1 = A + Bx_1$

so that $B = P_1(R-1)/x_2$

where: x_1 = ambient level exposure to benzene

x_2 = industrial level exposure to benzene

P_1 = the lifetime probability of dying of leukemia with no or negligible benzene exposure

To use this model estimates of R and x_2 must be obtained from the epidemiological studies. The exposure values x_1 are derived in the exposure study conducted by SRI dated May 1978 and will be discussed where they are utilized.

The estimate of the lifetime probability of death due to different types of leukemia, P_1, is discussed in detail in the next section.

B. Estimation of Lifetime Probability of Death Due to Various Forms of Leukemia for a Member of the U.S. Population

The data utilized to estimate the lifetime probability of death due to various forms of leukemia is shown in Table 1, which was taken from "Vital Statistics of the United States 1973 Volume II - Mortality Part A." The second and third columns (total deaths and total death rate in 1973) were taken from page 1-184 and 1-8, respectively and utilized to derive column four, total U.S. population in each of the age classes.

The total number of deaths in 1973 due to each of the types of leukemia listed by the 8th ICD code

204 - lymphatic

205 - myeloid

206 - monocytic

207 - other and unspecified

are shown in columns five through eight.

The age specific death rates for each type of leukemia are estimated by dividing the total number of deaths due to that type by the total number of people in that age class.

In the appendix of a 1978 CAG document on population risk due to coke ovens a method referred to as the "constant segmented model" is derived that allows one to estimate the lifetime probability of death due to a disease given the age-specific incidence rates for the disease and all sources of death. This model was employed using the data in Table 1 to obtain the lifetime probabilities of leukemia that approximate as closely as possible the type of leukemia that the relative risk estimates were based upon in each epidemiological study. These lifetime probabilities are shown in Table 2, and will be used subsequently to estimate lifetime probabilities of leukemia death for each unit of exposure to the general population for each of the epidemiological studies.

IV. Epidemiological Studies Utilized

Each of the epidemiological studies is discussed in general. The relative risks are modified in each of the studies to represent a most likely rather than a conservative lower limit as usually is the case where the primary aim of epidemiological workers is to establish with little doubt a "statistically significant" elevated relative risk. Estimates of the average lifetime exposure are also made using as much data as is available. This information is then utilized employing the previously discussed mathematical model to estimate the lifetime probability of leukemia for each unit of exposure.

A. Infante (1977)

1. Description of Infante Study (1977)

In a retrospective study of mortality in a cohort of 748 white male workers in two Ohio plants manufacturing a natural rubber cast film product, Infante et al., (1977) observed a statistically significant higher rate of leukemia than in either of two control groups. The leukemia mortality rate was 5.06 times higher than the general U.S. white male population standardized for age and time period of the cohort exposure, and 4.74 times higher than a cohort of 1447 white males employed at an Ohio fibrous-glass construction products factory. These results were based on a 75% follow-up of the vital status of the workers. A total of 160 deaths were observed and of these there were 7 leukemia deaths, four of which were acute myelogenous, one chronic myelogenous, and two monocytic leukemia.

As with virtually all epidemiological studies, the Infante study has various strengths and shortcomings. Among its strengths are; (1) the worker exposures are said to have been almost exclusively restricted to benzene, since it is used throughout the plant as the principal solvent in all major processes; (2) the individuals in the cohort all worked before 1950 and were followed until 1975, thus allowing long latency diseases to be observed, and (3) acute

myelogenous leukemia was observed, which is the same cell type of leukemia observed in other studies where workers have had known benzene exposures. The disadvantages of relying on this study for determining general population risks are: (1) the authors essentially give no estimate of worker exposures except to say that the levels were less than the prevailing recommended occupational limits at the time various monitoring surveys were made; (2) the members of the cohort study actually worked at two separate plants (Akron and St. Mary's, Ohio). Air monitoring information in the former plant is almost non-existent (Baier, 1977), and therefore the exposure to half of the members of the cohort is almost completely unknown. However, it is known (Young 1977) that the crude rates (leukemia cases/total people in the cohort) are similar in the two locations; (3) Warren et al., (1977) claimed that over 400 workers known to be exposed to low benzene levels were deliberately excluded from the cohort. In spite of these problems, it is felt that this study is the least flawed of the three utilized.

2. Estimation of the Relative Risk

In an update, published as a letter to the editor in Lancet (Benzene and Leukemia, October 14, 1977) Infante et al., note that:

(1) Sakol (1977) has supplied additional information that at least two more cases of leukemia known to exist, but not reported on death certificates, were probably in Infante's cohort;

(2) Due to a more complete follow-up, the expected number of deaths due to leukemia in their cohort was reduced from 1.38 to 1.25.

Using this supplemental information, the new relative risk due to total leukemia is estimated to be

$$R = (7+2)/1.25 = 7.20$$

3. Estimation of Average Occupational Exposure

Information about the plant benzene levels is contained in the Appendix to the testimony of Baier at the OSHA benzene hearings (Baier, 1977). From the opening of the factory in 1940 until 1946, no monitoring records were available. Following the installation of new ventilation equipment in 1946, a survey showed that levels in "most areas" in the plant ranged from 0 to 15 ppm and that all areas had less than the maximum safe limit of 100 ppm which prevailed at that time. From this information, one can guess that the average exposure to all people in the plant before 1946 is probably not much more than 100 ppm, and not less than 15 ppm.

Benzene levels were monitored after 1946 at various plant locations but they were all instantaneous samples and no reliable information is available about how many man-hours were spent at those locations or whether protective masks were worn. These are case reports of exposures to 1000 ppm for short time intervals. Since the average levels were generally close to the occupational standard, we will make the assumption that the average worker exposure was the same as the prevailing recommended occupational limits. These are tabulated below along with the time weighted average for the 36 years of the total exposure period.

Time Interval	No. of Cases	Average Exposure (ppm)	Time-Weighted Average (ppm)
1940-46	(7)	100-15	
1947	(1)	50	39.9-23.3
1948-56	(9)	35	
1957-68	(12)	25	
1969-75	(7)	10	

The actual levels to which the workers were exposed was a subject of heated debate at the OSHA benzene hearings. The CAG would like to see a realistic estimate of the population weighted average exposure and its uncertainty limits. The time-weighted averages for occupational exposure must be converted to a continuous exposure lifetime basis. It will be assumed that the maximum likely lifetime exposure would result if a worker entered the factory in 1940 and was exposed for 35 years to the occupational limit of benzene. This exposure would result in a time-weighted average of 40.36 ppm for 35 years. The least likely exposure is assumed to occur if a worker started in 1950 and was exposed to the occupational limit, which results in a time-weighted average of 23.7 ppm for 25 years. The equivalent continuous lifetime exposures corresponding to these work place exposure estimates are:

$$\text{High estimate: } 40.36 \times \frac{240}{365} \times \frac{1}{3} \times \frac{35}{70} = 4.4\text{ppm}$$

$$\text{Low estimate: } 23.7 \times \frac{240}{365} \times \frac{1}{3} \times \frac{25}{70} = 1.8\text{ppm}$$

The geometric mean of the high-low exposures, $\sqrt{4.4 \times 1.8} = 2.81$, is taken to be the best estimate of the lifetime average for workers in the cohort.

4. Estimation of Lifetime Probability of Leukemia Per Unit of Exposure

The change in the leukemia rate per lifetime average ppm in the atmosphere is derived from the previously discussed equation:

$$B = P_1 (R-1)/x_2$$

which gives us an estimate

$$B = .006732 \times (7.20-1)/2.81 = .014854$$

B. Askoy (1974, 1976, 1977)

1. Description of Askoy Studies

Askoy (1977, 1976, 1974) has reported his observations of the occurrence of leukemia and aplastic anemia cases at two medical institutions in Istanbul over a period from 1967 to 1975. He has compared the types of leukemia seen in shoe workers, who work with benzene solvents in small unventilated shops, with the types of leukemia observed in people with no known exposure to benzene. He has also tabulated the exposure duration of patients with different types of leukemia. He found that in shoe workers there were 34 cases of leukemia observed in the nine years from 1967 to 1975. Based on "official records" which show that in Istanbul there are 28,500 workers in the shoe, slipper and handbag industry, he calculates that the annual incidence

rate of leukemia is 13 per 100,000, which is significantly higher than 6 per 100,000, the rate in the general population. The calculation is based on crude rates with no age adjustment.

He also found that the types of leukemias occurring in people exposed to benzene are different than for those with no known exposure. In a sample of 50 non-exposed leukemia patients, approximately 50 percent had chronic leukemia, but in 40 benzene-exposed patients only 5 percent had chronic leukemia. Also, in the exposed group preleukemia and acute erythroleukemia accounted for 34 percent of the cases, whereas in the non-exposed group only 6% of the cases were of those types.

The concentration of benzene to which the workers were exposed was estimated only in terms of the maximum concentrations existing at the times when benzene was being used in the shops. At the OSHA benzene hearings in 1977 Dr. Askoy stated that the concentrations outside working hours ranged between 15 and 30 ppm and reached a maximum of between 150 and 210 ppm when adhesives containing benzene were being used.

2. Estimation of Relative Risk

A total of 26 patients with leukemia were observed in the 6 2/3 year period from 1966 to September 1973 in a group of 28,500 Instanbul shoe workers exposed chronically to benzene. This was felt to be an underestimate of the true number of leukemia cases among the shoe workers during the period with Askoy subsequently being aware of two additional cases. However, three of the twenty-eight total cases were lymphoblastic or lymphoid leukemia, not thought to be associated with benzene exposure. Eliminating these three cases, an estimate of the yearly incidence rate is

$$I = \frac{(26+2-3) \times 10^5}{28{,}500 \times 6.67} = 13.15 \text{ per } 100{,}000 \text{ per year}$$

The total incidence rate of leukemia in Turkey is thought to be about 2.5 to 3.0 per 100,000 Askoy (1977). However from Askoy's non-exposed patient group we estimate that 48% based on 24 out of 50 are non-lymphoblastic or lymphoid leukemia. In addition, the national rate which is based on the total population was felt by Cooke (1954) to be about twice that experienced for the relatively young group of benzene-exposed shoe workers who had a average age at diagnosis of 34.2 years. Using this information we estimate that the yearly incidence rate of non-lymphoblastic or lymphoid leukemia in the Turkish population of the same age structure as the benzene exposed shoe workers is

$$I = \frac{(2.5+3.0)}{2} \times \frac{24}{50} \times \frac{1}{2} = .66 \text{ per } 100,000$$

An estimate of the relative risk for benzene exposed shoe workers is thus

$$R = \frac{13.15}{.66} = 19.92$$

3. Estimation of Lifetime Average Exposure

It was noted that the benzene levels were 15 to 30 ppm outside working hours and 150 to 210 ppm during working hours when benzene was in use in the typical small shoe manufacturing shop. We will assume that the average working hour exposure to benzene was the geometric mean of the midpoint of the two intervals or

$$x_2 = \sqrt{\left(\frac{15 + 30}{2}\right) \times \left(\frac{150 + 210}{2}\right)} = 63.6 \text{ ppm}$$

In addition we will assume:

(1) A ten hour working day

(2) A 300 day working year

(3) An average age at the end of the observation period of 50 years

(4) An average of 9.7 years of exposure; this is the average length of exposure for the leukemia cases in Askoy's series.

These assumptions lead to a lifetime average exposure estimate of

$$x_2 = 63.6 \times \left(\frac{10}{24}\right) \times \left(\frac{300}{365}\right) \times \left(\frac{9.7}{50}\right) = 4.22 \text{ ppm}$$

4. Estimation of Lifetime Probability of Leukemia Per Unit of Exposure

The change in the leukemia rate per lifetime average ppm in the atmosphere is derived from the previously discussed equation:

$$B = P_1(R-1)/x_2$$

which gives us an estimate

$$B = .004517 \times (19.92-1)/4.22 = .020252$$

C. Ott, et al., (1977)

1. Description of Ott Study

The long-term mortality patterns and associated exposure estimation of a cohort of 594 workers exposed to benzene were reported by Ott, et al., (1977). The workers were employed in three production areas of the company, which had been in operation for varying times since 1920.

Each job category was assigned an average exposure range as accurately as the historical air monitoring data permitted. The concentrations ranged from less than 2 ppm (8-hour time weighted average) to greater than 25 ppm. The analysis covered employees with known benzene exposure who

worked from January 1, 1940 through 1973. A total of 53 employees with known exposure to arsenicals, vinyl chloride and asbestos in addition to their benzene exposure was omitted from the formal cohort of people exposed to benzene.

The benzene exposure of each person was evaluated and ex-pressed as the product of parts per million times months of exposure. For the 91 deceased people with exposure to benzene alone, 45% of them had exposures between 0 and 499 ppm-months and 35% had exposures greater than 1000 ppm-months. The results of the analysis of mortality by cause of death showed no statistically significant excess of mortality compared to the U.S. white male age-specific mortality rates. Three cases of leukemia were observed where 0.8 cases were expected, a situation of borderline statistical significance ($p<0.047$). All three were myelocytic leukemia, two of them acute, which latter is the type associated with benzene exposure of shoe workers (Askoy, 1976) and other occupations (Vigliani, .976).

2. Estimation of Relative Risk

In Ott's cohort 3 deaths due to non-lymphocytic non-monocytic leukemia were observed with only .8 expected. An estimation of the relative risk of non-lymphocytic - monocytic leukemia is thus

$$R = 3/.8 = 3.75$$

3. Estimation of Lifetime Average Exposure

Ott estimated the ppm-months of exposure of each individual in his cohort from work history data and plant hygiene benzene measurement surveys. The most complete presentation of this data is given in Ott's (1977) table 7 which is used to estimate the average level of exposure.

It is assumed that the average exposure in each of the exposure intervals is equal to the midpoint of the first two intervals and is equal to the lower limit plus 1/2 of the interval width for the open or third classification. The total average ppm-months is obtained by taking the average of the three classifications weighted by the expected value of the number of deaths in each classification giving the value

$(250 \times 65.1 + 750 \times 16.2 + 1250 \times 32.8)/(65.1 + 16.2 + 32.8)$

= 608.46 ppm x months

The average lifetime exposure is obtained by using the following assumptions:

(1) An eight hour working day

(2) A 240 day working year

(3) An average age at the end of the observation period of 65 years

which gives the lifetime estimate of

$$\left(\frac{608.46}{12}\right) \times \left(\frac{8}{24}\right) \times \left(\frac{240}{365}\right) \times \left(\frac{1}{65}\right) = .171 \text{ ppm}$$

4. Estimation of Lifetime Probability of Leukemia Per Unit of Exposure

The change in the leukemia rate per lifetime average ppm in the atmosphere is derived from the previously discussed equation:

$$B = P_1(R-1)/x_2$$

which gives us an estimate

$$B = .002884 \times (3.75-1.)/.17 = .04638$$

D. Summary of Results

The total leukemic response has been based on different classifications of leukemia for the three studies. A summary of the type of response utilized is given in Table 3. It would have been preferable to have applied a uniform method of classification for all the studies. However, due to the lack of specific detail in the presented papers this was not possible.

Even with this added source of variability the resulting slope estimates B, which have the physical meaning of the total probability of deaths due to 1 ppm of benzene in the air breathed over an individual lifetime, were remarkably consistent between studies. The geometric mean of the three estimates is

$$B = \sqrt[3]{.014854 \times .020252 \times .046380} = .024074$$

The estimated log mean is

$\log_{10}B = -1.618453$, with the estimated variance of this mean being $\sigma^2_{\log B} = .021785$

V. Estimation of Expected Number of Leukemia Deaths Due to Environmental Exposure to Benzene

The SRI in their exposure document expressed exposure to the U.S. population in two ways.

The first method assumed a static population living around the point sources and gave total ppb-person years for each of the point source classifications in Table I-1.

If exposure units in 10^6 ppb-person years are denoted as D, the expected number of leukemia deaths per year may be estimated approximately by the relationship

$$N_D = .024074 \times D \times 10^3/70.96 = .339262D$$

where .024074 is the geometric mean of the slope parameter taken from the three studies, and 70.96 is the average expected life of a randomly drawn person living in the United States based on 1973 vital statistics.

Using Table I-1 and the above equation the number of leukemia deaths per year are estimated for the various point sources and are shown in Table 4.

The second method employed to estimate exposure did not make the over-simplified assumption that the human population was static. Instead an attempt was made to follow a typical individual through a typical day in order to obtain his average exposure. The exposure estimates derived on this basis are shown in Table I-2 of the SRI document and were utilized in conjunction with the above equation to derive the estimated number of leukemia deaths per year shown in Table 5.

We note that approximately a total of 90 cases of leukemia per year could be expected due to benzene exposure. In a recent CAG document on POM's, a method was developed to obtain confidence intervals for estimates based upon the assumptions that each epidemiological study gave an unbiased estimate of the true slope parameter and the estimates were distributed log normally. Adding the additional assumption that the exposure estimates are also log normally distributed we derive the relationship that the 95% confidence interval for the log of the number of leukemia deaths per year is

$$1.953289 \pm \sqrt{.083689 + \log^2 u}$$

where we are 95% confident that the true exposure is between $(u-1) \times 100\%$ and $(u-1)^{-1} \times 100\%$ of the exposure estimate.

