R Le

TO: Safety Directors; Environmental Coordinators

RE: Benzene

FROM: T. G. Grumbles
DATE: March 19, 1987

Interoffice Communication

SUBJ: EEGL DOCUMENT FOR BENZENE

VISTA

As promised recently, enclosed is the documentation for the recent NRC recommendation for the 50 ppm emergency exposure guidance level for benzene.

T. G. Grumbles

djm
Attachment

cc: Mary Heller
Dr. Drumwright

EMERGENCY AND CONTINUOUS EXPOSURE GUIDANCE LEVELS FOR SELECTED AIRBORNE CONTAMINANTS

Volume 6

BENZENE AND ETHYLENE OXIDE

COMMITTEE ON TOXICOLOGY

Board on Environmental Studies and Toxicology
Commission on Life Sciences
National Research Council

National Academy Press
Washington, D.C. 1986

SUMMARY

Table 1 summarizes emergency exposure guidance levels (EEGLs) for up to 24 h for benzene and ethylene oxide.

TABLE 1

Recommended Emergency Exposure Guidance Levels for Benzene and Ethylene Oxide

Substance	Duration of Exposure	EEGL
Benzene	1 h	50 ppm
	24 h	2 ppm
Ethylene Oxide	1 h	20 ppm
	24 h	1 ppm

BENZENE

BACKGROUND INFORMATION

PHYSICAL AND CHEMICAL PROPERTIES

Synonyms:	Benzol, benzole, benzolene, bicarburet of hydrogen, carbon oil, coal naphtha, cyclohexatriene, mineral naphtha, motor benzol, phenyl hydride, pyrobenzol, pyrobenzole (IARC, 1982)
CAS number:	71-43-2
Molecular formula:	C_6H_6
Molecular weight:	78.11
Boiling point:	80.1°C
Density:	0.88 g/ml
Freezing point:	5.5°C
Flash point (closed cup):	11.1°C
Solubility:	Slightly soluble in water; soluble in all proportions in alcohol, acetone, ether, oils
General characteristics:	Colorless, nonpolar liquid with odor characteristic of aromatic hydrocarbons; odor threshold, 4.68 ppm
Conversion factors:	1 ppm = 3.2 mg/m^3 1 mg/m^3 = 0.31 ppm

OCCURRENCE AND USE

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. In the late nineteenth century, benzene facilitated the rapid development of the rubber industry, because of its ability to dissolve rubber and its ease of evaporation during the manufacture of formed or coated rubber products. It played a similar role in high-speed printing processes, because it is an excellent solvent for inks that must dry rapidly. Many other industries have used benzene as a solvent or as a starting material for chemical syntheses. Manufacturers of paints and plastics have been among the main users. Today, because of its antiknock properties, a mixture of benzene-rich aromatic substances is added to gasoline as a replacement for alkyl lead compounds to increase octane ratings.

Benzene is one of the most heavily used organic chemicals in industry. Its major source is petroleum, although large amounts are also recovered from coal in the coking process. According to the U.S. International Trade Commission (1985), approximately 1.34 billion gallons of benzene was produced in the United States in 1984.

SUMMARY OF TOXICITY INFORMATION

EFFECTS ON HUMANS

Acute Effects

Benzene has an appreciable vapor pressure at normal temperatures, so hazardous occupational exposure usually occurs via inhalation. Acute exposure to benzene at high concentrations can kill by depressing the central nervous system or by leading to fatal cardiac arrhythmic responses to circulating catecholamines (Snyder and Kocsis, 1975).

Hamilton (1931) reviewed the earlier literature on acute benzene poisoning, including nineteenth century autopsy findings, and noted a few instances in which central nervous system effects apparently persisted for at least 12 d after severe acute poisoning. Gerarde (1960) stated that exposure at 19,000-20,000 ppm for 5-10 min is fatal; exposure at 7,500 ppm for 30 min is dangerous; exposure at 1,500 ppm for 60 min induces serious symptoms; exposure at 500 ppm for 60 min leads to symptoms of illness; exposure at 50-150 ppm for 5 h produces headache, lassitude, and weakness; and exposure at 25 ppm for 8 h has no effect. A National Research Council (1976) review stated that exposure at about 25,000 ppm is rapidly fatal.

Chronic Effects

The major chronic toxic effect of benzene is hemopoietic toxicity, an effect peculiar to benzene among the simple aromatic hydrocarbons. Chronic exposure of humans to benzene at low concentrations in the workplace is associated with blood disorders, including aplastic anemia and leukemia (Browning, 1965; Snyder and Kocsis, 1975; Snyder et al., 1977). The bone marrow toxicity of benzene can be characterized by a progressive decrease in some or all circulating formed elements of the blood--erythrocytes, platelets, and the various types of leukocytes. The extent to which cells of each type are depleted varies with the individual and the degree of exposure to benzene. When blood cells of all three major types are substantially depleted, the effect is called pancytopenia. Pancytopenia with degeneration of the red bone marrow results in aplastic anemia. Benzene-induced damage to bone marrow can result in necrosis and fatty replacement of the cytogenic cells. In both human and animal studies, benzene induced a decrease in bone marrow function in a dose-dependent manner.

Signs of chronic benzene toxicity have been observed in humans since the turn of the century (Santesson, 1897; Selling, 1916; Helmer, 1944; Goldwater, 1941; Hunter, 1939). These studies generally showed that a decrease in number of circulating erythrocytes or leukocytes is a relatively good indication of early benzene toxicity; among leukocytes, decreases in both granulocytes and lymphocytes have been reported. Toxicity appears to be a function of both exposure rate and duration of exposure, although individual workers vary in their reactions to benzene.

The lowest average concentrations of benzene vapor reported to have been associated with human leukemia and aplastic anemia are 1 and 5 ppm, respectively (Ott et al., 1978; Bond et al., 1986).

Among studies that have shown a wide range of hematologic response are those of Goldwater and colleagues (Goldwater, 1941; Goldwater and Tewksbury, 1941; Greenburg et al., 1939), who evaluated over 300 rotogravure printers in New York. A change in the printing process reportedly had led to the exposure of these workers to benzene at 11-1,060 ppm for 6-60 mo. There were 23 cases of serious cytopenia; six of the workers required hospitalization. Wilson (1942) reported studies of 1,104 workers in a rubber factory in Ohio who were exposed to benzene at up to 500 ppm (average, about 100 ppm). Mild hematologic abnormalities were noted in 83 and more severe pancytopenia in 25, of whom nine were hospitalized and three of the nine died. Savilahti (1956) reported that 107 of 147 Finnish shoe factory workers had some hematologic abnormalities; they had been exposed to benzene, which had been in use for about 10 yr at concentrations as high as 400 ppm. Hernberg et al. (1966), in a followup study of 125 of these workers 9 yr later, noted some persistent cases of cytopenia; one worker had developed acute leukemia and died. Another study with a long followup is that of Guberan and Kocher (1971), who followed 216 of 282 workers for 10 yr after cessation of benzene exposure. Four were reported to have had persistent decreases in blood counts, and one had died of aplastic anemia 9 yr after cessation of exposure. Followup data suggesting mild persistent anemia in workers in the rubber coating industry were presented by the National Institute for Occupational Safety and Health (1974); they had been exposed to benzene at concentrations reported to be generally less than 25 ppm, but ranging up to 125 ppm before installation of control measures (Pagnotto et al., 1961).

