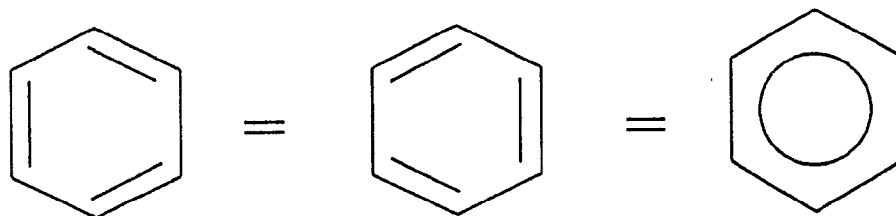


I. INTRODUCTION



BENZENE

Benzol, Cyclohexatriene, Pyrobenzol

C_6H_6 MW = 78.11

C = 92.25% H = 7.75%

CAS No. 71-43-2

STRUCTURE AND PROPERTIES

The representation of the benzene molecule has evolved from the resonance ring structures described by Kekule in 1865 to the structure in which all six carbon-to-carbon bonds are identical (see above representations).

Benzene at room temperature is a highly flammable, colorless, transparent liquid having a boiling point of 80° C and a melting point of 5.5° C. Slightly soluble in water (1.8 g/liter), benzene is miscible with a variety of organic solvents and with oils. Benzene has a vapor pressure of 13 mm Hg, a flashpoint of -11° C, and a flammability limit in air of 1.5%-8.0%. It is generally available in three grades--refined, nitration, and industrial. The differences are based on the content of nonaromatics specified (less than 0.15% for refined) and on the distillation range (less than 1° C for refined and nitration grades and less than 2° C for industrial grade).

PRODUCTION, USE, AND OCCURRENCE

Benzene ranks 16th in production volume for chemicals produced in the United States, with approximately 9.9 billion pounds being produced in 1984, 9.1 billion pounds in 1983, and 7.8 billion pounds in 1982 (Chem. & Eng. News, 1985). This simplest aromatic chemical is used primarily as a raw material in the synthesis of styrene (polystyrene plastics and synthetic rubber), phenol (phenolic resins), cyclohexane (nylon), aniline, maleic anhydride (polyester resins), alkylbenzenes (detergents), chlorobenzenes, and

other products used in the production of drugs, dyes, insecticides, and plastics (Purcell, 1978). Benzene, along with other light, high-octane aromatic hydrocarbons, such as toluene and xylenes, is a component of motor gasoline. Benzene is also used as a solvent, but for most applications, it has been replaced by less hazardous solvents.

Benzene has a long history of extensive use in industry, first as a volatile solvent and later as a starting material for the synthesis of other chemicals. An aromatic fraction containing benzene was known in the 18th century as a product of the distillation of coal (Purcell, 1978). Benzene, or bicarburet of hydrogen as it was then called, was first isolated in 1825 by Faraday, who obtained it from a liquid condensed by compressing oil gas. In 1833, Mitscherlich obtained bicarburet of hydrogen by distilling benzoic acid with lime and suggested the name "benzin" for the compound. Leibig objected and proposed "benzole." In 1845, benzene was found by Hoffman in light oil derived from coal tar. The commercial recovery of benzene from this source was developed and described by Mansfield in 1849. The synthesis of benzene by the polymerization of acetylene was first carried out by Berthelot in 1866. Discovery of benzene in coal gas after 1876 initiated the recovery of coal gas light oil as a source of benzene. Although petroleum was known to contain benzene, recovery of this material was not undertaken on a commercial scale until about 1941. Several years after the end of World War II, the demand for benzene by the rapidly expanding chemical

industry exceeded the total production by the coal carbonization industry. To help meet this deficit, benzene was produced in ever increasing amounts by the petroleum and petrochemical industries by the recovery from reformat and liquid byproducts of ethylene manufacture. Today, production from these sources far exceeds that from coal.

Benzene, the parent hydrocarbon of a series of aromatic compounds, should not be confused with the product benzoin or benzine, a comparatively low boiling petroleum fraction consisting of a mixture of hydrocarbons, predominately aliphatic in type. The term "benzol" is used to describe commercial products that are largely benzene; the term is rarely seen in the United States but is still found in British publications. The pure compound is now called benzene, the name approved by the International Union of Pure and Applied Chemistry and adopted by industries and nomenclature experts.

Benzene recovered from both petroleum and coal sources is extracted from catalytic reformat made in oil refineries, from pyrolysis gasoline made in steam-cracking olefin plants, from light oils in coking coal, and from dealkylation of toluene (Chem. & Eng. News, 1980). The major derivatives of benzene are ethylbenzene (50%), cumene (15%), cyclohexane (15%), and aniline (5%). Primary end uses include polystyrene (25%), nylon (20%), other styrenic polymers (10%), and rubber (5%). As an antiknock chemical, benzene and benzene-enriched aromatics are added to gasoline as a replacement for alkyl lead compounds. The benzene content of European motor fuel is about 5% (CGOS, 1982); U.S. gasoline contains an average of 0.8% (IARC, 1982).

Benzene is apparently ubiquitous in the environment. The main source of benzene in the environment is from industrial processes. However, benzene is a natural constituent of crude oil (Brief et al., 1980). Early uses of benzene as a solvent regularly resulted in workplace air concentrations of 1,600-3,200 mg/m³ (approximately 500-1,000 ppm) and above (IARC, 1982). In addition to the major source of benzene in the environment (industry), benzene is found in air, water, and sediments; soil and plants; food,

beverages, and feed; tobacco and tobacco smoke; and pyrolysis products.

HUMAN EXPOSURE

Hunter (1978) chronicles the history of long-term benzene exposure; a few early facts are given here for historic interest. In the last years of the 19th century, the use of benzene as a rubber solvent led to small outbreaks of "purpura hemorrhagica" with a number of deaths from aplastic anemia. Selling (1916) was the first to associate benzene poisoning with leukopenia. Unfortunately, this association led to using benzene in the treatment of leukemia; benzene was given in gelatin capsules starting with 3 g per day and increasing to 5 g per day. The result was a gain in weight, shrinkage of the spleen, and great reduction of the white cell count. After some months of treatment, however, most patients developed multiple hemorrhages, especially from the fauces and gums; women experienced troublesome menorrhagia. With advancing anemia (loss of white and red blood cells), fever, and bleeding, these patients died of chronic poisoning. In most industrial countries, the use of benzene as a solvent increased after World War I. In 1922, Alice Hamilton began her efforts to reduce the hazards of benzene in American industry.