The confidence limits derived from this relationship for various assumed values of u are shown in Table 6.

TABLE 1 - Data Utilized to Estimate Lifetime Probability of Death Due to Various Forms of Leukemia

Age Interval	Total Deaths TD	Total Death Rate x 10^5 TDR	Total People TP=TD/TDR x10^5	Total Number of Deaths Due to ICD CODE NUMBERS 204	205	206	207
0-1	55,581	1,805.2	30.7894	17	7	3	13
1-5	10,843	79.5	136.3899	157	74	4	93
5-9	7,514	41.5	181.0602	368	64	4	125
10-14	8,468	40.6	208.5714	211	88	6	92
15-19	22,908	111.9	204.7185	174	175	8	98
20-24	26,549	146.8	180.8515	76	141	4	65
25-29	22,205	143.5	154.7387	43	194	13	50
30-34	21,512	165.7	129.8250	28	181	9	54
35-39	26,374	235.1	112.1821	28	211	11	55
40-44	40,913	355.2	115.1830	36	241	19	68
45-49	67,349	563.3	119.5615	76	327	24	96
50-54	98,667	832.8	118.4762	131	407	28	134
55-59	133,604	1,314.5	101.6387	250	502	29	196
60-64	176,973	1,943.9	91.0402	370	576	42	262
65-69	213,495	2,804.7	76.1204	477	722	49	317
70-74	241,166	4,302.7	56.0499	515	794	70	376
75-79	263,251	6,722.4	39.1603	651	730	79	357
80-84	250,985	9,777.4	25.6699	576	522	39	311
85+	284,400	17,429.4	16.3173	469	364	47	254
TOTAL			209.8344 x 10^6				

TABLE 2 - Lifetime Probability of Death in U.S. Population Due to Leukemia Type Upon Which Relative Risk in Each of the Epidemiological Studies is Based

Epidemiological Study	ICD Codes Utilized	Type of Leukemia	Lifetime Probability of Death Due to Type of Leukemia
Infante	204-207	Total Leukemia	.006732
Ott	205	Myelogenous	.002884
Askoy	205-207	Non-Lymphatic	.004517

TABLE 3 - Summary of Data Used to Estimate Lifetime Probability of a Leukemia Death Per ppm Benzene Lifetime Exposure

Study	R Relative Risk Estimate	x_2 Estimate Average Lifetime Exposure	P_1 Lifetime Probability of Leukemia	B Estimated Slope Parameter
Infante	7.20	2.81	.006732	.014854
Askoy	19.92	4.22	.004517	.020252
Ott	3.75	.17	.002884	.046380

TABLE 4 - Source Specific Benzene Caused Leukemia Deaths/Year Based on Table I-1 of SRI Benzene Exposure Document[a]

Source of Exposure	Exposure in 10^6 x ppb-Person Years	Expected Number of Benzene Caused Leukemia Deaths/Year
Chemical Manufacturing	8.5	2.88
Coke Ovens	.2	.07
Petroleum Refineries	2.5	.85
Automobile Emissions	150.0	50.89
Gasoline Service Stations	19.0	6.44
Self Service Gasoline	1.6	.54
TOTAL	181.8	61.67

[a]Mara, Susan J. and Shonh S. Lee. Assessment of Human Exposures to Atmospheric Benzene. SRI International for U.S. Environmental Protection Agency, Research Triangle Park, NC. Publication No. EPA-450/3-78-031. June 1978.

TABLE 5 - Total Exposure of People Residing in Various Locations, and Resulting Estimated Benzene-Caused Leukemia Deaths/Year - Based on Table I-2 of SRI Benzene Exposure Document[a]

Vicinity of Residence	Exposure in 10^6 ppb-Person Years	Expected Number of Benzene-Caused Leukemia Deaths/Year
Chemical Manufacturing	10.0	3.39
Coke Ovens	.2	.07
Petroleum Refineries	4.5	1.53
Urban Areas	250.0	84.80
TOTAL	264.7	89.80

[a]Mara, Susan J. and Shonh S. Lee. Assessment of Human Exposures to Atmospheric Benzene. SRI International for U.S. Environmental Protection Agency, Research Triangle Park, NC. Publication No. EPA-450/3-78-031.

TABLE 6 - Confidence Limits on Total Benzene Caused Leukemia Deaths Per Year (Assumes "One-Hit" Model is the True Dose Response Relationship)

Level of Precision Assumed for Exposure Estimate (U-1) x 100%	95% Confidence Limits Lower Limit	Upper Limit
0%*	46.1	174.8
10%	45.8	176.0
50%	41.2	195.9
100%	34.3	234.9
1000%	7.5	1081.7

*Assumes no error in exposure estimate.

Bibliography

Cancer Risk of Benzene

Askoy, M. (1977) "Testimony of Mazaffer Askoy, M.D. to Occupational Safety and Health Administration, U.S. Department of Labor," July 13, 1977, 13 pp.

Askoy, M. (1976) "Types Leukemia in Chronic Benzene Poisoning. A Study in Thirty-Four Parts," Acta Haemat, 55, 65-72.

Askoy, M., Erdem, S., and Dincol, G. (1974) "Leukemia in Shoe-Workers Exposed Chronically to Benzene," Blood, 44 (6), 837-841.

Askoy. M., Erden, S., Dincol, K., Kepyuksel, T., and Dincol, G. (1974) "Chronic Exposure to Benzene as a possible Contributary Etiologic Factor in Hodgkin's Disease," Blut, 28, 293-298.

Baier, E.J. "Statement of the National Institutes of Occupational Safety and Health," Exhibit 84A, OSHA Benzene Hearings, July 19-Aug 10, 1977.

Cooke, J.V. (1954) "The Occurrence of Leukemia," Blood 9, 340.

Goldstein, B.D., Snyder, C.A., Snyder, R., and R. and S. R. Wolman, "Review of Benzene Toxicity," Prepared for EPA, August 18, 1977.

Infante, P.F., Rinsky, R.A., Wagoner, J.K., and Young, R.J. (1977) "Benzene and Leukaemia (Letter to the Editor)," Lancet, 867-869, October 22, 1977.

Infante, P.F., Rinsky, R.A., Wagoner, J.K., and Young, R.J. (1977) "Leukemia in Benzene Workers," Lancet, 76-78, July 9, 1977.

Ott, M.G., Townsend, J.C., Fishback, W.A., and Langer, R.A. (1977) "Mortality Among Individuals Occupationally Exposed to Benzene," Exhibit 154, OSHA Benzene Hearings, July 9-August 10, 1977.

Sakol, M.J. (1977) "Testimony of Marvin J. Sakol, M.D., to Occupational Safety and Health Administration, U.S. Department of Labor".

VII. APPENDIX

Mutagenic Risks of Benzene Exposure

Summary

In addition to the risk from leukemia, benzene exposure is also likely to induce inherited mutations. The magnitude of this risk can not be estimated because of the uncertain quantitative relationship between heritable mutations and chromosome aberrations which have been consistently observed in exposed workers.

Review of Experimental Results

Benzene was found to be non-mutagenic in the Ames' test for point mutational effects (Simmon et al., 1977; Shahin, 1977; and Lyon, 1975). However, it is possible that a human metabolic activation enzyme system or a mammalian body fluid activation system would cause it to be mutagenic.

Somatic chromosomal aberrations have been demonstrated in animals and humans. In rabbits, Kissling and Speck, 1971 reported the induction of cytogenetic damage *in vivo* by subcutaneous injection of 0.2 ng/kg day benzene. The frequency of metaphase spreads showing aberrations (mostly gaps and breaks) increased from 5.9% to 57.8% after an average exposure interval of 18 weeks. Two months after discontinuance of the benzene treatment, cytogenetic damage was still observed.

Dobrokhotov (1972) exposed rats to 0.2 g/kg day benzene and 0.8 g/kg day toluene, and found similar rates of chromosomal aberrations in the two chemicals given separately, and an additive effect when given together. Chromatic deletions in metaphase chromosomes of bone-marrow cells have been found in rats given single doses of benzene subcutaneously at 2 ml/kg (Philip and Jensen, 1970). Deletions have also been observed in rats given benzene at 1g/kg day, subcutaneously, for 12 days.

A dominant lethal and in vivo cytogenetics combined test has been performed with rats dosed intraperitoneally with 0.5 ml/kg benzene (Lyon; 1975). No dominant lethality was found but increases were found in chromatic and chromosomal aberrations. Lyon (1975) also found increased micronuclei counts 6 hours after the final dosing of rats at 0.05 and 0.25 ml/kg/day after two days of dosing intraperitoneally.

In patients with benzene-induced aplastic anemia, lymphocyte chromosome damage has been found (Pollini and Colombi, 1964). Polini et al., (1964) later found a 70% incidence of heteroploid chromosomal patterns in the blood lymphocytes and bone marrow parenchyma cells of each of four subjects with benzene-induced blood dyscrasias. Similar patient studies of benzene exposed individuals with

persistent chromosomal alterations associated with blood dyscrasias have also been reported by others (Forni and Moreo, 1967, 1969; Hartwich et al., 1969; Khan and Khan, 1973; Sellzei and Kelemen, 1971; Forni et al., (1971); Tough and Court Brown, 1965).

Vigiliani and Forni (1969) found a significant increase of chromosomal aberrations in pheripheral lymphocytes of workers exposed to benzene, but not in those exposed to xylene and toluene. Some of these aberrations persisted for several years after recovery from benzene hemopathy. They suggested that toxicity to the bone marrow might result in cells with an abnormal number of chromosomes and that proliferation of these cells could then give rise to an advantaged leukemic clone. Forni et al., (1971) examined chromosomal aberrations in 34 workers in a rotogravure plant and compared these to 24 matched controls, and found a significantly higher number of both stable and unstable aberrations in 10 benzene-exposed workers but a number comparable to controls in all of the 24 toluene-exposed workers.

A recent report (Kilian and Daniels, 1978) on 52 workers exposed to benzene for one month to 26 years (mean of 56.6 months) found chromosomal aberrations (chromosome breaks, dicentric chromosomes, translocations and exchange figures)

in peripheral lymphocytes at 2-3 times the rates found in controls. In this study, the 8 hour average time-weighted benzene exposure was 2-3 ppm, the average concentration determined by 15 minute sampling was 25 ppm and the peak concentration was 50 ppm.

The same laboratory reported on the monitoring of 471 peripheral lymphocyte cultures from 290 Texas Division benzene workers between 1965 and 1978 (Benge et al., 1978). A group of 972 "preemployment examinees" who were judged, on the basis of the history taken at the time, to have had negligible exposure to known chromosome-breaking agents were used as controls. Rates of chromosomal abnormalities were found not to be increased in the exposed group over the control group. The time-weighted average benzene concentrations were estimated to have been below 50 ppm prior to 1972 and well below 10 ppm from 1973 to the present time.

A report by Picciano (1978) which is a further analysis of the Kilian and Daniel (1978) and Benge (1978) study comparing the information on benzene exposed individuals to a 44-person group seen for preemployment examination. Workers exposed to three different levels of benzene at less than 10 ppm for several years showed a dose response relationship. The types of aberrations detected are similar to those reported for higher benzene exposures by (Tough et

al., 1970). The workers were monitored for urinary excretion of phenol which is a primary metabolite of benzene. All workers had no detectable phenol which indicated no recent exposure to benzene. Exposures for the dose-response relationship were 0, less than 1, 1-2.5 and greater than 2.5 ppm.

Fredga et al., (1978) performed a study on 65 workers, occupationally handling motor fuels. A moderate, but statistically significant, increase in frequency of chromosome aberrations was found in road tanker drivers and industrial workers, but not in ship tanker crews and gasoline station staff. The estimated exposure dose was 50 ppm or less. The dose absorbed will be reported in a subsequent study.

Conclusions

Ample evidence exists that benzene causes chromosomal aberrations in animals and humans exposed to benzene. This evidence was reviewed above. However, since this is a somatic cell effect as opposed to a germinal cell effect it is difficult to estimate the heritable risk to future generations from such evidence. These chromosomal aberrations probably involve breaks in DNA and therefore are heritable events if they occur in the germinal cells, although the experiments to prove that point have not been decisive.

It is generally recognized that rings, dicentrics, translocations and exchange figures are heritable, but chromosome breaks could be caused from toxicity of somatic cells and therefore may not be heritable. The former lesions should be used as indicators that genetic damage to future generations may have occurred. At the current time quantitative estimates of heritable genetic damage due to benzene cannot be made from data on the frequency of somatic mutations, although this damage may be occurring at concentrations as low as 1 ppm in air.

Bibliography

Mutagenicity: Benzene

Benge, M.C., J.R. Venable, D.J. Picciano and D.J. Killian (1977) "Cytogenetic study of 290 workers exposed to benzene." Report Dow Chemical U.S.A. 1977.

Dobrokhotov, V.B. (1972) "The mutagenic influence of benzene and toluene under experimental conditions," Gig. Sanit; 37:36-39.

Forni, A., and L. Moreo (1967) "Cytogenetic studies in a case of benzene leukaemia," Eur. Jour. Cancer, 3:251-255.

Forni, A., and L. Moreo. 1969. "Chromosome studies in a case of benzene-induced erythroleukaemia." Cancer, 3:251-255.

Forni, A., et al. (1971a) "Chromosome studies in workers exposed to benzene or toluene or both," Arch. Environ. Health, 22:373-378.

Forni, A., et al. (1971b) "Chromosome changes and their evolution in subjects with past exposure to benzene," Arch. Environ. Health, 23:385-391.

Fredga, K., Reitalu, J. and Berlin, M. Chromosome studies in workers exposed to benzene. Report #771018 of the Institute of Genetics, Univ. of Lund and Department of Env. Health, University of Lund, Sweden.

Haberlandt, W., and B. Mente 1971 "Deviation on number and structure of chromosomes in industrial workers exposed to benzene," Zbl. Arbeitsmed, 21:338-341.

Hartwich, G., and G. Schwanitz (1972) "Chromosomenuntersuchungen nach chronischer Benzol-Exposition." Dtsch. Med. Wschr. 97:45-49.

Hartwich, G., et al. (1969) "Chromsome anomalies in a case of benzene leukemia." Ger. Med. Monthly, 14:449-450.

Khan, H., and M. H. Khan (1973) "Cytogenetic studies following chronic exposure to benzene," Arch. Toxikol., 31:39-49.

Kilian, D. J., and R. C. Daniel (1978) "A cytogenetic study of workers exposed to benzene in the Texas Division of Dow Chemical, U.S.A.," Feb. 27, 1978, Dow Chemical, Freeport, Texas.

Kissling, M., and B. Speck (1971) "Chromosomal aberrations in experimental benzene intoxication," Helv. Med. Acta, 36:59-66.

Koizumi, A., et al. (1974) "Cytokinetic and cytogenetic changes in cultured human leucocytes and HeLa cells induced by benzene," Ind. Health (Japan), 12:23-29.

Ltapkalo, A.A. (1973) "Genetic activity of benzene and toluene," Gig Tr. Prof. Zabol., 17:24-28.

Lyon, J.P. (1975) "Mutagenicity Studies with Benzene," Ph.D. Thesis, University of California

Philip, P., and M.K. Jensen. (1970) "Benzene-induced chromosome abnormalities in rat bone marrow cells," Acta. Pathol. Microbiol. Scand. Sect. A., 78:489-490.

Picciano, D. (1978) Unpublished report.

Pollini, G., and R. Colombi (1964) "Lymphocyte chromosome damage in benzene blood dyscrasia," Med. Lav. 55:641-654.

Pollini, G., et al. (1964) "Relationship between chromosomal alterations and severity of benzol blood dyscrasia," Med. Lav. 55:735-751.2

Sellyei, M., and E. Kelemen (1971) Chromosome study in a case if granulocytic leukemia with "Pelgerisation" 7 years after benzene pancytopenia. Eur. Jour. Cancer, 7:83-85.

Shahin, M.M. (1977) Unpublished results. The University Alberta, Canada. Cited in Mutation Research 47:75-97 (1978).

Simmon, V.F., et al. (1977) "Mutagenic activity of chemicals identified in drinking water." Second International Conf. on Environmental Mutagens, Edinburgh, Scotland, July 1977.

Tough, I.M., and W.M. Court Brown (1965). "Chromosome aberrations and exposure to ambient benzene, "Lancet, 1:684.

Tough, I.M., et al. (1970) "Chromosome studies in workers exposed to atmospheric benzene. The possible influence of age," Eur. Jour. Cancer, 6:49-55.

Vigliani, E.C., and A. Forni. 1969. Benzene, chromosome changes and leukemia," Jour. Occup. Med., 11:148-149.

Vigliani, E.C., and A. Forni (1976) "Benzene and leukemia," Environ. Res., 11:122-127.

Vigliani, E.C., and G. Sanita (1964) "Benzene and leukemia." N.E. Jour. Med., 271:872-876.