Other large series of cases of aplastic anemia in benzene-exposed workers were studied by Vigliani and colleagues in Italy, who focused on leukemia (Vigliani and Forni, 1976; Saita and Vigliani, 1962), and by Aksoy et al. (1971, 1972) in Turkey. In the latter studies, the onset of hematologic toxicity in leather workers was temporally related to the use of a benzene adhesive that began around 1960. Aksoy et al. (1972) reported 32 cases of aplastic anemia in workers exposed to benzene at 150-650 ppm for 4 mo to 15 yr. In another study reported by this group (Aksoy et al., 1971), 51 of 217 apparently healthy workers

were found to have some hematologic abnormalities, including six cases of pancytopenia. These workers were described as having been exposed to benzene at 30-210 ppm for 3 mo to 17 yr. The reported exposures in this series represent occasional random measurements of what was in essence a cottage industry.

These studies of occupationally exposed groups are notable for the association of benzene with pancytopenia in workers in different countries and in different work settings where the only common factor appears to have been benzene. The clear temporal relationship between the onset and cessation of hematologic abnormalities and the use of benzene constitutes evidence of a causal relationship between benzene exposure and pancytopenia.

None of the aforementioned occupational studies, however, provided information on the lowest dose of benzene that might produce cytopenic effects in humans. Two studies of occupationally exposed groups that attempted to provide such information were by Doskin (1971) in the USSR and Chang (1972) in Korea. These studies were described in Assessment of Health Effects of Benzene Germane to Low-Level Exposure (USEPA, 1978), which did not provide details of exposure. Chang (1972) studied 119 workers exposed to benzene in an unspecified industrial area. Hematologic abnormalities were observed in 28--21 with anemia, two with leukopenia, and five with both. These workers were exposed to benzene concentrations as low as 20 ppm. The author suggested a benzene "threshold" of 10 ppm for cytopenic effects. However, no hematologic toxicity was observed in the 18 workers exposed to benzene at 10-20 ppm.

Doskin (1971) evaluated 365 workers employed for 3 yr in an apparently new chemical factory. The author stated that benzene exceeded the maximal permissible concentration (believed to be 5 ppm) by a factor of 2-8 in 64% of the measurements in the first year, 37% in the second year, and 3% in the third year. Approximately 40% of the workers exhibited mild hematologic abnormalities during the first year, and this percentage declined greatly later. The most common early sign of benzene hematotoxicity was mild thrombocytopenia followed by anemia. An initial increase in white-cell count was sometimes followed by leukopenia.

The leukemias are acute or chronic neoplastic diseases that are classified according to the cell type involved. The leukemia most commonly associated with benzene exposure is acute myelogenous leukemia, which is characterized by an increased number of cells morphologically similar to myeloblasts. Other types of leukemia have also been associated with benzene exposure.

Evidence that benzene is a human leukemogen has come primarily from epidemiologic studies and case reports. In 1964, Vigliani and Saita reported several cases of leukemia in workers exposed to benzene; the data suggested a risk for those workers 20 times greater than that in

the surrounding population (Vigliani and Saita, 1964). Aksoy et al. (1974, 1976) and Aksoy and Erdem (1978) reported an increased incidence of leukemia in shoe workers occupationally exposed to benzene. Ott et al. (1978) reported three deaths from leukemia, compared with 0.8 expected, among 594 workers exposed to benzene. Several investigators reported cancers of the lymphatic and hematopoietic systems in workers in the U.S. rubber industry (IARC, 1982).

Rinsky et al. (1981) conducted a retrospective mortality study of workers who had been exposed to benzene in the manufacture of rubber hydrochloride at two locations in Ohio. They noted that seven deaths from leukemia occurred among 748 workers who had at least 1 d of exposure to benzene between 1940 and 1950, compared with 1.25 expected in the same number of members of the U.S. population of comparable sex, age, and race. The standardized mortality ratio (SMR) of 560 is statistically significant. The mean exposure to benzene was brief; 437 (58%) of the cohort were exposed for less than 1 yr. Evaluation of leukemia mortality among workers exposed for 5 yr or more gave a significant SMR of 2,100. Reconstruction of past exposures to benzene at two locations indicated that airborne benzene concentrations in some areas of the plant rose occasionally to several hundred parts per million, but that for the most part the employee 8-h time-weighted average concentration was within the limits considered permissible at the time (10-100 ppm). The data corroborated an initial analysis of the same cohort by Infante et al. (1977) and indicated that benzene is a human leukemogen.

DeCoufle et al. (1983) performed a cohort mortality study of 259 male workers exposed to benzene (concentrations not specified). They observed four deaths from lymphoreticular cancers, compared with 1.1 expected. Three of the deaths were from leukemia (vs. 0.44 expected), and one from multiple myeloma. One worker with an initial diagnosis of multiple myeloma died from acute myelogenous leukemia.

Environmental Health Associates (1983) recently completed the largest U.S. epidemiologic study of benzene-exposed workers to date, which was sponsored by the Chemical Manufacturers Association. The study involved almost 8,000 workers in seven chemical plants of six companies. The results indicated an SMR slightly higher, but not statistically significant, for lymphatic and hematopoietic cancer among the exposed workers. The risk of lymphatic and hematopoietic cancer in the exposed group was significantly higher than that in an internal comparison group, which had an unusually low mortality rate. The study also showed a statistically significant dose-response relationship between cumulative exposure to benzene and mortality from leukemia and all lymphatic and hematopoietic cancers combined and mortality from leukemia. A relative risk of 3.4 for cohort members whose peak exposures to benzene were at less than 25 ppm was also demonstrated. The SMRs were also higher for cancers of bone, lung, kidney, prostate, and brain; for benign neoplasms; and for emphysema.

These reports show a strong correlation between benzene exposure and the development of leukemia and hematopoietic cancers. However, the dose-response relationship is not well established, largely because studies correlating exposure of workers and incidence of leukemia have been retrospective and data on duration and extent of benzene exposure have been deficient. Furthermore, benzene exposure in industry has not occurred in isolation from exposure to other solvents. However, experimental work with rats and mice that has demonstrated the capacity of benzene to cause cancers under controlled conditions supports the view that benzene causes cancer in humans. In 1981, the International Agency for Research on Cancer reviewed the epidemiologic data regarding the carcinogenic risk of benzene to humans and concluded that "there is sufficient evidence to conclude that benzene is carcinogenic" to humans (IARC, 1982).