Workplace and environmental exposure to benzene is widespread. As many as 2 million workers may be exposed (NIOSH, 1977; Brief et al., 1980). Nonoccupational exposure to benzene results primarily from environmental contamination, the chief sources being automobile and factory emissions and cigarette smoke. Benzene intake by urban residents in the United States is approximately 850 µg per day (NRC, 1980). Benzene is present in cigarette smoke at concentrations of 47-64 ppm (Lauwerys, 1979). Dietary intake may be as high as 250 µg per day (NRC, 1980). Benzene has been identified in bottled artesian water (Dowty et al., 1975) and in boiled beef and canned beef stew (Chang and Peterson, 1977).

Two recent studies have shown considerable benzene absorption via skin. Susten et al. (1985), using hairless mice, calculated that 20%-

I. INTRODUCTION

40% of the total benzene dose received by humans in tire-building operations could be absorbed dermally. Blank and McAuliffe (1985) calculated that an adult working in ambient air containing 10 ppm benzene would absorb 7.5 μ l per hour from inhalation and 1.5 μ l per hour from whole body (2 m²) exposure. They also calculated that 100 cm² glabrous skin in contact with gasoline containing 5% benzene would absorb 7.0 μ l per hour. These results are not too different from those decided by Susten et al. (1985) using mouse skin.

EXPOSURE STANDARD

Adopted in 1971, the present Occupational Safety and Health Administration (OSHA) standard for benzene is an 8-hour time-weighted average (TWA) of 10 ppm with a ceiling limit of 25 ppm and a maximum peak concentration of 50 ppm for a 10-minute period (Fed. Reg., 1985). The standard apparently was based on concern about the development of aplastic anemia and depression of various cellular elements in the blood, but not on concern about the development of cancer. In May 1977, OSHA issued an Emergency Temporary Standard that reduced exposures to benzene to 1 ppm and included other provisions. In February 1978, OSHA promulgated a new permanent standard for occupational exposure to benzene based on evidence that there was a causal connection between benzene exposure and leukemia. This standard limited employee exposure to 1 ppm as an 8-hour TWA, the level then estimated to be the lowest feasible. The standard included a ceiling limit of 5 ppm for any 15-minute period during an 8-hour day, limits on eye and skin contact with benzene, and industrial hygiene and medical surveillance provisions.

The United States Court of Appeals for the Fifth Circuit vacated the standard in 1978 and the Supreme Court affirmed that judgment in 1980. The Supreme Court held that OSHA's prior approach (reducing exposures to the lowest feasible level when there was strong qualitative evidence of carcinogenicity) was insufficient.

In December 1985, OSHA issued a newly proposed rule and notice of hearing on occupational exposure to benzene (Fed. Reg., 1985). In this

announcement, OSHA "proposes to reduce the existing benzene permissible exposure limit of 10 parts benzene per million parts of air (10 ppm) to an eight (8)-hour time-weighted average of 1 ppm to reduce substantially a significant health risk." The current 10-ppm exposure limit in the United States corresponds to that in most other industrialized countries, except for Sweden (5 ppm) and the USSR (5 mg/m³ approximately equivalent to 2 ppm) (Holmberg and Lundberg, 1985).

TOXICITY IN ANIMALS

The Proceedings of the International Collegium Ramazzini Conference on Benzene held in New York, New York, on November 3-4, 1983, have been published (Am. J. Ind. Med., 1985; Mehlman, 1985).

The following oral LD₅₀ values have been reported for benzene: 0.93 g/kg for Sprague-Dawley rats (Cornish and Ryan, 1965), 3.8 ml/kg for young adult male Sprague-Dawley rats (Kimura et al., 1971), and 5.6 g/kg for male Wistar rats (Wolf et al., 1956). The LC₅₀ value for a 7-hour exposure is 10,000 ppm for mice (Svirbely et al., 1943).

Depression of bone marrow function, resulting in leukopenia, was observed in rodents exposed to benzene: Sprague-Dawley rats (44 ppm, 5 hours per day, 4 days per week for 5 weeks); AKR/J mice (300 ppm, 6 hours per day for 28 days); and male CD-1 mice (103 ppm, 6 hours per day, 5 days per week, for 26 weeks). Similar results were also obtained in Wistar rats administered benzene orally (1 mg/kg for 14 days) and F344 rats administered benzene by subcutaneous injection (1 ml/kg per day for 10 days) (Gerarde, 1956; Deichmann et al., 1963; Irons et al., 1979; Green et al., 1981). Pretreatment of rats with Aroclor 1254^a resulted in decreased polyphenolic metabolites located in bone marrow and provided protection against benzene-induced lymphocytopenia (Greenlee et al., 1981). Relative spleen weights were reduced in DDF₁ mice administered benzene by inhalation (4,680 ppm, 8 hours per day for 3 days), and involution of the spleen occurred in Wistar rats administered 1 mg/kg by gavage for 14 days (Uyeki et al., 1977; Gerarde, 1956).

The hematotoxicity of benzene has been studied extensively, and a number of review articles have been published (IARC, 1982; Laskin and Goldstein, 1977; Snyder and Kocsis, 1975). The large literature on the effects of benzene on the hematopoietic system is not discussed in this report.

METABOLISM AND EXCRETION

After monosubstitution of benzene has occurred, three disubstitution positions are chemically possible: 1,2- (*ortho*-); 1,3- (*meta*-); or 1,4- (*para*-). Electron-withdrawing groups promote meta-substitutions and electron donating groups favor *ortho*- and *para*- substitutions. Since most benzene metabolites are hydroxyl additions, the *ortho*-/*para*- positions predominate (Purcell, 1978). Following metabolic formation of phenol (C_6H_5OH), further metabolism leads to both catechol (pyrocatechol; *ortho*-dihydroxybenzene) and hydroquinone (*para*-dihydroxybenzene). The third possible form, *meta*-dihydroxybenzene (resorcinol), probably does not occur. As described by Irons and Pfeifer (1982), the primary oxidation of benzene occurs via the cytochrome P-450-dependent monooxygenase system, resulting in biologically reactive intermediates (Figure 1). As generally agreed, benzene per se does not represent the principal structural moiety causing the identified toxic effects on the bone marrow or lymphoid system (Irons and Pfeifer, 1982; Bolcsak and Nerland, 1983). The metabolism and elimination of benzene in humans appear to be similar to those in rats and mice; the amounts of various metabolites, the extent of metabolism, and the nature of the phenol conjugates depend on the species, strain, and the route of administration (Rusch et al., 1977).