Warren, E.W., VanVoorhee, R.F., and Sampson, A.F., "Post Hearing Brief of API, NPRA, and Individual Member Companies Regarding the Proposed Revisec Permanent Standards for Occuopation Exposure to Benzene," OSHA Docket No. H-059 September 22, 1977.

Young, R.J. National Institute of Occupational Safety and Health, Personal Communication, October, 1977.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA-450/5-80-004		

4. TITLE AND SUBTITLE
"Carcinogen Assessment Group's Final Report on Population Risk to Ambient Benzene Exposures"

5. REPORT DATE
January 1979

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
Roy E. Albert

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
U.S. Environmental Protection Agency
Office of Research and Development
401 M Street, S.W.
Washington, D.C. 20460

10. PROGRAM ELEMENT NO.

11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS
Environmental Protection Agency
Office of Air Quality Planning and Standards
and Office of Research and Development
Research Triangle Park, N.C. 27711

13. TYPE OF REPORT AND PERIOD COVERED
Final

14. SPONSORING AGENCY CODE
EPA 200/04

15. SUPPLEMENTARY NOTES This report supercedes all findings and conclusions in the September 1978 report entitled "Carcinogen Assessment Group's Final Report on Population Risk to Ambient Benzene Exposures."

16. ABSTRACT

This report is one of three reports which were prepared by E.P.A. to determine what regulatory action should be taken by E.P.A. to control sources of atmospheric emissions of benzene. This report estimates from three epidemiological studies the leukemia risk associated with current general population exposures to benzene in the United States. These studies were conducted by Infante, et al., (1977), Ott et al., (1977), and Askoy et al., (1977, 1976, 1974). The original report has received extensive review by the interested public and E.P.A.'s Science Advisory Board. All comments received on this first report were reviewed and considered in preparation of this report.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Benzene Air pollution Populations Health effects Risk		13B

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
Unlimited	Unclassified	
	20. SECURITY CLASS (This page)	22. PRICE
	Unclassified	

EPA Form 2220-1 (9-73)

federal register

Wednesday
June 6, 1984

Part II

Environmental Protection Agency

40 CFR Part 61

National Emission Standards for Hazardous Air Pollutants; Regulation of Benzene; Response to Public Comments

ENVIRONMENTAL PROTECTION AGENCY

40 CFR Part 61

[AD-FRL-2523-7]

National Emission Standards for Hazardous Air Pollutants; Regulation of Benzene

AGENCY: Environmental Protection Agency (EPA).

ACTION: Response to public comments.

SUMMARY: The Environmental Protection Agency (EPA) listed benzene as a hazardous air pollutant under Section 112 of the Clean Air Act on June 8, 1977 (42 FR 29332). Standards were subsequently proposed for maleic anhydride process vents (45 FR 26660, April 18, 1980); ethylbenzene/styrene (EB/S) process vents (45 FR 83448, December 18, 1980); benzene fugitive emission sources (46 FR 1165, January 5, 1981); and benzene storage vessels (45 FR 83952, December 19, 1980). This Federal Register notice responds to public comments on the listing, health effects, and regulation of benzene as a hazardous air pollutant.

ADDRESSES: *Background Information Document.* The background information document (BID) may be obtained from the U.S. EPA Library (MD-35), Research Triangle Park, North Carolina 27711, telephone number (919) 541-2777. Please refer to "Response to Public Comments on EPA's Listing of Benzene Under Section 112," EPA-450/5-82-003, which contains a summary of all public comments on the health effects, listing, and regulatory approach for benzene.

Docket. Docket No. OAQPS 79-3 (Part I) contains information considered on the health effects, listing, and regulation of benzene. Other dockets contain... g public comments on the listing, health effects, and regulation of benzene are contained in Docket No. OAQPS 79-3 (Part II), for maleic anhydride plants; Docket No. A-79-27, for benzene fugitive emissions; Docket No. A-79-49, for EB/S plants; and Docket No. A-80-14, for benzene storage vessels. These dockets are available for public inspection between 8:00 a.m. and 4:00 p.m., Monday through Friday, at EPA's Central Docket Section (LE-131), West Tower Lobby, Gallery 1, 401 M Street, SW., Washington, D.C. 20460. A reasonable fee may be charged for copying.

FOR FURTHER INFORMATION CONTACT: For further information on the listing and health effects of benzene, contact Mr. Robert Kellam, Pollutant Assessment Branch, Strategies and Air Standards Division (MD-12), U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711, telephone number (919) 541-5645. For further information on the regulation of benzene, contact Mr. Gilbert H. Wood, Standards Development Branch, Emission Standards and Engineering Division (MD-13), U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711, telephone number (919) 541-5578.

SUPPLEMENTARY INFORMATION:

Overview of Benzene Regulation

This section provides background information and summarizes EPA's responses to the major public comments on the listing, health effects, and regulation of benzene. This section is intended to be an overview only. Subsequent sections and the BID contain more detailed responses to public comments.

Background

Based on studies linking occupational exposure to benzene with leukemia, EPA's general presumption that carcinogenic thresholds do not exist, the absence of a demonstrated threshold for benzene, and widespread exposure to large quantities of benzene emitted by stationary sources, EPA concluded that benzene could reasonably be anticipated to cause an increase in contracting leukemia for individuals exposed to benzene emissions from stationary sources. EPA therefore listed benzene as a hazardous air pollutant on June 8, 1977 (42 FR 29332).

Stationary sources of benzene are now estimated to emit at least 55,000 Megagrams (Mg) (about 120 million pounds) of benzene per year. The benzene sources have been divided into 12 source categories, based on technological considerations (such as control technology applicability) important in standards development. EPA decided to address the stationary source benzene problem by selecting for initial regulation five of these source categories: maleic anhydride process vents, ethylbenzene/styrene (EB/S) process vents, benzene fugitive emissions sources, benzene storage vessels, and coke oven by-product recovery plants.

EPA is collecting additional data on the remaining seven source categories to use in deciding whether or not standards development is warranted for them.

Benzene standards for four of the five source categories selected for initial regulation were proposed: maleic anhydride process vents (45 FR 26660, April 18, 1980); EB/S process vents (45 FR 83448, December 18, 1980); benzene storage vessels (45 FR 83952, December 19, 1980); and benzene fugitive emissions sources (46 FR 1165, January 5, 1981). The Agency intends to promulgate standards for benzene fugitive emission sources and propose standards for the fifth source category, coke by-product plants, in separate notices. In a third notice the Agency is withdrawing the proposed standards for maleic anhydride process vents, EB/S process vents, and benzene storage vessels, based on the conclusion that both the benzene health risks to the public from these source categories and potential reductions in health risks achievable with available control techniques are too small to warrant Federal regulatory action under section 112.

Summary of Responses to Major Comments

The primary comment received on the proposed standards was that benzene should not have been listed as a hazardous air pollutant. Commenters argued that benzene did not meet the criteria for listing under section 112 because they believe the health hazard posed by ambient levels of benzene is negligible, if not zero. Specifically, commenters, while generally agreeing with EPA that epidemiological studies have shown that a causal relationship exists between occupational benzene exposure and leukemia, maintained that the relationship had not been demonstrated at the much lower levels of benzene characteristic of the ambient air. In contending that EPA's nonthreshold presumption has been applied inappropriately in the case of benzene, commenters cited the lack of direct evidence that ambient levels pose leukamogenic risks as well as benzene research data and theoretical considerations compatible with the presence of a carcinogenic threshold for benzene.

Commenters asserted that the absence of data demonstrating that benzene reacts chemically with DNA supports the theory that benzene is likely to cause cancer by other than a direct genetic mechanism (the production of a transformed cell by direct interaction of a benzene molecule and the cellular genetic material). The nongenetic, or epigenetic, theory holds that such carcinogens must be present in sufficient quantities to induce toxic injury to the target tissue before cancer can occur. At levels below that required to cause "injury," body defense mechanisms are capable of protecting the tissues from a carcinogenic insult.

In support of a threshold for benzene, commenters maintained that benzene-induced leukemia was in most, if not all, cases preceded by evidence of injury to the blood-forming system (anemia, cytopenia, etc.). Commenters argued that because thresholds (10 to 35 ppm) exist for such effects, benzene exposure below these thresholds should not pose carcinogenic risks. Similarly, commenters cited epidemiological studies that did not show a positive correlation between benzene exposure and leukemia, as support for a risk threshold.

The EPA recognized at the time of listing that benzene at ambient levels, as with most other carcinogens, had not been demonstrated by epidemiologic studies to cause leukemia. The epidemiological methods that have successfully revealed associations between occupational exposure and cancer for substances such as benzene, asbestos, vinyl chloride, and ionizing radiation are not readily applied to the ambient environment with its increased number of confounding variables, a more diverse and mobile exposed population, a lack of consolidated medical records, and an almost total absence of historical exposure data. Given such uncertainties, EPA considers it improbable that any ambient association, short of a relationship of epidemic proportions or large increases in an extremely rare form of cancer, can be detected epidemiologically with any reasonable certainty.

Further, EPA agrees with the observations of the National Academy of Sciences (NAS) (*1*):

> In considering the possibility of thresholds for carcinogenesis, it is important to understand that there is no agent, chemical or physical, that induces a form of cancer in man that does not occur in the absence of that agent. In other words, when there is exposure to a material, we are not starting at an origin of zero cancers. Nor are we starting at an origin of zero carcinogenic agents in our environment. Thus, it is likely that any carcinogenic agent added to the environment will act by a particular mechanism on a particular cell population that is already being acted on by the same mechanism to induce cancers. This reasoning implies that only if it acted by a mechanism entirely different from that already operating on the tissue could a newly added carcinogen show a threshold in its dose response curve.

This view is consistent with evidence that any exposure may produce a change in the genetic material that can lead to cell transformation and that cancers may arise from a single transformed cell.

In addition to the support for a nonthreshold hypothesis, EPA notes the problems inherent in attempting to identify and to quantify real or practical carcinogenic thresholds. In this regard, EPA concurs with the NAS that theoretical evidence for the existence of carcinogenic thresholds must be tempered by the knowledge that the exposed human population is a "large, diverse, and genetically heterogeneous group exposed to a large variety of toxic agents. Genetic variability to carcinogenesis is well documented, and it is also known that individuals who are deficient in immunological competence (for genetic or environmental reasons) are particularly susceptible to some forms of cancer." (*1*)

For these reasons, EPA has taken the position, shared by other Federal regulatory agencies, that in the absence of sound scientific evidence to the contrary, carcinogens should be considered to pose finite health risks at any nonzero exposure levels. This nonthreshold presumption is based on the view that as little as one molecule of a carcinogenic substance may be sufficient to transform a normal cell into a cancer cell. Evidence is available from both the human and animal health literature that cancers may arise from a single transformed cell. Mutation research with ionizing radiation in cell cultures indicates that such a transformation can occur as the result of interaction with as little as a single cluster of ion pairs.

In the decision to list benzene under section 112 EPA found no reason to believe that the nonthreshold presumption did not apply to benzene. After reviewing the public comments, EPA believes that although they provide a comprehensive discussion of the scientific and theoretical support for a carcinogenic threshold for benzene, the evidence is inadequate to support a conclusion that ambient levels of benzene are without carcinogenic risk.

The EPA did not at listing and does not now believe that information such as the benzene exposure levels estimated from "negative" epidemiological studies can be regarded as the equivalent of no-effect levels. Because of the problems and uncertainties inherent in the design and conduct of such studies, they do not support the conclusion that the absence of a statistical correlation demonstrates the absence of a hazard.

While the epigenetic mechanism offers a possible explanation for the way in which cancers could arise in the absence of direct interaction with genetic material, this theory has not been substantiated by experimental evidence nor has applicability to the specific case of benzene been established beyond largely theoretical grounds.

The EPA does not agree with industry's conclusion that the absence or nondetection of covalent bonding with DNA indicates that benzene cannot directly interact with the genetic material. Evidence exists that benzene at levels as low as 1 to 2.5 ppm significantly increases chromosomal aberrations. (*2*) (*3*) Similarly, EPA does not regard as conclusive the evidence provided by commenters that leukemia or other adverse health effects do not occur in the absence of overt signs of blood toxicity. Again, studies are available demonstrating benzene-induced chromosomal aberrations following exposure to benzene at levels below those advanced as thresholds for blood toxicity.

Finally, commenters have argued that, below the benzene levels required to "injure" the blood-forming tissues, the body's defense mechanisms protect the tissues from low-level carcinogenic insults. EPA is not persuaded that such mechanisms are 100 percent effective. In addition, although the commenters do not regard chromosomal aberrations as evidence of blood toxicity, the presence of these effects indicates that benzene or an active metabolite has been able to overwhelm the protective mechanisms and enter the cellular nucleus.

In summary, EPA continues to believe that the nonthreshold presumption should apply in the case of benzene and that exposure to benzene via the ambient air should be regarded as posing carcinogenic risks. Although EPA recognizes that this finding is not without uncertainty, the Agency believes that it is consistent with the mandate of Section 112 requiring the protection of public health against air pollutants that "may reasonably be anticipated" to cause or contribute to the health effects of concern.

After reviewing the public comments, EPA also continues to believe that benzene emissions from some stationary sources represent a significant risk of leukemia to exposed populations. This judgment is based on the documented evidence that benzene is a leukemogen, on the magnitude of benzene emissions from stationary sources to the ambient air, on the observed and estimated ambient concentrations, on the proximity of large populations to emitting sources, on the estimates of the health risks to the exposed populations, and consideration of the uncertainties associated with quantitative risk estimates (including the effects of concurrent exposures to other

substances and to other benzene emissions).

Section 112 provides for the delisting of benzene only if it is found that benzene is clearly not a hazardous air pollutant. EPA judges the evidence, including that submitted by commenters, to be insufficient to support a conclusion that ambient levels of benzene do not pose carcinogenic risks or that the risks posed by ambient benzene emitted by stationary sources are insignificant. In conclusion, EPA continues to regard the listing of benzene on June 8, 1977, as appropriate and considers delisting at this time inappropriate.

A second major comment on the proposed standards contends that the individual source categories covered do not pose a significant health risk and, further, are already controlled adequately. In fact, several plants have installed controls or shut down since the basic information for standards development was obtained and, indeed, since standards were proposed. EPA has revised its emissions and health risk estimates based on the latest emissions information provided by the industry and has included in these estimates consideration of current controls. EPA has also adjusted its unit risk factor in response to public comments and is using a more detailed human exposure model. EPA has reassessed this new information for maleic anhydride process vents, EB/S process vents, benzene storage vessels, and benzene fugitive emission sources and concludes that in light of the health risks and potential reductions of these four source categories, only benzene fugitive emissions warrant Federal regulations under Section 112. Details regarding the new information and conclusions are included in the separate notices for these source categories.

Public Participation

The Science Advisory Board reviewed draft documents in December of 1977 on EPA's assessment of the health effects at low-level exposure, the extent of human exposure, and the estimation of population risks. Public comments were solicited at proposal of the maleic anhydride standard (April 18, 1980; 45 FR 26660) on the health effects, listing, and regulation of benzene. A public hearing was held on August 21, 1980, in Washington, D.C., to provide interested parties an opportunity for oral presentation of data, views, or arguments on the health effects, listing, and regulation of benzene. The hearing was open to the public, and each attendee was given an opportunity to comment. The public comment period was from April 28, 1980, to November 8, 1980.

Comments have been considered and changes made to the analysis and conclusions, where appropriate. Major comments received on the health effects, listing and regulation of benzene, and EPA's responses are summarized in this preamble. More detailed responses to the major comments and responses to the other comments not addressed in this preamble are contained in "Response to Public Comments on EPA's Listing of Benzene Under Section 112," EPA-450/5-82-003. Comments are identified by the docket item number in parentheses.

Listing of Benzene Under Section 112

The EPA listed benzene as a hazardous air pollutant based on "[s]cientific reports [which] strongly suggest an increased incidence of leukemia in workers exposed to benzene" (42 FR 29332, June 8, 1977). These reports included a review of benzene by NAS, (4) updated criteria published by the National Institute for Occupational Safety and Health (NIOSH), (5) and a proposal by the Occupational Safety and Health Administration (OSHA) for a revision downward of the existing workplace standard for benzene (42 FR 22516, May 3, 1977, and 42 FR 27452, May 27, 1977). While acknowledging that ambient exposure to benzene normally occurs at levels "substantially lower than those to which affected workers were exposed," EPA maintained that "there is reason to believe that ambient exposures may constitute a cancer risk and should be reduced" (42 FR 29332, June 8, 1977).

At the time of listing, EPA announced that it would review the scientific data to determine the health risks from exposure to ambient levels of benzene and invited public participation. The resulting EPA reports—"Assessment of Health Effects of Benzene Gasmans to Low Level Exposures," (6) "Assessment of Human Exposures to Atmospheric Benzene," (7) and "Carcinogen Assessment Group's Report on Population Risk to Ambient Benzene" (8)—form the basis for the majority of the public comments directed at the listing decision.