Chromosomal Effects

Cytogenetic changes indicate an alteration in the genetic material (DNA) of a cell. Evidence is increasing that latent diseases--such as cancer, birth defects, and genetic disease--can be initiated by alterations in cellular DNA.

Picciano (1979) found increased chromosomal aberrations in peripheral lymphocytes of 52 workers (compared with 44 controls) who were exposed to benzene for an average of 56.6 mo at less than 10 ppm.

Sarto et al. (1984) performed a cytogenetic evaluation of 22 healthy benzene production workers exposed at 0.2-12.4 ppm for a mean period of 11.4 $\pm$ 7 yr. Each exposed person was paired with a suitable control. No statistically significant increase in sister chromatid exchange (SCE) frequency was observed in the exposed group. However, statistically significant increases in chromosomal aberrations were found.

Morimoto and co-workers (Morimoto and Wolff, 1980; Morimoto, 1983; Morimoto et al., 1983) have shown that metabolism is necessary for production of benzene-induced SCEs. Working with whole-blood cultures from human donors, they found that benzene could induce SCEs, but only in the presence of microsomal enzymes. Addition of glutathione to blood cultures decreased benzene-induced SCEs. The benzene metabolites catechol and hydroquinone also induced SCEs. Significantly, addition of microsomal enzymes increased this response, and addition of glutathione inhibited it. Thus, the benzene metabolites are implicated in DNA damage induced by benzene. The capacity of glutathione to inhibit SCE induction suggests an electrophilic nature of the toxic metabolites.

Workers exposed to benzene at concentrations sufficient to disturb hemopoiesis have displayed a greater incidence of chromosomal aberrations than unexposed controls (Dabney, 1981). However, it is not

possible from the data provided to estimate a minimal exposure concentration or period needed to produce cytogenetic changes.

EFFECTS ON ANIMALS

The following acute oral LD_{50}s of benzene have been reported: 0.93 g/kg (95% confidence limits, 0.71-1.23 g/kg) for Sprague-Dawley rats (Cornish and Ryan, 1965), 3.8 ml/kg (2.9-4.8 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 LC_{50} for a 7-h exposure is 10,000 ppm for mice (Svirbely et al., 1943).

Carpenter et al. (1944) studied symptoms and reflexes of rabbits undergoing anesthesia with 3.5-4.5% benzene (35,000-45,000 ppm). Reflex actions and symptoms were variable in time of occurrence (Table 2).

TABLE 2

Symptoms or Reflexes of 10 Rabbits Undergoing Anesthesia with 3.5-4.5% Benzene Vapor in Air[a]

Symptom or Reflex	Average Time of Occurrence, min
Light anesthesia, relaxed	3.7
Excitation, running movements, tremors, chewing	5.0
Loss of pupillary reflex to strong light	6.5
Loss of blink reflex to tactile stimulus	11.4
Pupillary contraction	12.0
Involuntary blinking	15.6
Death	36.2

[a] Data from Carpenter et al., 1944.

In experimental studies, benzene has induced a decrease in bone marrow function in a number of species. Exposure to benzene caused significant leukopenia (Wolf et al., 1956; Deichmann et al., 1963; C. Snyder et al., 1978). In a National Toxicology Program (1985) study, dose-related leukopenia was observed in both rats and mice tested for 17 wk, beginning at the lowest dose (25 mg/kg).

Rozen et al. (1984), in a short-term dose-response study, exposed male C57BL mice to various concentrations of benzene by inhalation 6 h/d for 6 d. Mitogen-induced blastogenesis of both B and T lymphocytes was significantly decreased at concentrations as low as 10 ppm.

Baarson et al. (1984) showed that exposure of C57BL mice to benzene at 10 ppm 6 h/d for 5 d/wk, for up to 178 d, caused a progressive depression in the in vitro colony-forming ability of one of the erythroid progenitor cells. The benzene-exposed mice also had decreases in the number of splenic nucleated cells and circulating red cells and lymphocytes.

Coffin et al. (1977) exposed rats to benzene intermittently at 150 ppm. Each exposure was for 4 h. The toxic effect of intermittent exposure to benzene, as judged by chronaxie events and the appearance of leukopenia, was less than that of continuous exposure to benzene at the same concentration. Apparently, some recovery occurs after intermittent exposure.

Relative spleen weights were reduced in DDF_1 mice given benzene by inhalation (4,680 ppm, 8 h/d for 3 d), and involution of the spleen occurred in Wistar rats given 1 mg/kg by gavage daily for 14 d (Uyeki et al., 1977; Gerarde, 1960).

Mutagenicity and Other Short-Term Tests

Benzene is not mutagenic in bacterial systems. Lyon (1976) conducted a detailed mutagenicity study of benzene in S. typhimurium strains TA 98 and TA 100 at concentrations of 0.1-1 μg/plate with and without rat S-9. No evidence of mutagenicity, as demonstrated by increased reversion rate, was observed. Lyon (1976) also conducted a host-mediated assay with S. typhimurium TA 1950 in which mice were given two subcutaneous injections of benzene at 0.1 ml/kg. No increase in revertants was observed. Benzene's lack of mutagenicity in bacterial systems has since been confirmed in several other studies (Dean, 1978; Shahin and Fournier, 1978; Lebowitz et al., 1979).

Failure to demonstrate mutagenicity of benzene in the Salmonella assay could be due to the use of an open system that allowed evaporation of benzene. However, Jung et al. (1981) could not show benzene oxide to be mutagenic in the Salmonella assay in a closed system, although they did report that 12 of 17 structurally related oxiranes of benzene and its hydrogenated congeners were mutagenic to S. typhimurium TA 1535 and TA 100 in a desiccator (closed system). Benzene given to Drosophila melanogaster larvae in their food supply at 1 and 2% was not mutagenic (Nylander et al., 1978).

Benzene was not mutagenic in the mouse lymphoma forward-mutation assay with L5178Y cells (dose unspecified) (Lebowitz et al., 1979). Crespi and Penman (1984) have reported that benzene at 1 mg/ml (exposure time, 28 h) was mutagenic in a new human lymphoblastoma-cell assay that has an endogenous metabolic activation system. Amacher and Zelljadt (1983) noted that benzene induced morphologic transformation in Syrian hamster embryo cells when tested at 0.1, 1, 5, 19, and 35 μg/ml.