The National Institute of Environmental Health Sciences is conducting studies of the comparative metabolism and disposition of benzene and its metabolites in selected tissues in F344 rats and B6C3F₁ mice by the oral and inhalation routes (Sabourin et al., 1986). In both species, metabolism of benzene is nonlinear at all doses above 50 mg/kg. During 6-hour inhalation exposures, metabolism is exponentially related to

vapor concentration. One-half maximal saturation occurs at 280 ppm in rats and at 115 ppm in mice. Animals are exposed to ³H-benzene at 0.5-300 mg/kg per day (oral) or 1.0-300 ppm for 6 hours per day (inhalation) for 5 days. Following the final exposure, animals will be killed at multiple time points and benzene and its metabolites quantitated in urine, blood, feces, liver, lung, bone marrow, and Zymbal gland. Metabolites in oral cavity, stomach, skin around the mouth, and skin on the back will be analyzed only at the low and high doses. Metabolites to be analyzed by high-performance liquid chromatography include benzene, trihydroxybenzene, hydroquinone, catechol, phenol, muconic acid, and water soluble conjugates. To date, exposure of F344 rats and B6C3F₁ mice to ³H-benzene at 0.5, 5, 15, 50, 150, and 300 mg/kg per day by the oral route has been completed, and tissue samples are currently being analyzed for both water-soluble and unconjugated metabolites. To determine the actual exposure of the animal to a given dose of benzene requires that the amount of uptake be known. Therefore, the gastrointestinal and pulmonary uptake of ¹⁴C-benzene is being investigated. Gastrointestinal uptake studies indicate nearly 100% uptake of orally administered benzene by the gastrointestinal tract in all three animal strains. At 300 mg/kg, approximately 60% of the administered benzene is exhaled and the remainder is excreted in the urine. However, at a dose of 0.5 mg/kg, almost all of the ¹⁴C-benzene equivalents are excreted in the urine.

GENETIC TOXICOLOGY OF BENZENE

A summary of the genetic toxicology of benzene is shown in Table 2. Recent findings on the genetic toxicology of benzene and other solvents (toluene, xylenes, phenols) have been summarized by Dean (1985).

Bacterial Systems

Many studies have shown that benzene is not mutagenic in bacteria. Eight strains of *Salmonella* have been used, and rat liver S9 has been prepared from uninduced animals and from animals induced with 3-methylcholanthrene, phenobarbital, or Aroclor 1254 (Simmon et al., 1977;

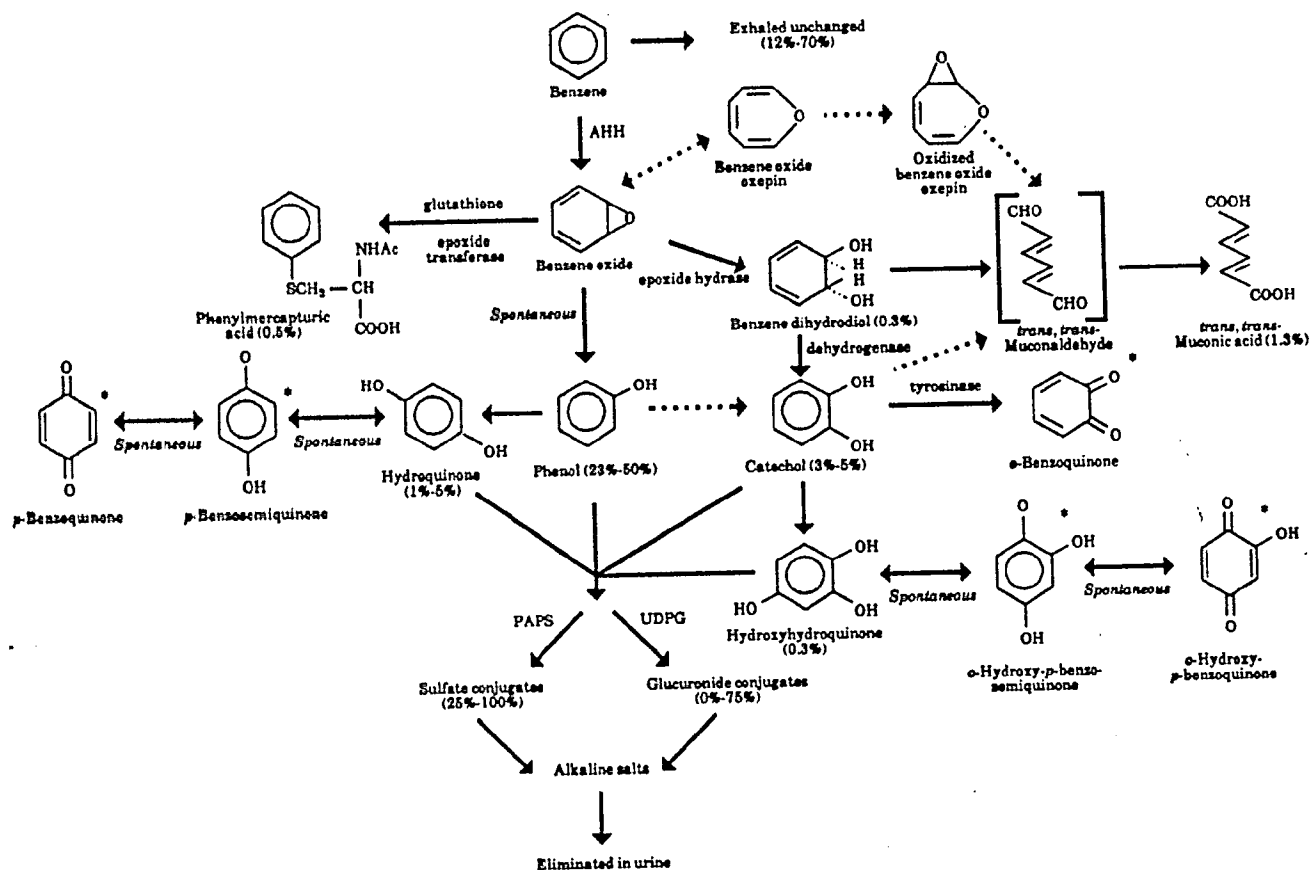


FIGURE 1. PATHWAYS OF BENZENE METABOLISM AND EXCRETION

Adapted from Parke and Williams, 1953; Laskin and Goldstein, 1977; Goldstein et al., 1982; Irons and Pfeifer, 1982; Pfeifer and Irons, 1983; Erexson et al., 1985; Sawahata et al., 1985. Values in parentheses are percentages of metabolic products detected in urine of animals (rabbits, rats, mice, dogs) or humans. Asterisks denote putative or demonstrated alkylating activity toward intracellular nucleophiles AHH = aryl hydrocarbon hydroxylase; UDPG = uridine diphosphate glucuronyl transferase; PAPS = 3'-phospho-adenosine-5'-phosphosulfate. Dashed lines indicate putative pathways. *trans,trans*-Muconaldehyde is a postulated intermediate.