Commenters, largely from potentially affected industries and trade associations, argued that the listing of benzene was ill-timed, unnecessary, and unjustified. The main thrusts of these arguments are that EPA failed to develop an adequate record in advance of listing and that the record subsequently prepared does not demonstrate that benzene at the levels encountered in the ambient air warrants designation as a hazardous air pollutant.

Timing of Benzene Listing Decision

Many commenters though benzene was listed improperly, or at least prematurely, citing what they believed to be an inadequate record (OAQPS-79-3 [Part I] IV-D-13 [Part II] IV-F-1, IV-F-9; A-79-49 IV-D-9, IV-D-11; A-79-27 IV-D-19) and EPA's reliance on a proposed policy regarding airborne carcinogens (44 FR 58642; October 10, 1979) (A-79-27 IV-D-8, IV-D-25, IV-D-26; OAQPS-79-3 [Part I] IV-D-1, IV-D-11; A-79-49 IV-D-7).

The Clean Air Act requires EPA to list under section 112 substances judged to cause or contribute to air pollution "which may reasonably be anticipated to result in an increase in mortality or an increase in serious, irreversible or incapacitating, reversible illness" (section 112(a)(1)). EPA based the decision to list benzene on a growing consensus in the scientific and regulatory community, evidenced by reports by NAS (4) and NIOSH (5) and proposed regulations issued by OSHA (42 FR 27452; May 27, 1977) that benzene was causally linked to the occurrence of leukemia in occupationally exposed populations. In EPA's view, leukemia clearly meets the criterion described in section 112 as resulting in an increase in mortality or "serious, irreversible or incapacitating, reversible illness."

The EPA's judgment that benzene present in the ambient air may "reasonably be anticipated" to pose a significant health hazard to the general population relied on two arguments advanced in the listing notice: first, that benzene was released to the air in 100 million pound quantities annually to which "large numbers of people are routinely exposed" and, second, that EPA had "adopted a regulatory policy which recognizes that some risk exists at any level of exposure to carcinogenic chemicals" (42 FR 29332; June 8, 1977). The latter referred to the "Interim Procedures and Guidelines for Health Risk and Economic Impact Assessments of Suspected Carcinogens" published by EPA May 25, 1976 (41 FR 21402).

Based on the above, EPA believes that the decision to list benzene was fully informed, timely, and therefore appropriate. The subsequent assessments of low-level exposure and carcinogenic risk were intended, as indicated in the listing notice, for use in "determining which sources of benzene emissions must be controlled, and the extent of control needed" (42 FR 29333, June 8, 1977). To the extent that these assessment documents addressed the

criteria for listing benzene under section 112, they have affirmed EPA's decision.

The EPA rejects the contention that the delay between listing and the proposal of emission standards for benzene sources suggests that EPA lacked the scientific evidence to justify the June 1977 listing. EPA's assessments of the health effects of low-level exposure,(6) the extent of human exposure,(7) and the estimation of population risks (8) were submitted for external review by EPA's Science Advisory Board in December 1977 and publicly released in September 1978, June 1978, and January 1979, respectively. The first emissions standard for benzene sources was not proposed until April 18, 1980 (45 FR 26660). Proposal was not delayd by "the evidence" for listing but rather the complex task of developing specific national emission standards for each source category.

Several commenters (A-79-27-IV-D-6, IV-D-25, IV-D-26; A-80-14-IV-D-4, IV-D-11; OAQPS-79-3 [Part I] IV-D-1, IV-D-11; A-79-49-IV-D-7; OAQPS-79-3 (Part II-IV-D-5) maintained that the listing and rulemaking proceedings for benzene were premature, arguing that they were based on a proposed policy regarding airborne carcinogens (44 FR 58642; October 10, 1979).

Neither the listing of benzene nor the proposed or promulgated standards are based on the proposed airborne carcinogen policy. They are based on section 112. As described above, EPA is persuaded that the decision to list benzene under section 112 was neither premature nor in excess of the Agency's legal authority.

Health Effects of Benzene

Public comments on the EPA report "Assessment of Health Effects of Benzene Germane to Low-Level Exposure" focused on areas of the benzene health literature relevant to evaluation of human health risks from ambient exposure. These include effects on reproduction and development (embryotoxicity and teratogenicity), effects on the cellular genetic material (mutagenicity and chromosome breakage), and carcinogenicity. The basis for listing benzene as a hazardous air pollutant is carcinogenicity. However, since comments were received on the report's discussions on the other effects, they are included for completeness.

Reproductive and Teratogenic Effects. EPA concluded in the benzene health assessment report that the health literature was inconclusive regarding potential effects of benzene on human reproduction and the fetus. Some commenters took a stronger position, asserting that no evidence was available linking benzene with reproductive or teratogenic effects (OAQPS-79-3 [Part I]-IV-D-9, IV-D-13; [Part II]-IV-D-22, IV-F-1, IV-F-8).

The EPA agrees with the commenters that the available data do not implicate benzene as a potential terstogen or embryotoxin in test species. The risks of adverse fetal developmental or reproductive effects, however, have not been studied adequately. No state-of-the-art multiple generation reproduction studies involving benzene have been done, without which it will not be possible to determine the levels at which benzene would have no observed effect.

From the available data concerning adverse reproductive effects of benzene in humans, it is *not* possible to conclude that *no* adverse human reproductive consequence results from ambient levels of benzene, since no well-designed and executed epidemiological studies have been conducted. It is not known if ambient levels of benzene have effects on the many areas of human reproduction, such as the processes of spermatogenesis and changes in menstrual cycle. Until such possibilities are explored, EPA believes that the evidence for benzene-induced reproductive effects in humans must be regarded as inconclusive.

Chromosomal Effects. Although commenters did not disagree with EPA's conclusion that benzene can cause chromosome breakage in humans, (6) they were divided on the exposure levels at which such damage occurs and on the implications of the observed changes (OAQPS-79-3 [Part I] IV-D-8, IV-D-13, [Part II] IV-F-1, IV-F-8; A-79-27, IV-D-27; A-79-49, IV-D-9). Several commenters asserted that these effects result only from high exposures, in excess of 10 ppm (A-79-27, IV-D-27, A-79-49, IV-D-9, OAQPS-79-3 [Part I] IV-D-13), and that "no reliable evidence" exists to link subclinical benzene exposure to chromosome aberrations or to relate the observation of chromosome breakage with human leukemia" (OAQPS-79-3 [Part I] IV-D-13, [Part II] IV-F-1, F-8; A-79-49 IV-D-9).

Conversely, one commenter challenged EPA's conclusion that a dose-dependent relationship between benzene exposure and chromosome damage had not been demonstrated, citing a study by Picciano (2) in benzene-exposed workers, and maintained that this study documented chromosomal effects at benzene exposure levels at and below 2.5 ppm (OAQPS-79-3 [Part I] IV-D-8).

The EPA does not agree that the data on human cytogenetic effects support a conclusion that benzene-induced chromosome damage occurs only after "excessive exposure." As described in the health assessment document, studies are available that relate increased chromosome breakage to benzene exposure well below the OSHA standard of 10 ppm time-weighted average (TWA). (3) (9)

With respect to a dose-response relationship, EPA agrees that the Picciano study indicates a dose-dependent relationship between exposure to benzene and the amount of chromosome damage. As noted in the EPA health assessment document, however, "[t]here is no correlation, * * *, between the degree or length of exposure, *the clinical symptons*, and persistence or extent of chromosomal aberrations" [emphasis added]. (6) EPA believes that this study and the study by Kilian and Daniel (3) are appropriately considered evidence of an association between benzene exposure and chromosome breakage and that the lowest benzene levels (1.0 to 2.5 ppm) where significant increases in breakage were found are considered properly to reflect exposures below those associated with clinical symptons of toxicity.

EPA also agrees that no direct evidence of a casual linkage between chromosomal aberrations and leukemia exists. EPA remains concerned, however, by the frequency of reports correlating chromosome abnormalities with cancer incidence. In addition to benzene workers and leukemia, this association has been pointed out in atomic bomb survivors with leukemia, (10) in uranium miners with lung cancer, in vinyl chloride workers with liver cancer, in luminous dial painters with bone cancer, and in individuals developing visceral cancers after methotrexate treatment for psoriasis. (11)

Carcinogenicity. Commenters did not challege EPA's conclusion that "there is substantial epidemiological evidence that benzene is a human leukemogen." (6) A number of commenters, however, disagreed with EPA's conclusion that benzene posed increased leukemia risk at the levels present in the ambient air. EPA addresses these comments below in "Health Issues Relevant to Benzene Listing Decision."

One commenter took issue with EPA's conclusion that "there is no convincing evidence that benzene causes neoplasias, including leukemia, in animals." (6) The commenter cited two studies, one by Maltoni and Scarnato

(12) and one by Snyder et al. (13) demonstrating benzene-induced tumors in rodents (OAQPS–79–3 (Part I) IV–D–8).

The carcinogenicity studies on benzene in animals reported by Maltoni and Scarnato (1979) (12) and Snyder et al. (1980) (13) support the comment that a positive tumorigenic effect of benzene is evident from these studies. The results of these studies are addressed in the following section.

Health Basis for Listing

As previously discussed, the Agency based the decision to list benzene on a growing consensus in the scientific and regulatory community, supported by reports by NAS, (4) NIOSH, (5) and emergency temporary standards issued by OSHA (42 FR 22516, May 3, 1977) that benzene was causally related to the occurrence of leukemia in occupationally exposed populations.

Although the association between human leukemia and benzene exposure is only one of several adverse health effects attributed to benzene, the serious consequences of this disease and the uncertainties regarding the existence of any no-effect levels of exposure combined to make it the basis for the decision to list. EPA's health basis for listing rested primarily on retrospective studies in occupationally exposed human populations. Of these, three reports documenting an association received greatest emphasis: Infante et al. (14) Aksoy et al., (15) and Ott et al. (16) In the interval since listing, animal data have become available that further support a causal relationship. (12) (13)

Commenters critical of EPA's decision to list benzene argued that these studies suffered from design and methodological flaws, the correction of which would tend to greatly reduce if not eliminate the observed association. Several commenters also thought EPA had misinterpreted the study results and ignored other well-conducted studies that reached significantly different conclusions.

Epidemiological Studies. The work by Infante et al., a retrospective cohort mortality study undertaken by NIOSH, was reported initially in 1977 with a completed follow up published in 1981. (17) The study found a greater than fivefold excess risk of leukemia among workers exposed to benzene during the period of 1940 to 1949 in the "Pliofilm" (rubber hydrochloride) production industry.

One commenter stated that the Infante work was "seriously flawed and largely discredited," citing testimony from the public hearings on the OSHA benzene standard (18) and the Supreme Court's plurality decision on the OSHA standard (19) (OAQPS–79–3 (Part II) IV–D–5; A–79–27 IV–D–8). More specifically, commenters asserted that the study was flawed in two respects: the exposed cohort was improperly defined; and the exposure levels assumed were erroneous (OAQPS–79–3 (Part I) IV–D–13, (Part II) IV–D–5, IV–F–1, IV–F–9; A–79–27 IV–D–6; A–79–49 IV–D–9; A–80–14 IV–D–4, IV–D–16).

Though EPA recognizes that the Infante et al., study has weaknesses, EPA believes that the characterization of the study as "seriously flawed and largely discredited" is inaccurate. Although the commenter does not provide explanation of his criticism beyond references to the OSHA benzene rulemaking, his remarks imply that the study is invalid due to erroneous reporting of the exposure concentrations. EPA acknowledges, as did the authors of the study, that the historical exposure levels cannot be determined with certainty. This fact, however, is irrelevant to the study's conclusion that exposed workers experienced a fivefold excess risk of leukemia over the general population.

Commenters thought the cohort selected for the study inappropriately excluded certain mechanical and "dry side" workers as well as an unknown number of workers who left the plant's employment before 1944.

The issue of cohort definition in Infante et al. was discussed in subsequent publications by the authors (20) (21) as well as the OSHA benzene rulemaking (43 FR 5918, February 10, 1978). The authors argue that "dry side" workers "were never intended for inclusion in the cohort following discussion with company personnel indicating there was no benzene exposure on the dry side" (43 FR 5927). Subsequent reports of benzene levels (three sample points) on the "dry side" by the University of North Carolina (22) were regarded as inadequately detailed "to permit a valid interpretation." (20) The authors also contend that maintenance personnel (pipefitters, mechanics, etc.) were appropriately excluded from the cohort "because company records did not show which men had responsibilities in pliofilm production." (20) Workers who left employment prior to 1944 "could not be included because their personnel records were not in a retrievable form." (20)

The EPA considers the rationale for the selection of the Infante et al. cohort appropriate. EPA notes further that, as described in the completed follow up by Rinsky et al. as well as expert testimony offered by Dr. Marvin Sakol at the OSHA benzene hearings, (18) the strict cohort definition excludes several additional cases of leukemia that "support further the notion that there existed a causal link between benzene exposure in those facilities and the occurrence of leukemia." (17)

Commenters also contended that the benzene concentrations to which the workers were exposed were much higher than assumed by EPA, supplying information from studies indicating that the workers could have been exposed to levels of 100 to 1,000 ppm in the 1940's and as high as 355 ppm in the 1970's with a mean of 30 ppm.

Rinsky et al. (17) provide a thorough discussion of the available information on the benzene levels to which workers may have been exposed in the subject facilities during the periods studied. The authors concluded that "for the most part, employees' 8-hour time-weighted averaged exposures were within the recommended [occupational] standard in effect at the time. However, as is characteristic of industrial processes, there were occasional excursions above these limits." EPA concludes that, while intermittent levels may have approached the values suggested by the commenters, the range of occupational standards for the periods studied (100 to 10 ppm) appears reasonable as an estimate of the chronic exposure pattern. In this regard, EPA agrees with the recent conclusion of the Benzene Work Group of the International Agency for Research on Cancer (IARC) that "the excessive mortality from myelogenous and monocytic leukemia had occurred among workers with occupational exposure to benzene that was generally within accepted limits," recognizing that "the possible contribution of the occasional excursions in exposure and of the employment of some workers in other areas of the plant must be noted; and * * * May have made some contribution to the observed excess in mortality from leukemia." (23)

Aksoy et al. studied the incidence of leukemia and other diseases among workers occupationally exposed to benzene in the Turkish shoeworking industry. (24) (25) (26) Based on case ascertainment by contact with medical care and comparison of leukemia incidence in the exposed population to estimates for the general population of Western nations, Aksoy et al. found a two fold exces leukemia risk among shoeworkers with chronic benzene exposure.

Although commenters generally agreed that the study was of value "in reaffirming, * * * that prolonged exposures to high concentrations of

benzene result in serious blood disorders including a small number of leukemias" (OAQPS-79-3 [Part II] IV-F-1, IV-F-9), several specific criticisms suggested that the excess risk observed was exaggerated. Two commenters argued that Aksoy et al. relied on inappropriate figures (6 per 100,000) for the background leukemia incidence and that when a more reasonable estimate derived from the experience of the European Standard Population (8 to 14 per 100,000) was used, the study no longer shows an excess incidence among the exposed workers (OAQPS-79-3 [Part I] IV-D-13 [Part II] IV-F-1, IV-F-9; A-79-49 IV-D-9). One commenter expressed concern that the age distribution of exposed workers was not available and speculated that the margin for error in the "official count" used as the denominator of the shoeworking population (28,500) was "probably substantial" (OAQPS-79-3 [Part II] IV-F-1, IV-F-9).

EPA agrees that Aksoy's choice of the 6-per-100,000 background leukemia incidence is subject to criticism since it is not easily attributed to the Turkish rural population. It is also reasonable that the "official count" of 28,500 shoeworkers may be an underestimate and therefore overestimates the excess leukemogenic risk in the exposed population. It is equally likely, however, that Aksoy's methodology leads to an underestimate of the excess risk. First, only leukemia cases of which the author was directly aware as a medical practitioner were counted in the study. As Aksoy testified before OSHA, "undoubtedly there were other additional patients among shoeworkers who were not included in our study." (*18*) Second, as EPA's health assessment points out, "the distribution of cases reported by Aksoy et al. strongly differs from that of leukemia in the general population. If the relative incidence were computed solely for acute myeloblastic leukemia and its variants [the forms of leukemia associated with benzene exposure], a magnification of the risk in benzene-exposed shoeworkers would be observed." (*6*) Finally, Aksoy has also testified that rural leukemia incidence in Turkey may be on the order of 3 per 100,000, or half of what he had estimated originally.(*18*) This fact would also increase the calulated excess risk.

Concerning the age distribution of the shoemaker population, the limited age information available led EPA to incorporate an age adjustment factor in the Agency's risk assessment. On the basis of better information on the age structure of Turkey's male population, (*27*) EPA now believes this adjustment was unnecessary and has revised the unit risk derivation accordingly.

Ott et al. (*16*) reported long-term mortality patterns and associated benzene exposure for a cohort of 594 chemical manufacturing workers. Three cases of leukemia were observed where 0.6 was expected, an excess risk of 3.75. The finding was statistically significant ($p=0.047$) in a one-tailed test of significance.