As with humans, exposure of experimental animals to benzene can result in chromosomal aberrations. Lebowitz et al. (1979) observed an increase in chromosomal aberrations in lymphocytes of animals subjected to subchronic benzene exposure for 5 d. Chromosomal gaps and deletions were reported at high exposure rates (Dean, 1978; Cortina et al., 1982). Tice et al. (1982) observed significant increases in SCEs after a lower rate of exposure (4-h inhalation exposure of male and female DBA/2 and C57BL/6 mice to benzene at 28 ppm). They concluded that production of SCEs in vivo depends on metabolism of benzene, inasmuch as toluene, a competitive inhibitor of benzene metabolism (Andrews et al., 1977), protected against the effect of benzene.

Erexson et al. (1986) exposed male DBA/2 mice to benzene at 0, 10, 100, and 1,000 ppm and male Sprague-Dawley rats at 0, 0.1, 0.3, 1, 3, 10, and 30 ppm for 6 h. Mouse peripheral blood lymphocytes revealed a significant concentration-related increase in SCEs after exposure to benzene at 10-1,000 ppm. Mouse bone marrow cells showed a significant concentration-related increase in micronuclei after exposure to benzene at 10-1,000 ppm. Rat peripheral blood lymphocytes showed a significant increase in the SCE frequency after exposure to benzene at 3-30 ppm. Rat bone marrow cells showed a significant concentration-related increase in micronuclei after inhalation of benzene at 1-30 ppm. These results show that benzene can induce statistically significant cytogenetic effects in both mice and rats after a 6-h exposure to benzene at relatively low concentrations (Erexson et al., 1986).

Styles and Richardson (1984) exposed male Wistar rats to benzene at nominal concentrations in air of 1, 10, 100, and 1,000 ppm for 6 h and then examined bone marrow cells for chromosomal abnormalities 24 h after the exposure. Their analysis showed a significant increase in the percentage of cells with chromosomal abnormalities, including gaps, in the groups of animals exposed at 100 and 1,000 ppm. In the 1-ppm and 10-ppm groups, there was an increase in the number of cells with chromosomal abnormalities, but it was not statistically significant.

Toft et al. (1982) exposed NMRI mice to benzene at various concentrations in air ranging from 1 to 200 ppm and found that continuous exposure at 14 ppm for 4-10 d resulted in significant increases in micronuclei in polychromatic erythrocytes. No effect was seen at 1-10 ppm, but at higher concentrations the effect seemed to be related to the product of concentration and exposure time. Intermittent exposure (8 h/d, 5 d/wk, for 2 wk) at 21 ppm and higher also resulted in increased frequency of micronuclei. No micronuclei were observed if mice were exposed at 14 ppm for up to 8 wk. Tunek and co-workers (Toft et al., 1982; Tunek et al., 1982) have related benzene's ability to induce micronuclei in bone marrow cells with its metabolism in vivo, as Tice et al. (1982) did for SCEs.

Carcinogenicity

Until recently, benzene-induced leukemia was observed only in humans, and no satisfactory animal model existed. However, recent investigations have reported that benzene is leukemogenic and carcinogenic in experimental animals. Cronkite et al. (1984) exposed 90 female C57BL mice to benzene at 300 ppm by inhalation 6 h/d, 5 d/wk, for 16 wk and then held them for lifetime observation. After 64 wk from commencement of the study, 10 of 90 benzene-exposed mice had died, compared with only one of 88 control animals. Of the 10 dead mice that had been exposed, six had thymic lymphomas and two had unspecified lymphomas. The single dead control animal did not have lymphoma or leukemia. The authors concluded that benzene is leukemogenic in female C57BL mice. Later studies with male CBA mice confirmed that about 30% of test animals developed leukemia when the above test was repeated (Cronkite, personal communication). (The Committee noted that, although lymphomas in animals are commonly referred to as leukemias, the presence of stem cells in the blood might not have been demonstrated in these studies.)

Maltoni et al. (1983) administered 99.9% pure benzene (impurities, 0.06% paraffin and 0.01% toluene) at 500 mg/kg to 40 male and 40 female Sprague-Dawley rats by gavage, using olive oil as the vehicle. This study was in progress at the date of publication (1983), but 92-wk interim results had shown a statistically significant increase in Zymbal gland tumors. Maltoni et al. (1983) also studied the effects of inhalation of benzene at 200-300 ppm for 4-7 h/d in rats exposed in utero and then exposed for 15 wk after birth. And they exposed 13-wk-old rats to benzene by inhalation for 4-7 h/d for 104 wk. The interim results after 92 wk showed dose-related statistically significant increases in Zymbal gland carcinomas, leukemias, and oral cavity carcinomas.

An update of the investigation (Maltoni et al., 1985) shows that benzene is a strong carcinogen, in that it produces a variety of neoplasias at different anatomic sites, including malignant tumors that rarely occur in tested animals (Zymbal gland carcinomas, carcinomas of the oral and nasal cavities, carcinomas of the skin, hepatocarcinomas, and liver angiosarcomas).

The National Toxicology Program (1985) conducted a carcinogenesis study of benzene given by gavage to F-344 rats and B6C3F1 mice (50 of each sex of each species per dose group). Male and female mice and female rats were given 99.7% pure benzene at 0, 25, 50, and 100 mg/kg in corn oil by gavage 5 d/wk for 103 wk. Male rats were given benzene at 0, 50, 100, and 200 mg/kg. This study resulted in increases in the incidence of Zymbal gland squamous cell carcinomas in male and female mice and rats. Oral carcinomas in rats, carcinomas of the skin in male rats, lymphomas in male and female mice, and tumors of the lung, Harderian gland, preputial gland, ovary, and mammary gland in mice were also found. The variety of types and sites of malignant tumors

produced in rats and mice by benzene exposure is remarkable. The findings demonstrating benzene's multicarcinogenic potential are consistent with the findings of Maltoni et al. (1983) and Environmental Health Associates (1983).

Teratogenicity

A single subcutaneous injection of benzene at 3 ml/kg into pregnant CF_1 mice on days 11-15 of gestation produced cleft palate, agnathia, and micrognathia in 2.7% of the fetuses (Watanabe and Yoshida, 1970). However, no control animals were used, and the relevance of the subcutaneous route to inhalation exposure is doubtful.