TABLE 2. SUMMARY OF THE GENETIC TOXICOLOGY OF BENZENE

Test System	Endpoint	Result	References
Bacterial Systems			
<i>Salmonella typhimurium</i>	Gene mutation	-	Lyon, 1975
		-	Cotruvo et al., 1977
		-	Simmon et al., 1977
		-	Shahin and Fournier, 1978
		-	Kaden et al., 1979
		-	Bartsch et al., 1980
		-	Florin et al., 1980
		-	Ho et al., 1981
-	Hermann, 1981		
<i>Bacillus subtilis</i>		-	Tanooka, 1977
<i>B. subtilis</i>	DNA damage	+	McCarroll et al., 1981a
<i>Escherichia coli</i>		+	McCarroll et al., 1981b
<i>E. coli</i>		-	Rosenkranz and Leifer, 1980
Nonmammalian Eukaryotes			
<i>Saccharomyces cerevisiae</i>	Gene mutation	-	Egilsson et al., 1979
<i>Tradescantia</i>		+	Schairer et al., 1978
		+	Schairer and Sautkulis, 1982
<i>Drosophila melanogaster</i>		-	Nylander et al., 1978
		-	Kale and Baum, 1983
Mammalian Cells (in vitro)			
Mouse lymphoma	Gene mutation	-	Lebowitz et al., 1979
Human leukocytes	Chromosomal aberrations	+	Koizumi et al., 1974
Human lymphocytes	Chromosomal aberrations	+	Morimoto, 1974
		-	Gerner-Smidt and Friedrich, 1978
	Sister-chromatid exchanges	-	Gerner-Smidt and Friedrich, 1978
		-	Morimoto and Wolff, 1980
		+	Morimoto, 1983
	+	Morimoto et al., 1983	
Chinese hamster ovary cells	Chromosomal aberrations	-	NTP (Appendix G)
Mammals (in vivo)			
Mice	Micronuclei	+	Diaz et al., 1980
		+	Hite et al., 1980
		+	Meyne and Legator, 1980
		+	Siou et al., 1981
		+	Tunek et al., 1982
		+	NTP (Appendix G)
	Chromosomal aberrations	+	Meyne and Legator, 1980
		+	Tice et al., 1980
		+	Siou et al., 1981
		+	Tice et al., 1982

TABLE 2. SUMMARY OF THE GENETIC TOXICOLOGY OF BENZENE (Continued)

Test System	Endpoint	Result	References
Mammals (in vivo)			
Mice (Continued)			
	Sister-chromatid exchanges	+	Tice et al., 1980
		+	Tice et al., 1982
	Sperm morphology	+	Topham, 1980
Rats	Micronuclei	+	Lyon, 1975
	Chromosomal aberrations	+	Lyon, 1975
		+	Dean, 1969
		+	Anderson and Richardson, 1981
	Sperm morphology	-	Lyon, 1975
Hamsters	Micronuclei	-	Siou et al., 1981
	Chromosomal aberrations	-	Siou et al., 1981
Rabbits	Chromosomal aberrations	+	Kissling and Speck, 1971
Humans	Chromosomal aberrations	+	Reviewed by IARC, 1982

Cotruvo et al., 1977; Shahin and Fournier, 1978; Florin et al., 1980; Ho et al., 1981; Hermann, 1981). Although all of these studies used the standard plate-incorporation assay described by Ames et al. (1975), negative results were obtained in a host-mediated assay that consisted of strain TA1950 injected intraperitoneally into Swiss albino mice; the mice then received two subcutaneous 0.1-ml injections of benzene at 1-hour intervals (Lyon, 1975). Bartsch et al. (1980) exposed strains TA98 and TA100 in inverted Petri dishes at concentrations less than or equal to 20% benzene (v/v) in air in a 10-liter desiccator for 4 or 12 hours at 37°C. The results were negative in the presence or absence of Aroclor-induced rat liver S9. Although all of these studies were reverse-mutation assays at a histidine gene in *Salmonella*, a forward-mutation assay in *Salmonella* for resistance to 8-azaguanine also failed to detect any mutagenic activity of benzene (Kaden et al., 1979). Benzene not only failed to revert a variety of strains of *Salmonella* but also did not revert a histidine auxotroph of *Bacillus subtilis* (Tanooka, 1977). Although McCarroll et al. (1981a,b) reported that benzene caused growth inhibition by producing DNA damage in *B. subtilis* and *Escherichia coli*, Rosenkranz and Leifer (1980) found

that benzene was negative in the *E. coli* pol A test.

Nonmammalian Eukaryotic Systems

Benzene failed to induce petites in yeast (Egilsson et al., 1979) and was negative for both somatic mutation (Nylander et al., 1978) and germ cell mutation (sex-linked recessive lethal) in *Drosophila* (Kale and Baum, 1983). However, exposure of vascular plant *Tradescantia* resulted in mutations (Schairer et al., 1978; Schairer and Sautkulis, 1982). Thus, it appears that this higher plant may be capable of metabolizing benzene to mutagenic metabolites.

Mammalian Cells in Vitro

Benzene was not mutagenic in the mouse lymphoma forward-mutation assay in L5178Y/TK⁺ cells (Lebowitz et al., 1979). However, the genotoxic effects of benzene have been studied in cultured human cells. Koizumi et al. (1974) showed that benzene inhibited DNA synthesis in HeLa cells and in human leukocytes in vitro. The authors also found that benzene induced chromosome breaks and gaps in cultured human leukocytes, but high doses (1.1 and 2.2 × 10⁻³ M) and long exposure times (72 hours) were

required to produce the chromosomal abnormalities. Morimoto (1974) reported that treatment of cultured human lymphocytes with benzene at high doses (2×10^{-4} to 3×10^{-3} M) for long exposure times (53 hours) caused chromosomal abnormalities, including acentric fragments, breaks, and gaps. These observations were not confirmed by Gerner-Smidt and Friedrich (1978), who treated cultured human lymphocytes with benzene (2.1×10^{-2} M) for 72 hours and found no increase in either chromosomal aberrations or in sister-chromatid exchanges (SCE's). Likewise, Morimoto and Wolff (1980) found that benzene (5×10^{-3} M for 72 hours) did not induce SCE's in cultured human lymphocytes; these authors did find, however, that two major metabolites, catechol and hydroquinone, induced SCE's at low concentrations (3 and 9×10^{-5} M, respectively). None of these studies in cultured human cells incorporated exogenous metabolic activation. Benzene did not induce SCE's or chromosomal aberrations in Chinese hamster ovary (CHO) cells in the presence or absence of Aroclor-induced rat liver S9 (Appendix G). Morimoto (1983) and Morimoto et al. (1983) reported that benzene induced SCE's in vitro, but high doses of benzene (5 mM) and of S9 (10%-20%) were required.