One commenter criticized the statement in EPA's health assessment (*6*) that excess leukemia incidence observed in the Ott et al. study was only of "borderline" statistical significance. The commenter noted that "[s]ince the p value observed (0.047) is less than the p value (0.050) commonly used to determine statistical significance, there is no basis for considering the value borderline" (OAPQS 79-3 [Part I] IV-D-8). Other commenters argued that the study should be appropriately regarded as "inconclusive" (OAQPS 79-3 [Part I] IV-D-9, IV-D-13, [Part II] IV-D-22, IV-F-1, IV-F-9; A-79-49 IV-D-8, IV-F-2). One commenter remarked that while the cases were too few to draw "solid statistical conclusions," the Ott et al. study was the "best documented study of chronic exposures to benzene in the literature to date" (OAQPS 79-3 [Part II] IV-F-1, F-9).

Commenters also contended that the exclusion of one decedent whose leukemia was identified as a "significant other condition" rather than the cause of death eliminated the significance (QAQPS 79-3 [Part I] IV-D-13). One commenter asserted that Ott et al. applied an "inappropriate one-tailed [statistical] test" to determine significance and that the use of an appropriate test (two-tailed) did not reveal a significant association between the leukemia cases and exposure to benzene (OAQPS 79-3 [Part I] IV-D-13).

The presence of confounding exposures to other potential carcinogens was also noted by commenters as evidence that the study should not be viewed as conclusive of a benzene-leukemia association. The same commenters noted that the cases of leukemia occurred in workers exposed to lower benzene levels (2 to 9 ppm) than those encountered by many other individuals in the study population (OAQPS 79-3 [Part I] IV-D-13, [Part II] IV-F-1, VI-F-9).

While EPA does not view the Ott et al. study, taken alone, as conclusive evidence of an association between low-level (2 to 9 ppm) occupational exposure to benzene and leukemia, the Agency believes that this work, combined with other findings in the benzene health literature, serves to reinforce the public health concerns regarding benzene exposure.

EPA does not agree that the use of "borderline" in describing the significance of the Ott et al. study is inappropriate since the value calculated (0.047) was very close to the predetermined limit (0.050) chosen for the test. EPA does agree that the test, as constructed, supports a finding of significance.

EPA disagrees that the use of a "two-tailed" test for significance would be more appropriate than the one-tailed test employed by Ott et al. The hypothesis to be tested in that benzene exposure increases the leukemia risk, not that risk may increase or decrease. The benzene health literature does not support a finding that benzene exerts a protective influence on exposed individuals.

Omitting from the study the individual for whom leukemia was not the immediate cause of death would not, in EPA's opinion, be an appropriate change. In view of the recognized causal relationship between benzene and nonlymphatic leukemias, EPA believes that a case of myelogenous leukemia, such as this, should not be ignored.

EPA does not view the extent of confounding exposures in Ott et al. as severe. The authors did exclude from their analysis persons known to have been exposed to levels of arsenicals, vinyl chloride, and asbestos, all of which have been associated with human health effects. This exclusion eliminated 53 persons from consideration including one leukemia victim. The remaining substances, which include the suspect carcinogen vinylidene chloride, have not been shown to be associated with a leukemia risk in either man or animals. Therefore, inclusion of such exposed persons would not be likely to affect the target organ site for benzene in terms of increased risk.

According to the authors' testimony before OSHA, the "low levels of potential benzene exposure relative to other employees in the cohort . . . made a retrospective assessment of the possible relationship to benzene exposure very judgmental." (*18*) EPA, while recognizing this uncertainty, agrees with the reservation expressed by OSHA in its benzene rulemaking that "because of the small population size as well as the possibility of sensitivity of those individuals developing leukemia, it cannot be concluded that these deaths are not caused by benzene exposure" (43 FR 5928).

Commenters cited other epidemiological studies, notably the work of Thorpe, (*28*) for which no correlation between leukemia and benzene exposure was demonstrated (OAQPS–79–3 [Part I] IV–D–9, IV–D–13, [Part II] IV–F–1, IV–F–9; A–79–27 IV–D–24, IV–F–1; A–79–49 IV–D–9, IV–F–2). The Thorpe study found "no excess incidence of leukemia among petroleum workers exposed to benzene levels estimated to range up to 20 ppm" (OAQPS–79–3 [Part I] IV–D–13).

EPA believes that deficiencies in the Thorpe study preclude a judgment that exposure to benzene below 20 ppm poses no risk of leukemia. The author of this study dwells on the shortcomings of the work, the most important of which are that (1) quantitative determinations of the extent of exposure could not be done, (2) follow up of members of the cohort was inadequate, and (3) problems existed with verifying the leukemia diagnosis.

Followup was left to each unit (plant) separately. Since the author did none of it, the follow up was poor. Many units had no mechanisms by which to notify the plant of the death of an annuitant and, where notification was made, often no cause of death was reported to the company. Cases reported among annuitants were included, although possible underreporting in the group was recognized. No mention was made concerning follow up efforts on former employees who did not qualify for an annuity. Unfortunately, no table on completeness of ascertainment of vital status was given.

Other problems with this study involve the questionable practice of reporting on the pooled results of a study of eight separate and perhaps considerably different plants. A significant risk that may be present in one or more of the plants could have been obscured by the inclusion of populations of nonexposed individuals. Additionally, no consideration of latent factors was presented; no effort was made by the author to require a minimum time since onset of employment of individuals in the study or to provide even cause-specific mortality by time since first employment. Furthermore, the study has been criticized by Brown (*29*) with respect to factors relating to underreporting of leukemia in the study population.

Animal Studies. EPA originally concluded in the benzene health assessment that "there is no convincing evidence that benzene causes neoplasias, including leukemia, in animals."(*6*) One commenter submitted that two animal studies, reports by Maltoni and Scarnato(*12*) and Synder et al.(*13*) had become available demonstrating benzene-induced tumors in rodents (OAQPS–79–3 [Part I] IV–D–8).

EPA agrees that the studies referenced support the finding of a positive tumorigenic effect of benzene in rodents. The study by Maltoni and Scarnato indicated an increased incidence of Zymbal gland carcinomas, mammary gland carcinomas, and leukemia in benzene-treated Sprague-Dawley rats. Snyder et al. observed a higher occurrence of hematopoietic neoplasms, bone marrow hyperplasia, and splenic hyperplasia in benzene-treated C57BL mice. The hematopoietic neoplasms were categorized as lymphocytic lymphoma with thymic involvement, plasmacytoma (myeloma), and leukemia with a hematocytoblast apparently as the predominant cell type. The finding of a tumorigenic effect of benzene in other mammalian species serves to strengthen the concern over benzene's effects on human populations.

Health Issues Relevant to Benzene Listing Decision

EPA listed benzene as a hazardous air pollutant based on evidence linking occupational benzene exposure with leukemia and on the knowledge that large numbers of people are exposed to and, therefore, may be at risk from, benzene emitted into the ambient air by a variety of stationary sources. This rationale assumes that (1) it is reasonable to conclude that a causal relationship continues to exist at the significantly lower exposure levels characteristic of the ambient air, and (2) that the magnitude of the relationship warrants efforts to reduce human exposure.

A number of commenters took issue with EPA's judgment, arguing that an exposure threshold for benezene-induced leukemia exists below which there is no health risk and that, even granting an association with leukemia at ambient levels, the magnitude of the health risks to exposed populations is negligible. (Due to the number of commenters on these subjects, the comment numbers are not listed.) The comments focus on EPA's presumption that effect thresholds do not exist for carcinogens (the nonthreshold hypothesis) and the methodology used by EPA's Carcinogen Assessment Group (CAG) in deriving quantitative estimates of benzene leukemogenic risks. A summary of public comments addressing the issue of a carcinogenic threshold for benzene follows the statement of EPA's position on carcinogenic thresholds.

EPA's Position on Carcinogenic Thresholds (Nonthreshold Hypothesis). In evaluating the public health hazards associated with exposure to known or potential carcinogens, EPA has maintained that, in the absence of sound scientific evidence to the contrary, such substances must be considered to pose some finite cancer risk at any exposure level above zero (41 FR 21402, May 25, 1976; 44 FR 58642, October 10, 1979; 44 FR 39858, July 6, 1979). OSHA (45 FR 5002, January 22, 1980), the Consumer Product Safety Commission (CPSC), the Food and Drug Administration (FDA), the Food Safety and Quality Service, and the President's Regulatory Council (44 FR 60038, October 17, 1979), among others, have shared this conviction.

Support for the nonthreshold hypothesis for carcinogens dervices from both scientific and practical considerations. As summarized by the Interagency Regulatory Liaison Group (IRLG): "[t]he self-replicating nature of cancer, the multiplicity of causative factors to which individuals can be exposed, the additive and possibly synergistic combination of effects, and the wide range of individual susceptibilities work together in making it currently unreliable to predict a threshold below which human population exposure to a carcinogen has no effect on cancer risk" (44 FR 39876).

The mechanism by which a carcinogen acts is of particular importance in postulating whether or not an effect threshold exists. NAS has observed:

> Whether or not a particular effect follows a dose-response relationship that has a threshold depends entirely on the mechanism of the effect. Many effects have thresholds. For example, the gastrointestinal-radiation syndrome, acute drug toxicity, and radiation or drug control of some tumors all have dose-response curves that show thresholds. The curves are sigmoid, and below a particular dose there is a zero probability of producing the effect, because the effect requires many independent events and will not occur until the number of such events exceeds some critical value. The gastrointestinal-radiation (or drug) syndrome is a case in point. An animal will not die until the number of intestinal crypt cells that have been killed exceeds a value that is critical to the integrity of the organ. Any radiation or drug dose that kills fewer cells than this critical number can be considered to be safe (at least for this one syndrome).

We are used to thinking in terms of thresholds and sigmoid dose-response curves. For example, if it costs $4,000 to buy an automobile, we do not imagine that we will have a 50% chance of buying the same vehicle for $2,000. If 100 aspirin tablets constitute a lethal dose, we do not calculate that we will have a 1% chance of dying if we

swallow a single tablet. Because we know the mechanisms underlying these events, we expect thresholds to the dose-response curves, and indeed they are evident.

However, other effects may well *not* have threshold dose-effect relationships. If an effect can be caused by a single hit, a single molecule, or a single unit of exposure, then the effect in question cannot have a threshold in the dose-response relationship, no matter how unlikely it is that the single hit or event will produce the effect. Mutations in prokaryotic and eukaryotic cells can be caused by a single cluster of ion pairs which were produced by a beam of ionizing radiation. We would expect that mutations can be caused by a single molecule or perhaps group of molecules in proximity to the DNA. The necessary conclusion from this result is that the dose-response relationship for radiation and chemical mutagenesis cannot have a threshold and must be linear, at least at low doses.

It is one step further to correlate mutagenesis with carcinogenesis. Nevertheless, the evidence is strong that there is a close relationship between the two [references].

We therefore conclude that, if there is evidence that a particular carcinogen acts by directly causing a mutation in the DNA, it is likely that the dose-response curve for carcinogenesis will not show a threshold and will be linear with dose at low doses.*(1)*

Evidence for a linear-carcinogenic response at low dose comes from studies suggesting cancers may arise from the "transformation" of a single cell. *(30)(31)* One study observed that in women with a genetic condition that leads to their body cells being of two recognizable types, tumors are characteristically of one cell type, while normal tissues are composed of a mixture of both types. Another described experimental efforts in which transformed cells were transplanted into whole animals. Both of these observations further support the theory that cancers may arise from single cells. A single cell origin of cancers implies that the statistical form of the carcinogenic dose response relationship may be highly influenced by the extreme tail of the distribution of cell transformations with dose. As Crump points out "the effect of this is to make virtually any process of discrete events approximately linear at low dose." *(18)*

EPA's presumption that any exposure to a carcinogen poses a health risk is not intended to foreclose discussion or ignore evidence of real or practical effect thresholds for such substances. In this regard, a number of theories postulate the existence of thresholds. These include consideration of the body's defense and repair capabilities (immunosurveillance, detoxification, and DNA repair) and reports of the regression of preneoplastic lesions with the cessation of exposure. Observations of an inverse relationship between dose and the latency period for tumor expression have been proposed as evidence of practical thresholds where the dose corresponds to a latency that exceeds the individual's lifespan. Proponents also have suggested, as indirect evidence of thresholds, the carcinogenicity at high doses of certain substances for which a biological requirement exists. Threshold levels have, in addition, been inferred from "negative" epidemiological and animal studies.

While EPA agrees that the evidence for real or practical carcinogenic thresholds should play a role in hazard evaluation, the Agency is persuaded that the utility of such information in establishing "no effect" levels is seriously limited. Although protective mechanisms such as DNA repair are reasonably effective, it is generally recognized that few, if any, biological processes are 100 percent efficient (45 FR 5128, 5129). Similarly, while decreased dose could increase the *median* time-to-tumor to greater than a lifespan, the typical distribution of tumors across age groups still would result in "early" cancers arising.

Evidence for practical thresholds is also questionable. There is no reason to believe that biologically required substances, which have been found to be carcinogenic at high levels, may not pose some cancer risk at levels where they are normally found in the body. In the same way, the failure to detect a positive association in the animal bioassay or epidemiological study does not constitute evidence of a no-effect level. NAS has noted that

> • • • the observation of no positive responses does not guarantee that the probability of response is actually zero. From a statistical viewpoint, zero responders out of a population of size N is consistent at the 5% significance level with an actual response probability between zero and approximately 3/N (e.g., when N = 100 and zero responders are observed, the true probability of response may be as high as 3%).*(1)*

Finally, EPA concurs with NAS that theoretical arguments for the existence of carcinogenic thresholds must be tempered by the knowledge that the exposed human population is a "• • • large, diverse, and genetically heterogeneous group exposed to a variety of toxic agents. Genetic variability to carcinogenesis is well documented (Strong), 1976, (32) and it is also known that individuals who are deficient in immunological competence (for genetic or environmental reasons) are particularly susceptible to some forms of cancer (Cottier et al., 1974)(33)." *(1)*

OSHA noted in its summary of public hearings on an occupational carcinogen policy:

> A number of witnesses testified that, even if thresholds could be established for the circumstances in which animals are exposed only to single carcinogens, this would have little or no relevance to risk assessment for humans, who are exposed to many carcinogens, either simultaneously or sequentially. Specifically, several witnesses pointed out that there is already a relatively high incidence of cancer in the human population. Hence many individuals are already at or close to the threshold for certain processes involved in cancer development, so that incremental exposure to even small quantities of an agent that accelerates these processes would be expected to lead to an increase in the frequency of cancer. (45 FR 5135)

NAS has further elaborated:

> In considering the possibility of thresholds for carcinogenesis, it is important to understand that there is no agent, chemical or physical, that induces a form of cancer in man that does not occur in the absence of that agent. In other words, when there is exposure to a material, we are not starting at an origin of zero cancers. Nor are we starting at an origin of zero carcinogenic agents in our environment. Thus, it is likely that any carcinogenic agent added to the environment will act by a particular mechanism on a particular cell population that is already being acted on by the same mechanism to induce cancers. This reasoning implies that only if it acted by a mechanism entirely different from that already operating on the tissue could a newly added carcinogen show a threshold in its dose-response curve. *(1)*

In summary, EPA's position has been that the nonthreshold hypothesis is, for carcinogens, a reasonable and appropriate presumption that must be overcome by sound scientific evidence before any exposure to such substances can be concluded to be without health risk. At the same time, however, EPA regards relevant evidence of the ability of biological systems to mitigate adverse health effects as important considerations in the evaluation of the health hazard.

Support for a Threshold for Benzene. Commenters challenged EPA's nonthreshold presumption for benzene, arguing that the Agency had failed to consider convincing evidence that a leukemogenic threshold for benzene does exist and that this threshold is well above any ambient levels that might be encountered by the general population. In support of this position, commenters cited studies of benzene metabolism, alternative mechanisms for cancer induction, and evidence derived from epidemiological studies.

One commenter cited the work of Richert and Irons (34) as evidence that

exposure to levels of benzene below 10 ppm does not produce any adverse health consequences in human cells (OAQPS-79-3 [Part I] IV-D-13, [Part II] IV-F-1, IV-F-2, IV-F-3).

Rickert studied benzene metabolism in rodents and human cells *in vitro* to determine the concentrations of toxic benzene metabolites that might occur in the bone marrow of humans exposed to benzene (OAQPS 79-3 [Part II] IV-F-2). He concluded that the metabolite concentrations in rats and human tissue are of the same order of magnitude at similar benzene doses. Irons used this information to compare the metabolite concentrations expected at various benzene exposures with those at which the first signs of hematotoxicity (lymphocytopenia) occurred. He found "that a significant difference exists between the projected concentration of benzene metabolities in bone marrow, as calculated for a 6 hour exposure to 10 ppm benzene *in vitro*, and the concentration of the same metabolites which produce a demonstrable effect on a sensitive population of human cells *in vitro*" (OAQPS 79-3 [Part II] IV-F-3).