Other studies in pregnant mice with other routes of administration--orally at 0.3-1.0 ml/kg on days 6-15 of gestation (Nawrot and Staples, 1979) and by inhalation at 500 ppm for 7 h/d on days 6-15 of gestation (Murray et al., 1979)--failed to show any teratogenic effect. No teratogenic effect has been observed in rabbits exposed at 500 ppm for 7 d during days 6-18 of pregnancy (Murray et al., 1979). Kuna and Kapp (1981) exposed Sprague-Dawley rats at 10, 50, and 500 ppm for 7 h/d during days 6-15 of gestation. Benzene vapor was fetotoxic at 50 and 500 ppm, and offspring of dams exposed at 500 ppm demonstrated exencephaly, angulated ribs, dilated lateral and third ventricles of the brain, and lagging ossification. CFY rats were exposed by inhalation to benzene at 313 ppm for 24 h/d on days 9-14 of gestation (Hudak and Ungvary, 1978). Benzene at this dosage was not teratogenic. Green et al. (1978) exposed pregnant Sprague-Dawley rats to benzene vapor at 100, 300, and 2,200 ppm for 6 h/d on days 6-15 of gestation. Soft tissue examination revealed no significant increase in the incidence of anomalies among the exposed animals. Skeletal examination showed a significant increase in the number of fetuses with delayed ossification of sternebrae in the 300-ppm and 2,200-ppm groups. The litter incidence of missing sternebrae was significantly increased in all three groups.

Reports of teratogenic effects of benzene in animals are few, and the concentrations used were high. Benzene has not been shown to be teratogenic at doses that are not lethal to embryos or fetuses.

MECHANISM OF ACTION AND PHARMACOKINETICS

To understand the mechanism of benzene toxicity, it is essential to study its disposition. Parke and Williams (1953) were among the first to suggest that one of its metabolites might be responsible for benzene toxicity. In several studies, modification of benzene metabolism has led to alteration in benzene toxicity. For example, Andrews et al. (1977) reported that toluene, a competitive inhibitor of benzene metabolism, protected mice against benzene toxicity. Ikeda and Ohtsuji (1971) reported that pretreatment of rats with phenobarbital resulted

in increased tolerance of the leukopenic action of benzene. Longacre et al. (1981a,b) demonstrated that benzene metabolites were found at higher concentrations in bone marrow of DBA/2 mice, which were sensitive to benzene, than of C57B1/6 mice, which were relatively resistant to benzene. On the basis of these and other studies, it is now accepted that benzene metabolites are responsible for its adverse hematologic effects.

Although the major route of human exposure is inhalation, benzene has been administered by other parenteral routes in many animal studies. There is no evidence that variations in the route of administration alter the toxicity of benzene qualitatively. A substantial portion of any dose of benzene is exhaled unchanged or stored in fat in both animals and humans (Schrenk et al., 1941; Srbova et al., 1950; Teisinger et al., 1952; Nomiyama and Nomiyama, 1974a,b; Rickert et al., 1979). Rickert et al. (1979) reported that, after inhalation of benzene by rats, excretion via the lung followed a biphasic pattern indicative of a two-compartment system.

The bulk of the evidence suggests that benzene toxicity is produced by one or more metabolites, rather than by benzene itself (Snyder et al., 1981). The broad outlines of benzene metabolism were best established by Parke and Williams (1953), who used ^{14}C-labeled benzene. Phenol, catechol, hydroquinone, and 1,2,4-trihydroxybenzene were recovered as ethereal sulfates and glucuronide conjugates in urine of treated animals. 1-Phenylmercapturic acid and trans-trans-muconic acid were other metabolites.

The mechanism of benzene hydroxylation remains a matter of discussion. The metabolic pathway (Snyder et al., 1981) involves first the conversion of benzene to benzene oxide (Jerina and Daly, 1974) by the hepatic microsomal mixed-function oxidase (Gonasun et al., 1973). The oxide might rearrange nonenzymatically to form phenol, which reacts with glutathione to form a premercapturic acid that is later converted to 1-phenylmercapturic acid; or it might react with epoxide hydrolase, which converts it to benzene dihydrodiol. The mixed-function oxidase and epoxide hydrolase are microsomal enzymes, but a cytosolic dehydrogenase oxidizes the dihydrodiol to catechol. Hydroquinone is the primary product of further hydroxylation of phenol.

Harper et al. (1975) compared the metabolism of benzene to phenol by lung and liver microsomal preparations from hamsters, rats, and rabbits. There were wide differences in the apparent V_{max} of benzene hydroxylation among the various species and tissues and smaller differences in the apparent K_m of benzene hydroxylase.

Jerina and Daly (1974) suggested that the hydroxylation involves the intermediate formation of an epoxide, and much of the theory of carcinogenesis by bay-region diol-epoxides is founded on this concept. Although the epoxide of benzene has never been isolated and identified during the enzymatic oxidation of benzene, Tunek et al. (1982) reported

that the addition of an excess of purified epoxide hydrolase to a rat liver microsomal system during benzene metabolism resulted in the production of the dihydrodiol. This must have resulted from the intermediate formation of the epoxide. However, Ingelman-Sundberg and Hagbjork (1982) suggested that hydroxylation could occur via the insertion of a hydroxyl free radical. They postulated that the free radicals are generated by an "iron catalyzed cytochrome P-450-dependent Haber Weiss reaction," and their idea is supported by the demonstration that several compounds that prevent free-radical formation or act as free-radical scavengers can inhibit the hydroxylation of benzene by an isolated, reconstituted rabbit liver microsomal mixed-function oxidase. These discrepant suggestions could be reconciled if (1) two different cytochromes P-450 were responsible for benzene hydroxylation, one generating free radicals and thereby hydroxylating benzene and another forming the epoxide, or (2) the cytochrome P-450 responsible for benzene oxide formation also functioned as an NADPH oxidase, thereby producing hydrogen peroxide and then hydroxyl radicals. In regard to the first possibility, Post and Snyder (1983) recently demonstrated that at least two different rat liver mixed-function oxidases are active in benzene hydroxylation, but their mechanisms of action have yet to be clarified. The second possibility would mean that the mixed-function oxidase is inefficient and that oxygen radical formation is an indication of uncoupling of NADPH oxidation from substrate epoxidation.

The dihydroxylated metabolites, hydroquinone and catechol, appear to be formed by different pathways. The dihydrodiol can be aromatized by a cytosolic dehydrogenase (Ayengar et al., 1959) to yield catechol. Hydroquinone can be formed from phenol (Gilmour and Snyder, 1983) in a further hydroxylation step. Small amounts of catechol have also been observed to be produced from phenol (Gilmour and Snyder, 1983; Sawahata and Neal, 1982). These compounds, and possibly the trihydroxy compound, have been postulated by Tunek et al. (1980) and Irons et al. (1982) to be formed via the intermediate formation of quinones or semiquinones. In each case of hydroxylation, a free-radical insertion can be postulated.

Still another mechanism must be developed to explain the formation of muconic acid. Muconic acid was first identified as a metabolite of benzene in the rabbit (Parke and Williams, 1953) and is a known metabolite of catechol degradation by plant dioxygenases. Goldstein et al. (1982) suggested, however, that muconic dialdehyde might be the primary metabolite that results from ring opening and is later converted to muconic acid.