Mammals in Vivo

Although benzene has given mixed results for the induction of SCE's and for chromosomal aberrations in vitro, it is one of the few compounds that has been tested extensively for a variety of cytogenetic effects in vivo. There are now a dozen studies that show that benzene causes cytogenetic damage in rodents in vivo; of these, eight are in mice (Diaz et al., 1980; Hite et al., 1980; Meyne and Legator, 1980; Tice et al., 1980; Siou et al., 1981; Tice et al., 1982; Tunek et al., 1982; NTP, Appendix G); three are in rats (Dean, 1969; Lyon, 1975; Anderson and Richardson, 1981); and one is in rabbits (Kissling and Speck, 1971). However, benzene failed to induce cytogenetic damage in hamsters (Siou et al., 1981).

Micronuclei. Micronuclei are chromosomes or small fragments of chromosomes that are not incorporated into daughter nuclei during cell division. They may be induced by agents that

break chromosomes (clastogens) or that affect the spindle apparatus. The ability of benzene to induce micronuclei in the bone marrow polychromatic erythrocytes of mice has been confirmed by five independent studies in five strains of mice. Diaz et al. (1980) and Tunek et al. (1982) administered benzene subcutaneously to male mice (the F₁ from the cross CSW $\times$ Cs No. 1 or outbred NMRI) and observed a dose-dependent increase in micronuclei. Benzene was administered by gavage to male and female mice in the following three studies. Hite et al. (1980) found that benzene induced similar dose-dependent increases in micronuclei in both sexes of Charles River (CD-1) mice. Meyne and Legator (1980) and Siou et al. (1981), who used Swiss (CD-1) and Swiss Lane Petter mice, respectively, also found that benzene induced micronuclei in both sexes, but with greater dose-dependent increases in micronuclei in males than in females. Siou et al. (1981) showed that castration of males reduced their sensitivity for micronuclei formation below that of the females. Meyne and Legator (1980) found that intraperitoneal injections of benzene produced a dose-dependent increase in micronuclei in males and yet caused no increase in micronuclei in females. The NTP (Appendix G) found that benzene administered by gavage induced micronuclei in male and female B6C3F₁ mice; males were more sensitive than females (Choy et al., 1985). In male Long-Evans rats, intraperitoneal injections of benzene induced a dose-dependent increase in micronuclei, and the doses were similar to those that induced micronuclei in mice (Lyon, 1975). Although benzene induced micronuclei in mice and rats, Siou et al. (1981) did not find an increase in micronuclei in hamsters that received benzene by gavage.

Chromosomal Aberrations. Meyne and Legator (1980) reported that exposure of Swiss (CD-1) mice to benzene by gavage or intraperitoneal injection increased the frequency of chromosomal aberrations and that males were more sensitive than females. However, Tice et al. (1980) found that exposure of DBA/2 mice to benzene by inhalation caused an increase in chromosomal aberrations only when the animals had been pretreated with phenobarbital. The authors also showed that the combination of benzene and phenobarbital inhibited cellular proliferation in

1. INTRODUCTION

the bone marrow; once again, males were more sensitive to this inhibition than were females. Subsequent studies (Tice et al., 1982) showed that the combination of benzene and phenobarbital enhanced the frequency of chromosomal aberrations in males more than in females. The aberrations were all of the chromatid-type, not of chromosome-type, and there were no increases in chromosomal rearrangements of any kind. Siou et al. (1981) confirmed the observations of Meyne and Legator (1980) by administering benzene by gavage to Swiss Lane Petter mice: chromosome gaps were enhanced in both sexes, and males were more sensitive than females.

Three reports show that benzene induces chromosomal aberrations in rats. Lyon (1975) injected benzene intraperitoneally into male Long-Evans rats and found a significant increase in chromosomal aberrations in bone marrow cells, including achromatic lesions, chromatid and chromosome deletions, and double minutes (small supernumerary chromosomal fragments). Dean (1969) reported that benzene injected intraperitoneally into Carworth CF₁ rats increased the number of chromatid gaps and chromosome fragments in bone marrow cells from both sexes. Anderson and Richardson (1981) examined the ability of benzene to induce chromosomal aberrations in the bone marrow cells of Wistar-derived male Alderly Park rats. Exposure by inhalation or injection increased the frequency of chromosomal aberrations. No dose response was observed, and a single exposure was as effective as multiple doses. A significant increase in chromosomal aberrations, mostly gaps and breaks, was found in rabbits injected subcutaneously with benzene (Kissling and Speck, 1971).

Although benzene induces chromosomal aberrations in mice, rats, and rabbits, a similar effect has not been seen in Chinese hamsters. Siou et al. (1981) administered benzene by gavage to Chinese hamsters and reported no increase in chromosomal aberrations. This result suggests that hamsters' sensitivity to benzene is different from that of other rodents.

Sister Chromatid Exchanges. The ability of benzene to induce SCE's in mice was reported by Tice et al. (1980, 1982): inhaled benzene

enhanced the frequency of SCE's in male and female DBA/2 mice, but more so in males. Three-month-old mice were more sensitive than were 10-month-old mice. The authors also compared effects resulting from two different routes of exposure and found that inhalation of 120 ppm of benzene for 4 hours equaled the effect of an intraperitoneal injection of 1 mmole/kg. Although additional experiments are still in progress, the authors have tentatively concluded that a greater increase in SCE's results from inhaled benzene than from injected benzene. In contrast to their other findings, these authors also found that treatment with phenobarbital before inhalation of benzene enhanced SCE's in male and female mice but more so in females. Inhaled benzene also caused a greater increase in SCE's in DBA/2 mice than in C57BL/6 mice. These two strains are isogenic and vary in that DBA/2 mice have no inducible aryl hydrocarbon hydroxylase (AHH) activity, whereas the C57BL/6 strain does.