Although EPA regards this work, published after the release of the health assessment document, as generally supportive of the concept of a threshold for lymphocytopenia and other hematotoxic effects that may result from benzene exposure, EPA does not agree with the inference drawn from this study that exposures below 10 ppm pose no health risk. The *in vitro* system used may not represent the most sensitive human population at risk of hematotoxic effects. Further, it is not clear that effects such as lymphocytopenia must precede the induction of leukemia, nor has it been established that the benzene metabolities studied are related to the onset of leukemia.

Several commenters submitted that EPA's presumption of low-level benzene risk ignored alternative mechanisms for carcinogenesis, applicable to benzene, for which effect thresholds appear likely. One commenter asserted that, while a substance's ability to directly alter genetic material could be viewed as support for a nonthreshold mechanism, there is "no evidence that [benzene] react[s] with DNA" (OAQPS-79-3 [Part I] IV-D-9, [Part II] IV-D-22). According to the commenter, "Benzene induces neoplasia through cell injury" to the bone marrow. The injury is "followed by regeneration of the bone marrow and myelogenous leukemia in a small number of cases." During exposures of humans to benzene levels in the air of 10 ppm or less, the metabolic detoxification reactions maintain the levels [of benzene] and its metabolites to be sufficiently low in the blood to be below the threshold for any effect on the bone marrow or metabolic effects on lymphocytes" (OAQPS 79-3 [Part I] IV-D-9, IV-D-13, [Part II] IV-D-22, IV-F-1, IV-F-9; A-79-27 IV-D-24, IV-D-27, IV-D-29; A-79-49 IV-D-9, IV-D-11, IV-D-12, IV-F-1, IV-F-2; A-80-14 IV-D-1, IV-D-3, IV-F-1).

Similarly, commenters argued that the documented association between hematotoxic effects (usually decreases in the levels of various formed elements in blood: cytopenia, pancytopenia, and lymphocytopenia) and leukemia supports the finding that such effects may be a necessary precondition for leukemia. In this regard, one commenter quotes Goldstein's observation that "there [do] not appear to be any proven cases in which leukemia began in the absence of previous cytopenia." (*35*) Commenters contend that because "pre-leukemic" changes such as cytopenia "do not occur below about 35 ppm," this exposure level or, more conservatively, a level of 20 or 10 ppm constitutes an effective threshold below which benzene "presents no health risk whatsoever."

While EPA agrees that the nongenetic, or "epigenetic," mechanism constitutes a possible explanation for the way in which cancers could arise in the absence of direct interaction with genetic material, the Agency is not persuaded, based on the largely theoretical nature of this position, that such a mechanism has been demonstrated in the case of benzene. For similar reasons, the Agency continues to regard as inconclusive the contention that hematotoxic effects must necessarily precede the development of leukemia in benzene-exposed individuals.

Covalent bonding (reaction) with DNA is generally regarded as evidence that an agent may have the ability to "transform" a normal cell into an abnormal, and possibly cancerous, cell via a somatic mutation. The absence of such bonding or its nondetection, however, does not demonstrate that substances such as benzene may not interact directly with genetic material to produce aberrant cells. In fact, there is good evidence that benzene, at levels as low as 1 to 2.5 ppm, significantly increases chromosome abnormalities in bone marrow cells including chromosome breaks and marker chromosomes (rings, dicentrics, translocations, and exchange figures).(*3*)(*9*) Whether such changes are appropriately considered mutations or simply toxic events depends on the fate of the affected cell. As OSHA has pointed out in its benzene rulemaking:

> If the alteration in the chromosomal material results in an inhibition of further cellular division, then in terms of its reproductive potential, the cell is dead and the damage inflicted may be classified as a toxic event. However, if the damage does not interfere with the reproductive ability of the cell, and the alteration is replicated, this may constitute a persistent gross mutation. The finding of gross chromosomal damage in bone marrow cells clearly demonstrates that despite competing detoxification reactions * * * benzene, or a reactive metabolite is able to overwhelm protective defense mechanisms and enter the nucleus of hematopoietic cells. (43 FR 5918)

The quote attributed to Goldstein noting that "there [do] not appear to be any proven cases in which leukemia began in the absence of previous cytopenia" is correct but incomplete. Later in the page Goldstein cautions that this interpretation is "open to speculation, especially in view of the paucity of routine laboratory data preceding the onset of leukemia."(*36*)

The lack of information, as well as the retrospective nature of most of the analysis, makes it difficult to substantiate a precedent relationship between hematotoxic effects and leukemia. In this regard, OSHA has observed:

> * * * since the mechanism by which benzene induces leukemia has not been elucidated it is possible that leukemia develops, not in response to the pancytopenic effects of benzene, but rather to the direct carcinogenic effect on the marrow hematopoietic stem cells not necessarily accompanied by any other evidence of marrow effect * * *. In such events, protection against non-neoplastic blood disorders would not rule out subsequent development of leukemia (43 FR 5929).

Similarly, Browning, in 1965, noted: "benzene leukemia is frequently superimposed upon a condition of aplastic anemia, but it can develop without a preceding peripheral blood picture characteristic of bone marrow aplasia."(*10*)

Finally, EPA is not persuaded that the "thresholds" identified by commenters for benzene-induced "injury" are sound. First, it is not clear that techniques such as peripheral blood counts and aspiration of bone marrow are capable of consistently detecting injury to the hematopoietic system, particularly when the normal ranges of such counts are broad.(*6*)

Second, injury may be occurring at levels below those at which cytopenia is observed. In its review of benzene, NAS commented on a report of benzene-induced chromosome abnormalities:

"Vigliani and Forni (*37*) reported chromosomal aberrations of both the stable and unstable type. In general, the chromosome aberrations were higher in peripheral blood lymphocytes of workers exposed to benzene than in those of controls. This was true *even in the absence of overt signs of bone marrow damage*" (*4*) [emphasis added]. As noted above, Picciano and Kilian and Daniel have also reported significant increases in chromosomal aberrations, an effect whose toxic potential cannot be ignored, in workers exposed to benzene at levels substantially below the 10 ppm submitted as the lowest level for a "threshold" for benzene-induced effects.

Commenters found support for a benzene carcinogenic threshold in epidemiological studies that did not find a significant association between benzene exposure and leukemia (citing work by Thorpe, (*28*) Tabershaw, (*38*) and Stallones (*39*)), in control or nonexposed populations for which a case for benzene exposure could be made (citing Infante et al. (*14*)), and among exposed populations following exposure reduction efforts (citing Infante et al. (*14*) and Askoy et al. (*15*)). (OAQPS–79–3 [Part I] IV–D–8, IV–D–11, IV–D–13, [Part II] IV–D–22, IV–F–1, IV–F–6; A–79–27 IV–D–24, IV–D–28; A–79–49 IV–D–10, IV–D–11, IV–D–12, IV–F–1, IV–F–2; A–80–14 IV–F–1).

As indicated in "Health Basis for Listing" above, EPA believes that the shortcomings of the Thorne study do not permit a firm conclusion regarding a carcinogenic threshold for benzene. In the larger context of the utility of negative epidemiological studies, EPA, as a member of the IRLG, concluded that studies not finding a positive statistical correlation do not demonstrate the absence of a hazard, due to the limitations of epidemiologic investigations and long cancer latency periods during which exposure to other potentially carcinogenic substances can occur (44 FR 39858; July 6, 1979). In addition, OSHA (45 FR 5001; January 2, 1980) and the National Cancer Advisory Board (*40*) contend that negative epidemiological data do not necessarily establish the safety of suspect materials.

Similarly, while EPA agrees that follow up studies such as those undertaken on the Infante et al. and Aksoy et al. populations may be useful in demonstrating risk reductions, they are not appropriate support for a position that risks have been eliminated. As with "negative" epidemiological studies, EPA does not agree that such findings demonstrate the absence of a hazard.

Having reviewed the public comments, EPA concludes that the evidence submitted in support of a real or practical threshold for benzene-induced leukemia is not sufficient to overcome EPA's presumption that benzene may pose a finite risk of leukemia at any exposure level greater than zero.

Although commenters have sought to demonstrate that benzene may cause leukemia via a nongenetic mechanism that requires threshold-governed tissue injury prior to leukemia induction and that levels of benzene below this threshold are noninjurious or otherwise detoxified, EPA regards this evidence as largely theoretical in nature and inconclusive.

EPA believes that the support for a "hematotoxic" threshold as protective against leukemia induction is speculative for two reasons: first, because neither the mechanism for benzene-induced leukemia nor that for blood disorders has been elucidated, and, second, because information is available that other effects of potential adverse health consequence have been shown to occur at levels lower than those postulated as hematotoxic thresholds. Finally, EPA does not accept the premise that the nonpositive epidemiological studies offer a means of establishing credible no-effect levels.

For these reasons, recognizing the uncertainties in the scientific data base, EPA believes that the nonthreshold presumption should continue to apply in the case of benzene and that benzene should be considered to pose a risk of cancer at any exposure level above zero. EPA believes that this finding is consistent with the mandate of Section 112 requiring the protection of public health against air pollutants that "may reasonably be anticipated" to cause or contribute to the health effects of concern.

Quantitative Risk Estimates of Carcinogens. EPA initially published interim guidelines for the conduct of quantitative risk assessments (QRA) for carcinogens on May 25, 1976 (41 FR 21402). In 1979, these were succeeded by the report of the Work Group on Risk Assessment of IRLG (44 FR 39858; July 6, 1979) of which EPA was a member.

EPA prepared, in conjunction with the listing of benzene under Section 112 and the development of emissions regulations, an assessment of the population risk to ambient benzene exposures.(*8*) The assessment was based on an extrapolation of the human leukemogenic risk drawn from available epidemiological evidence in combination with an assessment of human exposure to benzene emitted into the air by stationary sources. (*7*)

Although a few commenters objected to the performance of a risk assessment, arguing that the underlying uncertainties were too great to permit a meaningful result, most respondents favored attempting to estimate population risks. In an extensive critique of EPA's assessment, however, commenters disagreed with EPA on a number of scientific and technical grounds, ranging from the appropriateness of the dispersion model used in estimating ambient benzene levels to errors in the assumptions made in deriving an estimate of benzene's leukemogenic potency. Commenters argued that the correction of such errors would result in an overall leukemogenic risk from benzene sources substantially below that predicted by EPA, and, in fact, small enough to be regarded as a "statistical artifact" for which regulatory attention was unwarranted.

The original assessment of human exposure to benzene was performed by the Stanford Research Institute (SRI) under contract to EPA. (*7*) A number of commenters on the benzene listing and proposed standards criticized the SRI assessment as relying on outdated emissions estimates, employing an upwardly biased exposure model, omitting plant-specific information, and erroneously including plants no longer using benzene (OAQPS–79–3 [Part II] IV–F–1, IV–F; A–79–49 IV–D–9). One commenter questioned the use of a 20-kilometer radius in developing the exposure estimates (OAQPS–79–3 [Part I] IV–D–8). Several commenters were supportive of an alternative methodology submitted by Systems Applications, Inc. (SAI) (OAQPS–79–3 [Part I] IV–D–9, IV–D–13, [Part II] IV–D–22, IV–F–1, IV–F–6, IV–F–9; A–79–49 IV–D–9).

EPA agrees that the SAI exposure methodology offers some improvements over the exposure methodology used by SRI for the benzene assessment. SAI developed its methodology under contract to EPA in response to a need for a rapid, computer-efficient method for conducting national-level exposure assessments. This methodology, with the additional data submitted in the course of the comment periods on the benzene proposals, has been used to revise the exposure estimates and risk assessments for the promulgated standards.

Although the SAI methodology has supplanted the methodology initially used by EPA to estimate benzene exposures, EPA does not agree that the

SRI report, for the purposes intended, is grossly inaccurate or upwardly biased.

The SRI report was intended to be an initial rough estimate of national-level exposures to ambient air concentrations of benzene caused by air emissions from various types of sources. The purpose of the report was to help EPA decide which benzene sources to study in more depth and thereby determine the extent of regulation needed under the Clean Air Act. The report also helped EPA determine the order in which the studies would be conducted. Those studies, which accompany the development of regulations under section 112 of the Clean Air Act, address far more explicitly the sources of benzene selected for regulation and the public exposures to benzene associated with those sources. The nature of many of the comments suggests that the commenters did not understand EPA's intended use of the report and of the intentionally rough-cut approach considered appropriate for that use.

EPA agrees that much of the SRI report is difficult to evaluate. This report was one of EPA's first attempts at estimating nationwide exposure, and the methodologies were not yet fully described. As explained, the report was not meant to be a definite statement on exposure to benzene, but to be a guide to follow-on studies. All deficiencies considered, EPA considers the report adequate for its intended use.

The selection of a 20-kilometer limit on exposure estimation in the vicinity of stationary sources is based on modeling considerations. Twenty kilometers was chosen as a practical modeling stop-point. The results of dispersion models are considered reasonably accurate within that distance. The dispersion coefficients used in modeling are based on empirical measurements made within 10 kilometers of sources. These coefficients become less applicable at long distances from the source, and the modeling results become more uncertain.

Comments were generally critical of the use by CAG of a linear, nonthreshold model to derive a benzene unit risk factor. One commenter (OAQPS-79-3 [Part II] IV-D-9) rejected the assumptions used by CAG of no threshold and the validity of the linear model extrapolated toward zero. Other commenters viewed the model as "inherently conservative" and likely to yield an upper limit of the health risks (OAQPS-79-3 [Part I] IV-D-13; A-79-27-IV-D-27; A-80-14-IV-D-10a, IV-D-13).

While EPA agrees that the linear, nonthreshold model is conservative and would tend to provide an upper bound to the statistical range for the unit risk factor, the Agency does not believe that the assumptions upon which it is based are unreasonable or that the results of its use are exaggerated. IRLG agreed that although the mathematical model identifies an upper limit estimate of risk from a statistical standpoint, "[t]he risk estimates as applied to humans should not be regarded as upper limit estimates because of large biological uncertainties."[7]

The dose-response model with linearity at low dose has been adopted for low-dose extrapolation by EPA because it has the best, albeit limited, scientific basis of any current mathematical extrapolation model.[41] This basis is supported by EPA's conclusions in a Federal Register notice (45 FR 79358; November 28, 1980) announcing the availability of Water Quality Criteria documents. The Agency concluded that, "[t]he linear non-threshold dose-response relationship is . . . consistent with the relatively few epidemiological studies of cancer responses to specific agents that contain enough information to make the evaluation possible . . . There is also some evidence from animal experiments that is consistent with the linear non-threshold hypothesis . . . ."

Commenters argue that, in addition to the conservative nature of the model used, the assumptions made by EPA (CAG) in the derivation of a unit leukemia risk factor for benzene represented "serious misinterpretation" of the underlying epidemiological evidence (OAQPS-79-3 [Part I] IV-D-13, [Part II] IV-F-1, IV-F-9; A-79-27-IV-D-27; IV-D-24; A-80-14-IV-D-10a, IV-D-21). Among the specific criticisms were: CAG (1) inappropriately included in its evaluation of the Infante et al. study two cases of leukemia from outside the cohort, inappropriately excluded a population of workers that had been exposed to benzene, and improperly assumed that exposure levels were comparable with prevailing occupational standards; (2) accepted, in the Aksoy et al. studies, an unreasonable undercount of the background leukemia incidence in rural Turkey, made a false adjustment for age, and underestimated the exposure duration; and (3) included the Ott et al. study in the analysis despite a lack of statistical significance.

As previously discussed in "Health Basis for Listing," EPA has reexamined and reevaluated each of the three studies. In summary, EPA concluded that one case of leukemia was inappropriately included from the Infante et al. study in computing the original unit risk factor. Additionally, EPA reaffirmed its decision to exclude dry-side workers from that study in developing the risk factor. The Agency with the commenters that the Aksoy et al. study was adjusted improperly for age; however, the exposures and durations of exposures are still considered reasonable estimates. The Ott et al. study was not eliminated from the risk assessment because the findings meet the test of statistical significance and because it provides the best documented exposure data available from the three epidemiological studies.

Based on these findings, the unit risk factor (the probability of an individual contracting leukemia after a lifetime of exposure to a benzene concentration of one part benzene per million parts air) was recalculated. The revised estimate resulted in a reduction of about 7 percent from the original estimate of the geometric mean, from a probability of leukemia of 0.024/ppm to a probability of leukemia of 0.022/ppm.

Significance of Estimated Carcinogenic Risks from Benzene Exposure. Based on EPA's estimates of carcinogenic risk or on the alternative calculations submitted to the Agency for consideration, a number of commenters asserted that the risk of developing leukemia from exposure to benzene in the ambient air was too small to warrant regulatory consideration under section 112. Specifically, commenters argued that the regulation of benzene under section 112 would have "no meaningful impact on the occurrence of leukemia in the general population" (OAQPS-79-3 [Part I] IV-D-9, [Part II] IV-F-1, IV-F-9). In support of this position, commenters cited EPA's estimate that roughly 80 percent of ambient benzene emissions were attributable to mobile sources that would not be regulated under section 112 and noted that the number of leukemia cases predicted by the EPA assessment to occur as the result of benzene emissions from stationary source categories represented "less than one-tenth of one percent [of] the normal leukemia mortality risk in the U.S. population. . . . a result so small as to be indistinguishable from a risk of zero" (OAQPS-79-3 [Part I] IV-D-13, [Part II] IV-F-1, IV-F-9; A-79-49-IV-D-9; A-79-27-IV-D-18, IV-D-10, IV-F-1; A-80-14-IV-D-10a, IV-F-1).