An alternative fate for benzene metabolites is covalent binding to cellular macromolecules, which many investigators believe is related to the mechanism of benzene toxicity or carcinogenicity. Radiolabeled benzene must be used to detect covalent binding. R. Snyder et al. (1978) and Longacre et al. (1981a,b) reported that benzene metabolites bind to proteins in mouse liver, bone marrow, kidney, spleen, blood,

and muscle. Less covalent binding was observed in bone marrow, blood, and spleen in mice that are relatively resistant to benzene toxicity (C57Bl/6) than in more sensitive mice (DBA2). Irons et al. (1980) found covalent binding to protein in perfused bone marrow preparations, and Lutz and Schlatter (1977) reported that liver DNA of rats exposed to benzene vapor contained labeled benzene residues. Tunek et al. (1978) have argued that the covalent binding comes principally from a metabolite of phenol, rather than from benzene oxide.

Gill and Ahmed (1981) have suggested that the mitochondria represent an important site of covalent binding for benzene. Kalf et al. (1982) have demonstrated that inhibition of RNA synthesis in mitochondria from both liver and bone marrow was correlated with covalent binding of benzene metabolites to DNA. It appears that phenol, hydroquinone, catechol, benzoquinone, and 1,2,4-trihydroxybenzene can lead to adduct formation in bone marrow mitochondria. Inhibition of RNA synthesis in mitochondria decreases the synthesis of critical mitochondrial proteins and thereby impairs mitochondrial function.

The search for the ultimate mechanism of benzene-induced bone marrow depression or leukemia is complicated by the fact that neither the specific target cells nor the intracellular target has been clearly identified. The data suggest that benzene metabolites can damage both the pluripotential stem cell and the early-proliferating committed cell in either the erythroid or the myeloid line. Thus, Lee et al. (1981) suggested that early-proliferating and maturing cells in red marrow, such as the pronormoblast and the normoblast in the erythroid line, are particularly sensitive to benzene. Tunek et al. (1982) and Boyd et al. (1982) have reported effects of benzene and its metabolites on the formation of colonies of granulocyte-producing cells in vitro. Uyeki et al. (1977), who studied inhibition of the in vitro formation of colonies by splenic cells from benzene-exposed animals, were the first to report effects of benzene on stem cells.

In humans, the chronic adverse effects of benzene are variants of aplastic anemia or leukemia. It is likely in each case that metabolites of benzene initiate the disease process and are also implicated in mutational events, such as increases in SCEs and micronuclei. These effects could be produced as a result of any of several benzene metabolites in bone marrow cells. Multiple sites have been identified as potential targets for benzene metabolites. Irons et al. (1982) and Irons and Neptun (1980) have shown that benzene metabolites inhibit microtubule assembly, a critical process for cell replication. The data cited above demonstrate that benzene metabolites can covalently bind to DNA, RNA, and protein. Benzene metabolites might also inhibit specific enzymes. It can be argued that one of these events is responsible for the inhibition of cell replication. However, it might not be necessary to attempt to exclude any of these as contributing events. The final disease process could be the result of the sum of adverse effects of benzene metabolites on several events in the reproduction of bone marrow cells.

INHALATION EXPOSURE LIMITS

The current ACGIH TLV-TWA for benzene is 10 ppm, with a TLV-STEL of 25 ppm (ACGIH, 1986a,b). The present OSHA standard for benzene is an 8-h time-weighted average (TWA) concentration of 10 ppm, with a 15-min ceiling limit of 25 ppm and a peak concentration of 50 ppm (U.S. Department of Labor, 1985). In 1978, OSHA promulgated a permanent standard for occupational exposure to benzene. The standard limited employee exposure to benzene to an 8-h TWA concentration of 1 ppm, with a 15-min ceiling of 5 ppm for any period during an 8-h workday (U.S. Department of Labor, 1978). The rule-making is not yet completed. Other limits have been recommended: American National Standards Institute, 10 ppm; Czechoslovakia, 16 ppm; Soviet Union, 1.6 ppm; German Democratic Republic and Sweden, 10 ppm; and Federal Republic of Germany, 0 ppm (cited in ACGIH, 1986a).

COMMITTEE EVALUATION AND RECOMMENDATIONS

From the summary of the toxicity information, it can be concluded that thresholds (exposures at which the several toxic effects of benzene are not observed) have not been well established. The lower ranges of exposure at which the major categories of effects have been observed, however, can be tabulated as follows:

Acute neurotoxicity	50-150 ppm for 5 h
Subacute hematopoietic toxicity	25-100 ppm for 10 yr
Chromosomal aberrations	1 ppm for more than 1 yr
Chromosomal aberrations, animals	1-10 ppm for 6 h
Fetotoxicity, animals	50 ppm for 7 h/d on days 6-15 of gestation
Teratogenicity, animals	500 ppm for 7 h/d on days 6-15 of gestation
Leukemia	1-10 ppm for many years
Cancer, animals	300 ppm for 16 wk 25 mg/kg orally for 103 wk 108 ppm for 1 h/d for 103 wk

After considering the acute neurotoxicity of benzene in humans and the carcinogenic effects of chronic exposure to benzene in animals and humans, the Committee concluded that EEGLs should be based on the acute neurotoxic effects in humans. Gerarde (1960) showed that exposure at 50-150 ppm for 5 h produced headache, lassitude, and weakness and exposure at 25 ppm for 8 h had no observed effect. Exposure at 50 ppm for 1 h is thus expected to have mild, but not incapacitating, effects. On the basis of a linear extrapolation, the following EEGLs are recommended:

Exposure Time	EEGL
1 h	50 ppm
24 h	2 ppm

Exposure of mice to benzene at 1 ppm for 6 h resulted in increased micronuclei, so the possibility of chromosomal aberrations at 6 ppm for 1 h cannot be excluded. The significance of such effects, however, is unknown. The available experimental evidence that single exposures might have greater deleterious effects on germ cells (sperm and ova) than fractionated exposures suggests that women exposed at more than 10 ppm for 1 h should be cautioned to postpone conceiving until the next reproductive cycle after the exposure. Full recovery of sperm might theoretically require 3 mo, but the data are insufficient for this recommendation. It should be noted that these recommendations are based on few data and therefore could change appreciably as new information becomes available.

The proposed EEGL of 2 ppm for 24 h is estimated to yield a cancer risk of no greater than 1×10^{-4}.

CANCER RISK ASSESSMENT

Benzene is a carcinogen. Therefore, a separate evaluation for carcinogenic risk was performed.

The U.S. Environmental Protection Agency's Carcinogen Assessment Group (CAG) (1979) did a risk analysis for benzene exposure. It used a linear nonthreshold model to estimate the leukemia risk that would result from exposure to benzene at the low ambient concentrations at which the general population is exposed. 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 length of exposure or age at exposure. To use this model, 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 estimated. The background leukemia rate was based on vital-statistics data for the entire U.S. population (Infante et al., 1977), whereas specific data from the studies of Aksoy et al. (1974, 1976) were used to estimate values for the relative-risk and exposure parameters of the model.