Other Endpoints. An additional endpoint, sperm-head abnormalities, has been studied by Topham (1980), who showed that hybrid mice (CBA × BALB/c) given intraperitoneal injections of benzene exhibited small but reproducible and significant increases in sperm-head abnormalities. Earlier, Lyon (1975) found that intraperitoneal injections of benzene in Long-Evans rats at 0.5 ml/kg (one-fifth the LD₅₀ value) did not increase the frequency of dominant lethals.

Cytogenetic Studies in Humans

Benzene is one of the few agents whose cytogenetic effects have been studied in humans. The populations studied can be divided into two general groups: (1) people with a current or past history of benzene-associated blood dyscrasias and (2) workers with current or past exposure to benzene but with no apparent clinical signs of blood dyscrasias. In many of the 23 studies (IARC, 1982), significant increases in chromosomal aberrations were observed, which in some cases persisted for years after exposure ceased. Most of these studies involved small numbers of humans exposed to benzene and did not present detailed information about the concentration and length of exposure. Although the data do not

support a clear association between exposure to benzene and persistent chromosomal aberrations, the data do indicate an association between benzene-associated hemopathies and chromosomal aberrations.

In summary, these studies show that benzene is clearly a clastogen and that benzene causes other chromosomal changes. The data further suggest that benzene is not a gene mutagen, an agent that causes a molecular change in a gene as opposed to a chromosome. In addition, benzene must be metabolized *in vivo* to cause chromosomal changes, suggesting that metabolites of benzene are responsible for any observed cytogenetic changes. In general, male rodents are more sensitive than females to the genotoxic effects of benzene, and the route of administration influences the ability of benzene to induce chromosomal damage. Cytogenetic damage also has been observed in humans who have developed benzene-associated hemopathies, especially leukemia.

FETOTOXICITY AND TERATOGENICITY

Cleft palate, agnathia, and micronathia were associated with a single subcutaneous injection of 3 ml/kg to CF-1 mice on day 13 of gestation (Watanabe and Yoshida, 1970). This study was considered to be less than adequate, however, because no controls were used. Delayed ossification of sternebrae was observed in the offspring of Sprague-Dawley rats exposed to air containing 300 or 2,200 ppm benzene for 6 hours per day on days 6-15 of gestation (Green et al., 1978). Significantly increased incidences of missing sternebrae were observed in the female offspring after exposures of the mothers at 2,200 ppm. Delayed ossification was also found in the fetuses of Sprague-Dawley rats exposed to air containing 50 or 500 ppm benzene, 7 hours per day on day 6-15 of gestation. The mean number of caudals (vertebrae) in the fetuses of the rats that were exposed was significantly less than that in the fetuses of controls (Kuna and Kapp, 1981). Skeletal retardation and abnormalities (but not malformations) were observed in the offspring of CFY rats exposed to air containing 313 ppm benzene, 24 hours per day on days 9-14 of gestation (Hudak and Ungvary, 1978).

No teratogenic effects were observed in offspring following 6-hour daily exposure of pregnant Sprague-Dawley rats to benzene vapor at 0, 1, 10, 40, or 100 ppm on days 6-15 of gestation (API, 1982). A slight fetotoxic effect (reduced mean fetal body weights) was reported for both sexes of offspring of the mothers that were exposed to benzene at 100 ppm. The available data on teratologic studies have been summarized (Schwetz, 1983; Mehlman et al., 1981). Schwetz (1983) concluded that benzene has not been found to be teratogenic in laboratory animals exposed during the critical period of development of the embryo or fetus. After review of the teratogenic studies, the author stated that exposure to benzene at levels that do not cause other forms of toxicity would not be expected to cause adverse developmental effects. Davis (1986) reviewed the literature on the reproductive risks of benzene and concluded that more animal and epidemiologic studies are needed before more definitive conclusions can be made.

CARCINOGENICITY

Bone marrow hyperplasia, thymic lymphoma (6/40), plasmacytoma (1/40), and leukemia (1/40) were reported in C57BL/6J mice exposed to air containing 300 ppm benzene for 6 hours per day, 5 days per week, for 488 days as compared with an incidence of 2/40 lymphoma (nonthymic) in the controls (Snyder et al., 1980). (Only thymuses appearing to be abnormal at necropsy were examined microscopically.) Myelogenous leukemia occurred in 2/40 CD-1 mice exposed to air containing 300 ppm benzene for 6 hours per day, 5 days per week, for life, but these cannot be clearly related to chemical exposure (Goldstein et al., 1982). The reported findings from these studies do not allow any positive associations because of the marginal increases together with the small numbers of animals used.

Zymbal gland carcinomas were observed in 8/32 (25%) female Sprague-Dawley rats administered 250 mg/kg benzene in olive oil by gavage four or five times per week for 52 weeks, followed by 92 weeks of observation (Maltoni and Scarnato, 1979; Maltoni et al., 1982c, 1983). Squamous cell carcinomas of the oral cavity were associated with the administration by gavage of 500

1. INTRODUCTION

mg/kg benzene to Sprague-Dawley rats once a day for 104 weeks (Maltoni et al., 1982a). Increased incidences of hepatocarcinomas were found in Sprague-Dawley rats exposed to air containing 200-300 ppm benzene, 4-7 hours per day for up to 104 weeks (Maltoni et al., 1982b). IARC (1982) summarized the 15 available studies on benzene (1932-1981), and Van Raalte and Grasso (1982) reviewed the available carcinogenesis data on benzene through mid-1982.

Cronkite et al. (1985) reported that exposure of male and female C57BL/6 BNL mice to benzene vapor at or above 100 ppm for 10 exposures (6 hours per day, 5 days per week) reduced bone marrow cellularity and the number of pluripotent stem cells. Exposure at 300 ppm for 2, 4, 8, and 16 weeks showed a time-related decrease in stem cell levels and extended recovery periods. Cronkite et al. (1984, 1985) showed that female mice exposed at 300 ppm (6 hours per day, 5 days per week for 16 weeks after which exposure was stopped) exhibited increased incidences of leukemia (8/88 versus 20/89) largely due to thymic lymphomata (1/88 versus 10/89). In addition, Zymbal gland neoplasia (1/88 versus 16/89) and ovarian tumors (0/88 versus 8/89) were increased. These authors suggest that continuous exposure either suppresses the incidence of lymphoma in mice or shortens the life span, so lymphomas cannot be observed. Thus, shorter term or fractionated exposures of laboratory animals may result in higher incidences of neoplasia than longer term (2-year) continuous exposures.