Several commenters referenced, as evidence of the insignificance of the ambient benzene risk, the comparable or higher risks associated with activities such as skiing, hunting, and sky diving (OAQPS-79-3 [Part I] IV-D-19) and with involuntary hazards such as drowning

and electrocution (OAQPS–79–3 [Part I] IV–D–13, [Part II] IV–F–1, IV–F–9).

Commenters also maintained that the estimated risks posed by benzene emissions were at or below levels recognized by EPA and other Federal agencies as acceptable goals or targets for regulation (OAQPS–79–3 [Part I] IV–D–13).

EPA does not agree with the commenter's assertions that the health risks posed by benzene emissions from all stationary sources are insignificant or that the regulation of benzene under Section 112 is, therefore, unwarranted. EPA continues to believe that the well-documented evidence of benzene's leukemogenicity, the quantity of stationary source emissions, the observed and estimated ambient concentrations, the proximity of large populations to emitting sources, and the numerical estimates of health risks (including consideration of the uncertainties of such estimates) support the judgement that benzene is an air pollutant that "causes or contributes to air pollution which may reasonably be anticipated to result in an increase in mortality or an increase in serious irreversible, or incapacitating reversible, illness" (section 112(a)(1) of the Clean Air Act).

With an estimated 9.9 billion pounds (4.5 million megagrams) produced in 1981, benzene ranks 16th among all chemicals in terms of production volume in the United States. (*42*) Benzene is the largest production chemical that has been causally linked to cancer in humans.

EPA estimates that more than 120 million pounds (55,000 megagrams) of benzene are emitted annually to the ambient air from stationary industrial sources. The sources are primarily plants involved in benzene production, other chemical manufacturing, and the storage and distribution of benzene and gasoline. At these sources, benzene is emitted from the process vents, storage tanks, and liquid transfer operations as well as from leaks in process components such as pumps and valves. According to EPA estimates, at least 30 to 50 million people live within 20 kilometers of stationary sources (excluding gasoline marketing sources) that emit benzene. Levels of benzene have been monitored in the vicinity of benzene-emitting facilities at levels as high as 350 ppb (1,117 $\mu g/m^3$) with median values of 3.0 ppb (9.6 $\mu g/m^3$). (*43*)

EPA regards benzene emissions from some stationary source categories and potential human exposure to these emissions as significant. The fact that mobile sources emit more benzene than do stationary sources has no bearing on the significance of the benzene emissions from stationary sources, since these sources also emit large quantities of benzene. The fact that specific standards have not been proposed for mobile sources does not imply that the Agency has reached a conclusion on the significance of the health risks associated with these sources. As commenters pointed out, mobile sources are not regulated under section 112, but under Title II of the Clean Air Act. A control technology applicable for benzene emissions from mobile sources, as for other hydrocarbon compounds, is installation of a catalytic converter. In fact, benzene emissions from mobile sources are reduced substantially (along with other hydrocarbon compounds) by catalytic converters, installed in response to standards established under Title II of the Clean Air Act. EPA projects that by 1985, mobile source benzene emissions will have been reduced by 69 percent compared with those in the baseline year when the Clean Air Act was enacted (1970), and by 1990 they will have been reduced by 83 percent.

EPA disagrees that benzene does not warrant regulation because such regulation will not have a meaningful impact on the occurrence of leukemia in the general population. Except for established causal relationships with benzene and certain hereditary factors, the causes of leukemia are not known. Because it is estimated that only a small proportion of leukemias may, at present, be preventable does not argue that reasonable control measures should not be taken.

Furthermore, EPA does not agree that the presence of other unregulated or tolerated health risks, equal or greater in magnitude than those estimated for benzene exposure, obviates the need for regulation. Activities such as hunting and skiing are essentially voluntary in nature with well-advertised risks. The risk of someone being struck by lightning, while largely involuntary, would be difficult to reduce effectively. For benzene, however, a large component of the health risk is involuntary. At the same time, reasonable actions are available that can reduce the risks from benzene exposure. EPA questions the appropriateness of weighing risks that are accepted voluntarily or that have little opportunity for mitigation against risks largely beyond the individual's control but for which societal remedies are readily available.

Finally, commenters have chosen to make comparisons based on the "average" lifetime risks or the expected number of leukemia cases attributable to benzene emissions, arguing that an "average" lifetime risk of leukemia from ambient levels of benzene of 1 per 100,000 (10^{-5}) does not constitute a significant hazard and has, in fact, been accepted by EPA and other Federal agencies as an appropriate goal for regulation. Aside from the technical and philosophical difficulties inherent in the selection and verification of such goals described above, EPA has not selected a specific "goal" for carcinogenic risks from hazardous air pollutants and, further, disagrees with the choice of the "average" lifetime risk as an appropriate measure of individual risk. EPA believes that the determination that a substance poses a significant health risk via the ambient air must include consideration of the magnitude of the hazard to those individuals and subpopulations most expose to emissions of the substance. In the case of benzene, the estimated maximum lifetime risks for these populations are generally higher than are "average" risks cited by commenters. Current EPA estimates for the most exposed individuals living in the vicinity of source categories for which standards are being developed range from a leukemia risk of 150 per 100,000 for benzene fugitive sources to 640 per 100,000 for coke by-product plants (OAQPS A–79–16). The reader should recognize that any time leukemia risk numbers are cited, they are subject to considerable uncertainty. These uncertainties are explained in the next section of this preamble, titled "Selection of Benzene Source Categories for Regulations."

In conclusion, EPA continues to believe that benzene emissions from some stationary source categories represent a significant risk of leukemia to exposed populations, particularly to those individuals and subpopulations residing near major point sources. This belief rests on the documented evidence that benzene is a human leukemogen, on the magnitude of benzene emissions to the ambient air, on the observed and estimated ambient concentrations, on the proximity of large populations to emitting sources, and on estimates of the health risks to exposed populations, including consideration of the uncertainties associated with quantitative risk estimates (including the effects of concurrent exposures to other substances and to other benzene emissions).

Thus, EPA still believes that the listing of benzene on June 8, 1977, was appropriate and that delisting is inappropriate. The evidence submitted by commenters is judged insufficient to

support a conclusion that ambient levels of benzene do not pose carcinogenic risks or that the risks posed by benzene emitted by all stationary source categories are insignificant.

Other Issues Relevant to Listing of Benzene

Several commenters asserted that the listing of benzene was unnecessary in view of the "network of regulatory programs already put into effect to control ambient benzene exposures," thus taking benzene out of the statutory definition of "hazardous air pollutant" under section 112 (OAQPS-79-3 [Part I] IV-D-10, IV-D-13, [Part II] IV-F-1, IV-F-9; A-79-49 IV-D-10, IV-F-1, IV-F-2; A-80-14-IV-D-13, IV-D-10a, IV-F-1).

The regulatory programs to which the commenters refer were put into effect to attain and maintain the national ambient air quality standard (NAAQS) for ozone, not to control ambient benzene exposures. The health effects from exposure to ozone are very different from the health effects from exposure to benzene; ozone-caused health effects are serious, but there is no evidence that exposure to ozone causes cancer. Therefore, no scientific or technical basis exists for believing that attaining and maintaining NAAQS for ozone will ensure that the public is amply protected from benzene exposure.

It is true that controlling VOC emissions to attain and maintain the ozone standard often results in a degree of control over benzene emissions, because benzene is often emitted with the VOC's being controlled. EPA did not, as one commenter suggests, "ignore" this fact. The effectiveness of existing State standards and control devices in place for any other reason has been considered when emissions from existing plants have been estimated. In fact, the amount of control currently in place for three benzene source categories for which standards were previously proposed, maleic anhydride and EB/S process vents and benzene storage vessels, is relevant to the Agency's proposed conclusion that benzene emissions from these source categories no longer warrant federal regulatory action. One cannot reasonably assume, however, that the extent and stringency of the control of VOC emissions equates to adequate control of all benzene emissions nationwide. For example, the State regulations that control VOC emissions are federally required only for areas of the State where they are needed to attain and maintain the ozone standard; in areas of the State where such regulations are required, the regulations need be applied only to enough VOC sources with enough regulatory stringency to attain and maintain the ozone standard. Such regulations do not necessarily control all stationary benzene sources adequately. Consequently, the Agency disagrees with the commenters' assertions that existing regulatory programs for ozone/VOC's make it unnecessary to regulate any benzene sources.

Commenters suggested that EPA should adopt an acceptable carcinogenic risk target for benzene and other airborne carcinogens, citing precedents in other EPA and Federal rulemakings (OAQPS-79-3) [Part I] IV-D-13, [Part II] IV-F-1, IV-F-9; A-79-49 IV-F-1, IV-F-2).

EPA agrees that it can identify a lower *range* of risk estimates (incidence and maximum risk) where it is judged that the health risks do not pose such a public health problem as to warrant federal regulation. This, in conjunction with other factors such as achievable emissions and health risk reductions, can convince the Administrator that a source category is not appropriate to regulate under section 112. This is the case for the proposed withdrawal of the proposed benzene standards for maleic anhydride and EB/S process vents and benzene storage vessels.

Selection of Benzene Source Categories for Regulation

EPA proposed standards for four source categories of benzene emissions: maleic anhydride process vents, ethylbenzene/styrene process vents, fugitive emission sources, and benzene storage vessels. A standard will be proposed for a fifth source category, coke by-product plants. Comments submitted on each of the four proposed standards contended that each of the source categories regulated does not pose a significant risk to public health and therefore does not warrant regulation (OAQPS-79-3 [Part II] IV-D-9, IV-D-22, IV-F-1, IV-F-9; A-79-27 IV-D-24, IV-D-27, IV-D-28, IV-F-1, IV-K-1; A-79-49 IV-D-7, IV-D-10, IV-D-12; A-80-14 IV-D-10a, IV-D-13, IV-D-16, IV-F-1). Similar preproposal comments have been received on the coke by-product source category. Arguments advanced in support of this position include the relative insignificance of stationary source emissions of benzene versus mobile source emissions; the low level of estimated benzene risks compared to other public health risks; and the negligible impact of benzene control on the total U.S. leukemia incidence. EPA's response to these comments appears in the section entitled "Significance of the Estimated Carcinogenic Risks from Benzene Exposures." Additionally, commenters maintained that, even if the source categories regulated could be considered significant at proposal, emissions from these source categories are now actually much lower than projected at proposal and, thus, no longer pose significant risk.

Selection of Five Source Categories for Initial Regulation

Following the listing of benzene as a hazardous air pollutant, EPA divided the stationary sources of benzene emissions into 12 source categories. After evaluating these 12 source categories, EPA selected five source categories of benzene for initial regulation: process vents at maleic anhydride and EB/S plants, benzene fugitive emission sources, benzene storage vessels, and coke by-product plants.

EPA is collecting additional data on the remaining seven source categories to use in deciding whether or not standards development is warranted for them.

Proposal of Standards: Significant Risk Judgment

The information used in selecting the five source categories for initial regulation was preliminary information, based on screening studies of the identified source categories. During standards development prior to proposal, EPA gathered more detailed and refined information. The new information necessitated revisions in emissions estimates for the five source categories with some estimates increasing and others decreasing. Examples of the information used to upgrade emissions estimates include emissions test data, updated status on the number of operating plants, and more precise information on the control devices already installed on these plants.

In addition to upgrading the emissions estimates, EPA used the more precise emissions data to revise the quantitative risk estimates. At the time that standards for maleic anhydride process vents, EB/S process vents, benzene fugitive emissions sources, and benzene storage vessels were proposed, EPA made a judgment that the emissions from each of these source categories pose a significant leukemia risk. EPA based this judgment on the upgraded emissions and risk estimates available at that time.

Table I presents information for each source category, based on the emissions status of that source category at the time the standards were proposed. The

uncertainties in the risk estimates are described in the following paragraphs.

The ranges of maximum lifetime risk and annual leukemia incidence at proposal presented in Table I represent the uncertainty of estimates concerning benzene concentrations to which workers were exposed in the occupational studies of Infante, Aksoy, and Ott that served as the basis for developing the benzene unit risk factor. The ranges presented in this table represent 95 percent confidence limits on two sources of uncertainty in the benzene risk estimates. One source derives from the variations in dose/response among the three occupational studies upon which the benzene unit risk factor is based. A second source involves the uncertainties in the estimates of ambient exposure. In the former case, the confidence limits are based on the assumption that the slopes of the dose/response relationships are unbiased estimates of the true slope and that the estimates are log normally distributed. In the latter case, the limits are based on the assumption that actual exposure levels may vary by a factor of two from the estimates obtained by dispersion modeling (assuming that the source-specific input data are accurate).

BILLING CODE 6560-50-M

TABLE 1. BASELINE IMPACTS OF BENZENE SOURCE CATEGORIES AT PROPOSAL AND NOW

Standard	Benzene emissions (Mg/year)	Benzene and other VOC emissions (Mg/year)	Number of affected plants	Maximum lifetime risk[2] [3]	Leukemia incidence/year [3] (Cases per year)
Benzene Fugitive					
At proposal	8,300	13,200	130	1.7×10^{-4} to 1.2×10^{-3}	0.15 to 1.1
Current	7,900	12,600	128	1.5×10^{-3}	0.45
Maleic Anhydride					
At proposal	5,800	7,400	7 (5)[4]	2.3×10^{-4}	0.46
Current	960	1,250	7 (1)[4]	7.6×10^{-5}	0.029
Ethylbenzene/Styrene					
At proposal	2,400	6,240	13 (12)[4]	6.2×10^{-4} to 4.4×10^{-3}	0.027 to 0.20
Current	210	330	13 (3)[4]	1.4×10^{-4}	0.0057
Benzene Storage					
At proposal	2,200	2,200	126	1.5×10^{-4} to 1.0×10^{-3}	0.12 to 0.82
Current	620	620	126	3.6×10^{-5}	0.043

Footnotes for Table 1.

[1] Within 20 kilometers of plant.

[2] Maximum lifetime risk is the estimated probability that the people exposed continuously for 70 years to the highest maximum annual average ambient concentration of benzene will contract leukemia as a result of that exposure.

[3] The ranges of maximum lifetime risk and annual leukemia incidence at proposal presented in this table represent the uncertainty of estimates concerning benzene concentrations to which workers were exposed in the occupational studies of Infante, Abbey, and Ott that served as the basis for developing the benzene unit risk factor. The ranges presented in this table represent 95 percent confidence limits on two sources of uncertainty in the benzene risk estimates. One source derives from the variations in dose/response among the three occupational studies upon which the benzene unit risk factor is based. A second source involves the uncertainties in the estimates of ambient exposure. In the former case, the confidence limits are based on the assumption that the slopes of the dose/response relationships are unbiased estimates of the true slope and that the estimates are lognormally distributed. In the latter case, the limits are based on the assumption that actual exposure levels may vary by a factor of two from the estimates obtained by dispersion modeling. Several other uncertainties are associated with the estimated health numbers and are not quantified here. EPA has extrapolated the leukemia risks identified for occupationally exposed populations (generally healthy, white males) to the general population for whom susceptibility to a carcinogenic insult could differ. The presence of more or less susceptible subgroups within the general population would result in an occupationally-derived risk factor that may underestimate or overestimate actual risks. To the extent that there are more susceptible subgroups within the general population, the maximum individual lifetime risks are underestimated.

On the other hand, general population exposures to benzene are much lower than those experienced by the exposed workers in the occupational studies, often by several orders of magnitude. In relating the occupational experience to the general population, EPA has applied a linear, nonthreshold model that assumes that the leukemia response is linearly related to benzene dose, even at very low levels of exposure. There are biological data supporting this approach, particularly for carcinogens. However, there are also data which suggest that, for some toxic chemicals, dose/response curves are not linear, with response decreasing faster than dose at low levels of exposure. At such levels, the nonlinear models tend to produce smaller risk factors than the linear model. The data for benzene do not conclusively support either hypothesis. EPA has elected to use the linear model for benzene because this model is generally considered to be conservative compared to the nonlinear alternatives. This choice may result in an overestimate of the actual leukemia risks.

EPA estimates ambient benzene concentrations in the vicinity of emitting sources through the use of atmospheric dispersion models. EPA believes that its ambient dispersion modeling provides a reasonable estimate of the maximum ambient levels of benzene to which the public could be exposed. The models accept emission estimates, plant parameters, and meteorology as inputs and predicts ambient concentrations at specified locations, conditional upon certain assumptions. For example, emissions and plant parameters often must be estimated rather than measured, particularly in determining the magnitude of fugitive emissions and where there are large numbers of sources. This can lead to overestimates or underestimates of exposure. Similarly, meteorological data often are not available at the plant site but only from distant weather stations that may not be representative of the meteorology of the plant vicinity.