CAG reported that the excess risk for lifetime (70-yr) exposure to benzene at 1 ppb by inhalation is 24×10^{-6}. The Committee believes that the CAG risk assessment is reasonable. The dose yielding a lifetime (70-yr) risk of 1×10^{-4} is then:

$$(1 \times 10^{-4})/(24 \times 10^{-6}) \times 1 \text{ ppb} = 4.2 \text{ ppb}.$$

For a 1-d (24-h) exposure (for an EEGL) yielding the same risk, to reach the same cumulative dose and to allow for possible variability in the stage of the cancer process at which benzene is operating by using a factor of 2.8 (Crump and Howe, 1984), the value of 4.2 ppb needs to be multiplied by:

$$[70 \times 365 \text{ (days in a lifetime)}]/2.8 \text{ (multistage factor)} = 9{,}125.$$

The 24-h EEGL is 4.2 ppb x 9,125 = 38,015 ppb or 38 ppm. On the basis of Haber's law, this implies an EEGL of 912 ppm for a 1-h exposure--18 times higher than the EEGL based on neurotoxicity.

Several animal studies (NTP, 1985; Maltoni et al., 1983) have indicated increased incidences of various forms of cancer. The National Toxicology Program study found increases in leukemias and lymphomas--neoplasias that resemble those reported in epidemiologic studies of humans. As estimated by the California Air Resources Board and California Department of Health Services (1984), the maximal-likelihood estimate from the multistage model for the human equivalent-lifetime cancer risk (adjusted for life-shortening, if any) associated with benzene administered by gavage to the male B6C3F1 mouse at 17.9 mg/kg is 170×10^{-6} ppm for leukemia or lymphoma. The upper 95% confidence limit (UCL) of this estimate is 230×10^{-6} ppm (California Air Resources Board and California Dept. of Health Services, 1984, p. 101). Exposure was for 5 d/wk for 103 wk or a full lifetime.

The dose estimated to yield a 1×10^{-4} risk is

$$(1 \times 10^{-4})/(170 \times 10^{-6}) = 0.59 \text{ ppb.}$$

To convert this dose to the appropriate human 24-h dose requires multiplication (as above) by (70 x 365)/2.8 = 9,125.

Modifying the computation above by multiplying the dose by 170/230 (the risk x 10^{-6} for the maximal-likelihood estimate is 170; the risk x 10^{-6} for the 95% UCL is 230) gives 0.59 ppb x 9,125 x 170/230 = 4 ppm. This estimate now needs to be multiplied by 5/7, the animals having been treated 5 d/wk. This gives, for the 24-h EEGL, 2.8 ppm. The 95%-UCL approach takes into account the steepness of the dose-response curve and the number of animals in the experiment.

This estimate for a 24-h EEGL of 2.8 ppm based on animal data is approximately one-tenth that derived from human data. The reason lies in the elements that entered into the computation. The dose-response factor (increase in response per unit increase in dose) for the animal data is approximately 10 times that derived from the human data (230/24 = 9.58).

With respect to data from human exposures, the EEGLs based on carcinogenicity exceed those based on acute toxicity. It appears that the EEGL of 50 ppm for 1 h, which was based on neurotoxicity, will result in a cancer risk of no greater than 1×10^{-4} over the background rate.

REFERENCES

Aksoy, M., K. Dincol, T. Akgun, S. Erdem, and G. Dincol. 1971. Haematological effects of chronic benzene poisoning in 217 workers. Br. J. Ind. Med. 28:296-302.

Aksoy, M., K. Dincol, S. Erdem, T. Akgun, and G. Dincol. 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., and S. Erdem. 1978. Followup study on the mortality and the development of leukemia in 44 pancytopenic patients with chronic exposure to benzene. Blood 52:285-292.

Aksoy, M., S. Erdem, and G. Dincol. 1974. Leukemia in shoe-workers exposed chronically to benzene. Blood 44:837-841.

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

Amacher, D.E., and I. Zelljadt. 1983. The morphological transformation of Syrian hamster embryo cells by chemicals reportedly nonmutagenic to Salmonella typhimurium. Carcinogenesis 4:291-295.

American Conference of Governmental Industrial Hygienists. 1986a. Benzene, pp. 50-52. Documentation of the Threshold Limit Values and Biological Exposure Indices, 5th ed. Cincinnati, Ohio: American Conference of Governmental Industrial Hygienists, Inc.

American Conference of Governmental Industrial Hygienists. 1986b. TLVs®: Threshold Limit Values and Biological Exposure Indices for 1986-1987. Cincinnati, Ohio: American Conference of Governmental Industrial Hygienists, Inc.

Andrews, L.S., E.W. Lee, C.M. Witmer, J.J. Kocsis, and R. Snyder. 1977. Effects of toluene on the metabolism, disposition and hemopoietic toxicity of [^{3}H]benzene. Biochem. Pharmacol. 26:293-300.

Ayengar, P.K., O. Hayaishi, M. Nakajima, and I. Tomida. 1959. Enzymic aromatization of 3,5-cyclohexadiene-1,2-diol. Biochim. Biophys. Acta 33:111-119.

Baarson, K.A., C.A. Snyder, and R.E. Albert. 1984. Repeated exposure of C57B1 mice to inhaled benzene at 10 ppm markedly depressed erythropoietic colony formation. Toxicol. Lett. 20:337-342.

Bond, G.G., McLaren, E.A., Baldwin, C.L., and R.R. Cook. An update of Mortality Among Chemical Workers Exposed to Benzene. Unpublished manuscript. Submitted to Dept. of Labor. From Richard D. Olson, Dow Chemical. OSHA Docket No. H-059C Exhibit 201-28. March 3, 1986.

Boyd, R., J. Griffiths, V. Kindt, R. Snyder, J. Caro, and A. Erslev. 1982. Relative toxicity of five benzene metabolites on CFU-GM cultures. The Toxicologist 2(1):121. (Abstract No. 429)

Browning, E. 1965. Toxicity and Metabolism of Industrial Solvents. New York: Elsevier Publishing Co.

California Air Resources Board and California Department of Health Services. 1984. Report to the Scientific Review Panel on Benzene. Prepared by the staffs of the Air Resources Board and the Department of Health Services. [540 pp.]

Carpenter, C.P., C.B. Shaffer, C.S. Weil and H.F. Smyth, Jr. 1944. Studies on the inhalation of 1:3-butadiene; with a comparison of its narcotic effect with benzol, toluol, and styrene, and a note on the elimination of styrene by the human. J. Ind. Hyg. Toxicol. 26:69-78.