Maltoni et al. (1985) summarized a series of long-term gavage and inhalation studies conducted or ongoing at the Bologna Institute of Oncology, Italy. These authors reported an association of benzene exposure with Zymbal gland carcinomas, oral and nasal cavity carcinomas, skin carcinomas, acanthomas, forestomach carcinomas, mammary malignant tumors, hepatomas, liver angiosarcomas, hemolymphoreticular neoplasms, and pulmonary tumors. Details about these experiments are given in Maltoni et al. (1985) as well as in numerous other publications on these data from this laboratory.

CARCINOGENESIS STUDIES ON BENZENE METABOLITES

Phenol (CAS No. 108-92-2): Carcinogenesis studies of phenol were conducted by providing drinking water containing 0, 2,500, or 5,000 ppm to F344 rats and to B6C3F₁ mice for 103 weeks (NCI, 1980; Huff, 1983). An increased incidence of neoplasms was detected in low dose male rats (leukemia--18/50, 30/50 [$P < 0.05$], 25/50; pheochromocytoma of the adrenal glands--13/50, 22/50 [$P < 0.05$], 9/50; and C-cell carcinoma of the thyroid gland--0/50, 5/49 [$P < 0.05$], 1/50). No chemical-related neoplasms were observed in female rats or in male and female mice. Salaman and Glendenning (1957), Boutwell and Bosch (1959), Wynder and Hoffmann (1961), Van Duuren et al. (1968), and Van Duuren and Goldschmidt (1976) have shown that phenol in acetone or in benzene promotes skin cancer in mice pretreated with 7,12-dimethylbenz(a)anthracene (DMBA) or 3,4-benzo(a)pyrene (B[a]P). Phenol did not exhibit any cocarcinogenic effects when given with B[a]P (Van Duuren et al., 1973; Van Duuren and Goldschmidt, 1976).

Catechol (CAS No. 120-80-9): In 1951, Lehman et al. reported a study in which groups of 12-18 rats were exposed to 0.0625%-1.0% catechol in the diet for 2 years. The only response recorded was "beginning hepatic cell hyperplasia" in the 0.25% group. In a short-term skin tumor-promoting study, 30 female Sutter mice received 0.3 ml DMBA in benzene and, beginning 1 week later for 15 weeks, a single drop of 15% catechol in benzene. No promoter activity was observed (Boutwell and Bosch, 1959). Van Duuren and Goldschmidt (1976) applied 150 µg B[a]P topically to the dorsal skin of 50 female Swiss mice and 14 days later applied 2 mg catechol in 0.1 ml acetone three times per week until day 448. No tumor-promoting activity was found. Hecht et al. (1975) applied 75 µg DMBA in 0.1 ml acetone to groups of 30 female Swiss mice one time only, followed 10 days later with 0.1 ml of a 1% catechol/acetone (1 mg of catechol) solution five times per week for 67 weeks. In these studies, catechol was inactive as a tumor promoter. Van Duuren et al. (1973) and Van Duuren and

Goldschmidt (1976) applied 2 mg catechol with and without 5 µg B[a]P in 0.1 ml acetone to groups of 50 female Swiss mice three times per week for 368 days. Of the mice dosed with catechol and B[a]P in combination, 36 had skin papillomas (2.5 per mouse) and 31 had skin squamous cell carcinomas. In the group receiving only catechol, one mouse had a skin papilloma and one had a squamous cell papilloma. Thirteen mice receiving B[a]P had skin papillomas (1.1 per mouse), and 10 had squamous cell carcinomas. In these experiments, catechol increased the carcinogenic activity of B[a]P. Subsequently, Hecht et al. (1981) tested a subfraction of cigarette smoke condensate containing 97% catechol on groups of 30 female Swiss mice. A 0.25% catechol-acetone solution (0.1 ml) alone or with 0.003% B[a]P was applied five times per week for 52 weeks (this dose was about one-sixth the dose used by Van Duuren and Goldschmidt, 1976). The combination caused increased incidences of neoplasms per mouse (1.4 versus 0.1) and greater percentages of mice with total skin tumors (73% versus 14%) and squamous cell carcinomas (64% versus 11%). This study confirms that catechol possesses cocarcinogenic effects. Boyland et al. (1964) implanted 10-mg cholesterol pellets alone or containing 20% catechol into the urinary bladders of mice. Of the 19 mice that received the combination pellet and survived the 25-week experiment, 1 (5.3%) had a papilloma and 3 (15.8%) had carcinomas. Of 77 mice with cholesterol pellets, 4 (5%) had adenomas or papillomas and 5 (6.5%) had carcinomas. A marginal decrease ($P=0.03$) was observed for the catechol group (4/19, 21.1%, versus 9/77, 11.7%).

Hydroquinone (CAS No. 123-31-9): Lehman et al. (1951) fed hydroquinone at 0.125%-2.0% in the diet to groups of 12-18 rats for 2 years. The authors reported a "suggestion" of gastrointestinal ulceration and renal tumors in the 2% group. Carlson and Brewer (1953) offered diets containing 0%-0.5% or 0%-1.0% (four concentrations) with 0.1% citric acid to groups of 10-23 male and female rats for 103 weeks. No adverse hematologic or histopathologic effects were recorded. Roe and Salaman (1955) applied 0.3 ml of a 6.7% hydroquinone-acetone solution (20 mg dose) to the backs of male "S"-strain mice. Three weeks later, croton oil (0.3 ml of a 0.5%

acetone solution) was applied dermally once a week for 18 weeks. One of the 22 survivors had a skin papilloma. Hydroquinone was inactive as an initiator of skin carcinogenesis.

Using experimental conditions similar to those described above for catechol, Boutwell and Bosch (1959) and Van Duuren and Goldschmidt (1976) reported that hydroquinone had no promoter activity with DMBA or B[a]P. Boyland et al. (1964) tested hydroquinone as 20% in 10-mg cholesterol pellets implanted in the urinary bladder of mice. Six of the 19 survivors (32%) at week 25 had bladder carcinomas, whereas four benign and five malignant neoplasms were found in 77 cholesterol controls (11.7%). In this study, hydroquinone was considered carcinogenic.

In cocarcinogenesis studies, Van Duuren and Goldschmidt (1976) applied hydroquinone in doses of 5 mg with and without 5 µg B[a]P in acetone (see details under catechol). Hydroquinone with B[a]P induced fewer skin neoplasms than B[a]P alone (7 mice with 11 papillomas and 3 with squamous cell carcinomas versus 14 mice with 16 papillomas and 10 with squamous cell carcinomas).