EPA's dispersion models normally assume that the terrain in the vicinity of the sources is flat. For sources located in complex terrain, this assumption would tend to underestimate the maximum annual concentration although estimates of aggregate population exposure would be less affected. On the other hand, EPA's benzene exposure models assume that the exposed population is immobile and outdoors at their residence, continuously exposed for a lifetime to the predicted concentrations. To the extent that benzene levels indoors are lower and that people do not reside in the same area for a lifetime, these assumptions will tend to overpredict exposure.

Upon reconsideration, EPA has concluded that the presentation of the risk estimates as ranges does not offer significant advantages over the presentation as the associated point estimates of the risk. Further, the proposal ranges for benzene data risk comparisons among source categories more difficult and tend to create a false impression that the bounds of the risks are known with certainty. For these reasons, the benzene risks in this rulemaking are presented as point estimates of the leukemia risk. EPA believes that these risk numbers represent plausible, if conservative, estimates of the magnitude of the actual human cancer risk posed by benzene emitted from the source categories evaluated. For comparison, the proposal ranges may be converted into rough point estimates by multiplying the lower end of the range by a factor of 2.6.

[4] Includes all plants; number in parentheses denotes number of plants with uncontrolled emissions which would be controlled by the standard.

BILLING CODE 6560-50-C

Several other uncertainties are associated with the estimated health numbers and not quantified in the proposal ranges in Table I. EPA has extrapolated the leukemia risks identified for occupationally exposed populations (generally healthy, white males) to the general population for whom susceptibility to a carcinogenic insult could differ. The presence of more or less susceptible subgroups within the general population would result in an occupationally-derived risk factor that may underestimate or overestimate actual risks. To the extent that there are more susceptible subgroups within the general population, the maximum individual lifetime risks are underestimated.

On the other hand, general population exposures to benzene are much lower than those experienced by the exposed workers in the occupational studies, often by several orders of magnitude. In relating the occupational experience to the general population, EPA has applied a linear, nonthreshold model that assumes that the leukemia response is linearly related to benzene dose, even at very low levels of exposure. There are biological data supporting this approach, particularly for carcinogens. However, there are also data which suggest that, for some toxic chemicals, dose/response curves are not linear, with response decreasing faster than dose at low levels of exposure. At such levels, the nonlinear models tend to produce smaller risk factors than the linear model. The data for benzene do not conclusively support either hypothesis. EPA has elected to use the linear model for benzene because this model is generally considered to be conservative compared to the nonlinear alternatives. This choice may result in an overestimate of the actual leukemia risks.

EPA estimates ambient benzene concentrations in the vicinity of emitting sources through the use of atmospheric dispersion models. EPA believes that its ambient dispersion modeling provides a reasonable estimate of the maximum ambient levels of benzene to which the public could be exposed. The models accept emission estimates, plant parameters, and meteorology as inputs and predicts ambient concentrations at specified locations, conditional upon certain assumptions. For example, emissions and plant parameters often must be estimated rather than measured, particularly in determining the magnitude of fugitive emissions and where there are large numbers of sources. This can lead to overestimates or underestimates of exposure. Similarly, meteorological data often are not available at the plant site but only from distant weather stations that may not be representative of the meteorology of the plant vicinity.

EPA's dispersion models normally assume that the terrain in the vicinity of the sources is flat. For sources located in complex terrain, this assumption would tend to underestimate the maximum annual concentration although estimates of aggregate population exposure would be less affected. On the other hand, EPA's benzene exposure models assume that the exposed population is immobile and outdoors at their residence, continuously exposed for a lifetime to the predicted concentrations. To the extent that benzene levels indoors are lower and that people do not reside in the same area for a lifetime, these assumptions will tend to overpredict exposure.

Upon reconsideration, EPA has concluded that the presentation of the risk estimates as ranges does not offer significant advantages over the presentation as the associated point estimates of the risk. Further, the proposal ranges for benzene make risk comparisons among source categories more difficult and tend to create a false impression that the bounds of the risks are known with certainty. For these reasons, the benzene risks in this rulemaking are presented as point estimates of the leukemia risk. EPA believes that these risk numbers represent plausible, if conservative, estimates of the magnitude of the actual human cancer risk posed by benzene emitted from the source categories evaluated. For comparison, the proposal ranges may be converted into rough point estimates by multiplying the lower end of the range by a factor of 2.6.

Post-Proposal Review of Significant Risk Judgment

Some commenters on the proposed standards indicated that benzene emissions were actually much lower than estimated at proposal, citing factors such as increased controls, plant closures, reduced production capacity, and lower emission factors. In support of their contentions, they submitted detailed plant-specific information and results of emission test programs.

Based on this updated information, EPA has revised benzene emissions for the various source categories (see Table I). The maleic anhydride emissions estimates now include consideration of all new controls, plant closures, and changes in feedstock. The EB/S emissions estimates are those provided by the industry, based on plant-specific information. (In addition, EPA-assumed flare efficiency has been revised to 98 percent from 60 percent.) New benzene emission factors have been developed for benzene storage tanks and refined for benzene fugitive sources.

Based on these revised emissions estimates, EPA reconsidered whether benzene emissions from maleic anhydride process vents, EB/S process vents, benzene fugitive emission sources, and benzene storage vessels still warrant Federal regulation under Section 112. The factors considered by EPA are described in the following paragraphs. (The selection of coke by-product recovery plants for regulation is discussed in the preamble to the proposed standard for that source category and is not discussed further here).

Benzene fugitive emissions, which are not substantially different than they were when judged to be significant at proposal, contribute 7,900 Mg/yr; this figure reflects current controls. (EPA adjusted the control level for petroleum refineries in nonattainment areas to reflect controls required by States in accordance with EPA's Control Techniques Guideline (CTG) document. This adjustment reduced emissions, but the reduction was offset to some extent by refinements in emissions factors.) Approximately 20 to 30 million people live within 20 kilometers of the 128 plants with these fugitive emissions. These people are exposed to higher levels of benzene than is the general population. Due to the lack of a demonstrated threshold for benzene's carcinogenic effects, these people not only incur a higher benzene exposure but also run greater risk of contracting leukemia due to that exposure.

EPA revised the quantitative risk assessments for this source category based on the updated emissions estimates, the revised risk factor, and the more detailed SAI human exposure model. The lifetime risk of contracting leukemia for the most exposed individuals is estimated to be about 1.5×10^{-3} for benzene fugitive emission sources, and the increased leukemia incidence as a result of exposure to the current fugitive emissions is estimated to be about 0.45 cases per year. As explained earlier in this section, there is considerable uncertainty associated with the calculation of leukemia incidence and maximum lifetime risk numbers.

The number of process units emitting benzene fugitive emissions is anticipated to grow from about 240 to 310 units. These new sources probably would increase the number of people exposed to benzene emitted from this

source category and increase the estimated leukemia incidence accordingly.

Based on the human carcinogenicity of benzene, the magnitude of benzene fugitive emissions, the estimated ambient benzene concentrations in the vicinity of the plants with fugitive emissions, the proximity of people to these plants, the resulting estimated maximum individual risks and estimated incidence of leukemia cases in the exposed population, the projected increase in benzene emissions as a result of new sources, the estimated reductions in emissions and health risks that can be achieved, and consideration of the uncertainties associated with the quantitative risk estimates (including effects of concurrent exposures to other substances and to other benzene emissions), EPA finds that benzene emissions from benzene fugitive emission sources pose a significant cancer risk and that the establishment of a national emission standard under Section 112 is warranted. These factors will be discussed in more detail in the forthcoming document, "Benzene Fugitive Emissions—Background Information for Promulgated Standards," EPA–450/3–80–032b.

Several other factors were also considered which support this finding. First, if no standards were promulgated, several existing plants would remain uncontrolled or poorly controlled. Some benzene fugitive emissions sources are located in nonattainment areas and are controlled to some extent in accordance with the CTG; others are in attainment areas where no control is required. Control techniques are readily available to reduce uncontrolled emissions from benzene fugitive emission sources at reasonable costs. Second, nationwide standards would ensure that existing sources are controlled on a continuing basis. Third, if no standard were promulgated, new sources could remain uncontrolled or poorly controlled, thereby increasing cancer risks.

The revised estimated baseline emission and health impacts for maleic anhydride and EB/S process vents and benzene storage vessels have decreased significantly since proposal of the standards for these source categories. These impacts are presented in Table 1. Because of this decrease and the small additional reduction in health risks that could be achieved, the Agency has concluded that these source categories no longer warrant federal regulation under section 112. The basis for this decision is discussed in an accompanying Federal Register notice that proposes withdrawal of the proposed benzene standards for these three source categories.

Docket

The dockets are organized and complete files of all the information submitted to, or otherwise considered by, EPA in the development of this proposal. The principal purposes of the docket are to allow interested parties to effectively participate in the rulemaking process; and (2) to serve as the record in case of judicial review except for interagency review materials [307(d)(7)(A)].

Miscellaneous

This proposal was submitted to the Office of Managment and Budget (OMB) for review as required by Executive Order 12291. Any comments from OMB to EPA responses to those comments are available for inspection in Docket Number OAQPS–79–3 (maleic anhydride), A–79–49 (EB/S), or A–80–14 (benzene storage), Central Docket Section, at the address given in the ADDRESSES section of this preamble.

References

(1) Safe Drinking Water Committee. National Research Council. "Drinking Water and Health." National Academy of Sciences. Washington, D.C., 1977.

(2) Picciano, D. "Monitoring Industrial Populations by Cytogenetic Procedures" in *Proceedings of a Workshop on Methodology for Assessing Reproductive Hazards in the Workplace*. P. F. Infante and M. S. Legator, eds. April 19–22, 1978.

(3) Kilian, D. J., and Daniel, R. C. "A Cytogenetic Study of Workers Exposed to Benzene in the Texas Division of Dow Chemical, U.S.A." February 27, 1978.

(4) National Academy of Sciences–National Research Council "Health Effects of Benzene: A Review" for U.S. EPA (EPA–560/5–76–003).

(5) National Institute for Occupational Safety and Health "Update Criteria and Recommendations for a Revised Benzene Standard." September 1976.

(6) U.S. EPA "Assessment of Health Effects of Benzene Germane to Low-Level Exposures." Office of Health and Ecological Effects, Office of Research and Development. September 1978 (EPA–600/1–78–061).

(7) U.S. EPA. "Assessment of Human Exposures to Atmospheric Benzene." Office of Air Quality Planning and Standards. June 1978. EPA–450/3–78–031.

(8) U.S. EPA. "Carcinogen Assessment Group's Final Report on Population Risk to Ambient Benzene Exposures." Roy Albert, Chairman, Carcinogen Assessment Group. January 10, 1979 (EPA–450/5–80–004).

(9) Picciano, D. "Cytogenetic Study of Workers Exposed to Benzene." *Env. Res.* 19:33–38, 1979.

(10) Bloom, A. D., Y. Nakagone, A. Awa, and S. Nenishi. "Chromosome aberrations and malignant disease among A-bomb survivors." *Am. J. Public Health*, 60:641–644, 1970.

(11) Mulvihill, J. J. In *Persons at High Risk of Cancer*, ed. Fraumeni, J. F., Jr., Academic Press, N.Y., 1975, pp 3–37.

(12) Maltoni, C., and C. Scarnato. "First Experimental Demonstration of the Carcinogenic Effects of Benzene." Estratto da "La Medicina del Lavoro" 70:5, 1979.

(13) Snyder, Carroll A. et al. "The Inhalation Toxocology of Benzene: Incidence of Hematopoietic Neoplasms and Hematotoxicity in AKR/J and C57BL/6J Mice" Tox. and Appl. Pharm. 54:323–331, 1980.

(14) Infante, P. F., R. Rinsky, J. Wagoner, and R. Young. "Leukemia in Benzene Workers." Lancet, 2:76–78, 1977a.

(15) Aksoy, M., S. Erdem, and G. Dincol. "Types of Leukemia in Chronic Benzene Poisoning, A Study in Thirty-Four Patients. Acta Haematol. 55:65–72, 1976.

(16) Ott, M. G., J. C. Townsend, W. A. Fishback, and R. A. Langner. "Mortality Among Individuals Occupationally Exposed to Benzene." Exhibit 154, OSHA Benzene Hearings, July 19–August 10, 1977.

(17) Rinsky, R. A., R. Young, and A. Smith. "Leukemia in Benzene Workers." American Journal of Industrial Medicine, 2:217–245, 1981.

(18) Occupational Safety and Health Administration, Docket #H–059, Occupational Exposure to Benzene, Proposed Standard, Transcript of Public Hearing, July 19–August 10, 1977b.

(19) Supreme Court of the United States. *Industrial Union Department v. American Petroleum Institute et al.*, 448 U.S. 607 (1980).

(20) Infante, P. F., R. Rinsky, J. Wagoner, and R. Young. "Benzene and Leukemia." Lancet, October 22, 1977b.

(21) White, M. C., P. Infante, and B. Walker, Jr. "Occupational Exposure to Benzene: A Review of Carcinogenic and Related Health Effects Following the U.S. Supreme Court Decision." Am. J. Indust. Med., 1:233–243, 1980.

(22) Environmental Survey, Occupational Health Study Group, University of North Carolina, School of Public Health, 1974.

(23) International Agency for Research on Cancer, Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, 29:93–148, May 1982.

(24) Aksoy, M. K. Dincol, T. Akgun, E. Erdem, and G. Dincol. "Haematological effects of chronic benzene poisoning in 217 workers." Br. J. Ind. Med. 28:296–302, 1971.

(25) Aksoy, M. K. Dincol, S. Erdem, and G. Dincol. "Acute leukemia due to chronic exposure to benzene." Am. J. Med., 52:160–166, 1972.

(26) Aksoy, M., S. Erdem, and G. Dincol. "Leukemia in shoeworkers exposed chronically to benzene." Blood, 44(6):837–841, 1974.

(27) U.S. Department of Labor, Bureau of the Census, "County Demographic Profiles, Turkey," ISP–DP–25, August 1980.

(28) Thorpe, J. J. "Epidemiologic survey of leukemia in persons potentially exposed to benzene." J. Occup. Med. 16(6):375–382, 1974.

(29) Brown, S. "Letter to the editor concerning Thorpe article on leukemia and potential benzene exposure," JOM 17(1):5–6, 1975.

(30) Flaklow, P. J. "The origin and development of human tumors: studies with cell markers." New Eng. J. Med. 291:26–35. 1974.

(31) Gartler, S. M. "Utilization of mosaic systems in the study of the origin and progression of tumors" in *Chromosomes and Cancer*. J. German, ed. Wiley Interscience. New York. p. 313–334. 1974.

(32) Strong, L. C. "Susceptible subgroups." presented at NIEHS Conference on the Problems of Extrapolating the Results of Laboratory Animal Data to Man and of Extrapolating the Results from High-Dose Level Experiments to Low Dose Level Exposures. Pinehurst, N.C., March 10–12, 1978.

(33) Cottier, H., M. W. Hess, H. U. Keller, P. Lusciett, and B. Sordat "Immunological deficiency states and malignacy." In: *Interaction of Radiation and Host Immune Defense Mechanisms in Malignancy*. Proceedings of a conference at Greenbrier, W. Va., March 1974. p. 30–44.

(34) Rickert, D., and R. Irons (oral statements) from U.S. EPA. "Public Hearing: National Emission Standards for Hazardous Air Pollutants: Benzene Emissions from Maleic Anhydride Plants." August 21, 1980 (Transcript pp. 5–24).

(35) Goldstein, B. "Hematotoxicity in Man." *A Critical Evaluation of Benzene Toxicity*. S. Laskin, and B. Goldstein, ed., 1977. p. 165.

(36) Goldstein, p. 165.

(37) Vigliani, E. C., and A. Forni. "Benzene, chromosome changes, and leukemia." J. Occup. Med. 11:146–149. 1969.

(38) Tabershaw Cooper Associates. *A Mortality Study of Petroleum Refinery Workers Project OH–1* (1974) (OSHA Benzene Record, Ex. 2–59).

(39) Stallones, R. A., and D. Syblik. *Report on Mortality from Leukemia* (1977) (OSHA Benzene Record, Ex. 113, C.2).

(40) National Cancer Advisory Board. "General Criteria for Assessing the Evidence for Carcinogenicity of Chemical Substances: *Report of the Subcommittee on Environmental Carcinogens*." J.N.C.I. 58:481–485. 1977.

(41) Crump, K., D. Hoel, C. Langley, and R. Peto. "Fundamental carcinogenic processes and their implications for low-dose risk assessment." Cancer Res. 36:2, pp. 2973–2979. 1976.

(42) Chemical and Engineering News, May 3, 1976, p. 11.

(43) U.S. EPA. "Volatile Organic Chemicals in the Atmosphere: An Assessment of Available Data" Office of Research and Development. 1983 (EPA–600/3–83–027(A)).

Dated: May 23, 1984.

William D. Ruckelshaus,

Administrator.

List of Subjects in 40 CFR Part 61

Air pollution control, Asbestos, Beryllium, Hazardous substances, Mercury, Reporting and recordkeeping requirements, Vinyl chloride.

[FR Doc. 84-14679 Filed 6-5-84; 8:45 am]

BILLING CODE 6560-50-M