The National Toxicology Program has conducted 2-year carcinogenesis studies of hydroquinone in male and female F344/N rats and B6C3F₁ mice. Hydroquinone was given in water by gavage at doses of 0, 25, or 50 mg/kg for rats and 0, 50, or 100 mg/kg for mice. The experiments began in August 1982, the exposure phase was completed in August/September 1984, and the initial histopathology diagnoses are being evaluated.

p-Quinone (CAS No. 106-51-4): Three carcinogenesis studies have been reported (IARC, 1977). In 1940, Takizawa painted the skin of mice every 1 or 2 days for about 200 days with 0% (benzene), 0.1%, or 1.25% *p*-benzoquinone in benzene. Of 46 controls, 1 had a skin papilloma and 2 had adenocarcinomas of the lung. Among the 41 mice at 0.1%, 6 had skin papillomas, 2 had skin carcinomas, and 10 developed lung adenocarcinomas. In the 0.25% group (44 mice), 3 had skin papillomas, 1 had a skin carcinoma, and 5 had lung adenocarcinomas. In a series of three inhalation experiments, Kishizawa (1954, 1955, 1956) exposed groups of 25 mice to air

I. INTRODUCTION

containing 0 or 5 mg *p*-quinone, once per day, six times per week. The numbers of mice with neoplasms of the lung were not different between control and dosed groups. Umeda (1957) conducted studies in 24 rats by subcutaneously injecting 0.5 ml of propylene glycol with *p*-quinone once per week for 394 days (concentrations were 1% from days 1 to 53, 0.2% from days 54 to 173, and 0.4% from days 173 to 394). Of 17 surviving rats receiving 32 injections (81 mg *p*-quinone and 16.5 ml propylene glycol), 2 developed injection-site fibrosarcomas.

EFFECTS ON HUMANS

Humans are susceptible to benzene myelotoxicity as shown by the high incidence of pancytopenia among workers with significant exposure (Goldstein, 1983). The connection between myelotoxicity and acute myelogenous leukemia remains a subject of considerable discussion. Blood diseases associated with benzene exposure include pancytopenia (most frequently cited), leukopenia, bone marrow hypoplasia or aplasia, thrombocytopenia, granulocytopenia, and lymphocytopenia (IARC, 1982; Infante, 1978; Infante and White, 1983; Grossenbacher and Lob, 1982). Benzene, a myelotoxic chemical, causes pancytopenia and eventual aplastic anemia in most animal species exposed.

Ever since Santesson (1897) and Selling (1916) recorded that benzene could cause aplastic anemia, numerous supporting reports have been published (IARC, 1982). The correlation of exposure levels to specific hematologic toxicity has been well documented; however, it is not possible to reliably predict effects produced at specific exposure levels. Likewise, it has not been possible to establish with certainty the degree of exposure below which no adverse hematologic effects in humans would occur.

Effects in humans from benzene exposure have been well characterized and described in medical, toxicology, and poisoning treatment manuals and texts. Although the benzene-associated signs and symptoms in humans cannot be related exactly or with critical accuracy, the biologic events that occur in humans from increasing relatively short-term exposure to benzene appear to follow a pattern from effects on the hematopoietic system, through narcosis,

and death. Loss of consciousness, irregular heartbeat, dizziness, headache, and nausea were observed in workers exposed to benzene at concentrations below 20,000 ppm (Deutsche Forschungsgemeinschaft, 1974). Reports that single exposures at concentrations of 20,000 ppm were fatal within 5-10 minutes have been made (Flury, 1928). Continued exposure of workers to benzene has been associated with decreased concentrations of circulating erythrocytes, leukocytes, and thrombocytes (Snyder and Kocsis, 1975). The incidence of sister-chromatid exchanges was not significantly increased in the lymphocytes of 22 workers in Italy exposed to benzene at 0.2-12.4 ppm (mean exposure time, 11.4 years) in air as compared with persons living in the same area of similar age and smoking habits (Sarto, 1984). The incidence of chromosome-type chromosomal aberrations was significantly greater among exposed workers compared with controls.

Although a link between benzene exposure and hematologic disorders was suggested 80 years ago, a connection with leukemia was not clearly established at that time. In early epidemiologic studies, workers were exposed to other chemicals in addition to benzene (Infante, 1978; IARC, 1982; Grossenbacher and Lob, 1982).

An association between long-term exposure to benzene and the occurrence of leukemia was suggested as early as 1928 by Delore and Borgomano, who described acute lymphoblastic leukemia in a worker exposed to benzene for 5 years. IARC (1982) gives a chronology of published literature. Goldstein (1977) describes a number of additional case reports. Most malignancies in which an association with exposure to benzene has been reported have been leukemias, particularly those of the myelogenous type. A critical issue in benzene risk assessment seems to center on the interpretation of the shape associated with the dose-response curve relating benzene exposure to acute myelogenous leukemia and variants.

More than 100 occurrences of leukemia in humans have been associated with benzene exposure since 1928 (Delore and Borgomano, 1928; Vigliani, 1976). More recent epidemiologic studies of small cohorts exposed to benzene have

demonstrated a causal association for leukemia (IARC, 1982; Infante et al, 1977; Rinsky et al. 1986; Ott et al., 1978; Decoufle et al., 1983; Aksoy, 1985; Infante and White, 1985). Rinsky et al. (1986) examined the updated mortality of a cohort with occupational exposure to benzene and calculated a cumulative benzene exposure index (parts per million $\times$ years) for each cohort member. These authors found that the standard mortality ratio (SMR) for leukemia was 328 and for multiple myeloma was 398. With stratification of the cohort by cumulative exposure, the SMR's for leukemia increased from 105 in workers with less than 40-ppm-years' exposure to 314 in workers with 40- to 199-ppm-years, to 1,757 in those with from 200- to 399-ppm-years, and to 4,535 in those with 400-ppm-years or more. Most of these studies are summarized in the December 1985 Federal Register notice on occupational exposure to benzene (Fed. Reg., 1985) and to a lesser degree by Goldstein (1985).

STUDY RATIONALE

Benzene was studied for long-term effects in rodents by the NTP Carcinogenesis Program because of its large production volume, because of epidemiologic evidence that exposure of humans to benzene is associated with an increased incidence of leukemia, and because previous experimental studies for carcinogenicity in laboratory animals were considered to be inconclusive or inadequate. In the studies reported in this Technical Report, benzene was given by gavage in corn oil because the chemical was only slightly soluble in water, because benzene was considered too volatile to be administered by feed, and to make certain that the animals would be exposed to a sufficient and accurately calculated amount of the chemical. In retrospect, the inhalation route may have been a more appropriate mimic of human exposure.