Chang, I.W. 1972. Study on the threshold limit value of benzene and early diagnosis of benzene poisoning. J. Cathol. Med. Coll. 23:429-434.

Coffin, D.L., D.E. Gardner, G.I. Sidorenko, and M.A. Pinigin. 1977. Role of time as a factor in the toxicity of chemical compounds in intermittent and continuous exposures. Part. II. Effects of intermittent exposure. J. Toxicol. Environ. Health 3:821-828.

Cornish, H.H., and R.C. Ryan. 1965. Metabolism of benzene in nonfasted, fasted, and aryl-hydroxylase inhibited rats. Toxicol. Appl. Pharmacol. 7:767-771.

Cortina, T.A., E.W. Sica, N.E. McCarroll, W. Coate, A. Thakur, and M.G. Farrow. 1982. Inhalation cytogenetics in mice and rats exposed to benzene, pp. 81-88. In H.N. MacFarland, E.E. Holdsworth, J.A. MacGregor, R.W. Call, and M.L. Kane, Eds. The Toxicology of Petroleum Hydrocarbons. Washington, D.C. American Petroleum Institute.

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

Cronkite, E.P., J.E. Bullis, T. Inoue, and R.T. Drew. 1984. Benzene inhalation produces leukemia in mice. Toxicol. Appl. Pharmacol. 75:358-361.

Crump, K.S., and R.B. Howe. 1984. The multistage model with a time dependent dose pattern: Applications to carcinogenic risk assessment. Risk Analysis 4:163-176.

Dabney, B.J. 1981. The role of human genetic monitoring in the workplace. J. Occup. Med. 23:626-631.

Dean, B.J. 1978. Genetic toxicology of benzene, toluene, xylenes and phenols. Mutat. Res. 47:75-97.

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

Deichmann, W.B., W.E. MacDonald, and E. Bernal. 1963. The hemopoietic tissue toxicity of benzene vapors. Toxicol. Appl. Pharmacol. 5:201-224.

Doskin, V.A. 1971. Effect of age on the reaction to a combination of hydrocarbons. Hyg. Sanit. 36(3):379-384.

Environmental Health Associates. 1983. An industry-wide mortality study of chemical workers occupationally exposed to benzene. Berkeley, Calif.: Environmental Health Associates. 130 pp.

Erexson, G., J.L. Wilmer, W.H. Steinhagen, and A.D. Kligerman. 1986. Induction of cytogenetic damage in rodents after short-term inhalation of benzene. Env. Mutagen. 8:29-40.

Gerarde, H.W. 1960. Toxicology and Biochemistry of Aromatic Hydrocarbons. New York, N.Y.: Elsevier Publishing Company. 329 pp.

Gill, D.P., and A.E. Ahmed. 1981. Covalent binding of [^{14}C]benzene to cellular organelles and bone marrow nucleic acids. Biochem. Pharmacol. 30:1127-1131.

Gilmour, S., and R. Snyder. 1983. Metabolism of benzene and phenol in rat liver microsomes. Fed. Proc., Fed. Am. Soc. Exp. Biol. 42:1136. (Abstract No. 4948)

Goldstein, B.D., G. Witz, J. Javid, M.A. Amoruso, T. Rossman, and B. Wolder. 1982. Muconaldehyde, a potential toxic intermediate of benzene metabolism, pp. 331-339. In R. Snyder, D.V. Parke, J.J. Kocsis, D.J. Jollow, G.G. Gibson, and C.M. Witmer, Eds. Biological Reactive Intermediates. II. Chemical Mechanisms and Biological Effects. New York, N.Y.: Plenum Press.

Goldwater, L.J. 1941. Disturbances in the blood following exposure to benzol. J. Lab. Clin. Med. 26:957-973.

Goldwater, L.J., and M.P. Tewksbury. 1941. Recovery following exposure to benzene (benzol). J. Ind. Hyg. Toxicol. 23:217-231.

Gonasun, L.M., C.M. Witmer, J.J. Kocsis, and R. Snyder. 1973. Benzene metabolism in mouse liver microsomes. Toxicol. Appl. Pharmacol. 26:398-406.

Green, J.D., B.K.J. Leong, and S. Laskin. 1978. Inhaled benzene fetotoxicity in rats. Toxicol. Appl. Pharmacol. 46:9-18.

Greenburg, L., M.R. Mayers, L. Goldwater, and A.R. Smith. 1939. Benzene (benzol) poisoning in the rotogravure printing industry in New York City. J. Ind. Hyg. Toxicol. 21:395-420.

Guberan, E., and P. Kocher. 1971. Pronostic lointain de l'intoxication benzolique chronique: control d'une population 10 ans apres l'exposition. Schweiz. Med. Wochenschr. 101:1789-1790.

Hamilton, A. 1931. General review. Benzene (benzol) poisoning. Arch. Pathol. 11:434-454, 602-637.

Harper, C., R.T. Drew, and J.R. Fouts. 1975. Species differences in benzene hydroxylation to phenol by pulmonary and hepatic microsomes. Drug. Metab. Dispos. 3:381-388.

Helmer, K.J. 1944. Accumulated cases of chronic benzene poisoning in the rubber industry. Acta Med. Scand. 118:354-375.

Hernberg, S., M. Savilahti, K. Ahlman, and S. Asp. 1966. Prognostic aspects of benzene poisoning. Br. J. Ind. Med. 23:204-209.

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

Hunter, F.T. 1939. Chronic exposure to benzene (benzol). II. The clinical effects. J. Ind. Hyg. Toxicol. 21:331-393.

Ikeda, M., and H. Ohtsuji. 1971. Phenobarbital-induced protection against toxicity of toluene and benzene in the rat. Toxicol. Appl. Pharmacol. 20:30-43.

Infante, P.F., R.A. Rinsky, J.K. Wagoner, and R.J. Young. 1977. Leukemia in benzene workers. Lancet 2:76-78.

Ingelman-Sundberg, M., and A.L. Hagbjork. 1982. On the significance of the cytochrome P-450-dependent hydroxyl radical-mediated oxygenation mechanism. Xenobiotica 12:673-686.

International Agency for Research on Cancer. 1982. Benzene, pp. 93-148. In IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Vol. 29. Some Industrial Chemicals and Dyestuffs. Lyon, France: International Agency for Research on Cancer.

Irons, R.D., J.G. Dent, T.S. Baker, and D.E. Rickert. 1980. Benzene is metabolized and covalently bound in bone marrow in situ. Chem. Biol. Interact. 30:241-245.

Irons, R.D., W.F. Greenlee, D. Wierda, and J.S. Bus. 1982. Relationship between benzene metabolism and toxicity. A proposed mechanism for the formation of reactive intermediates from polyphenol metabolites, pp. 229-43. In R. Snyder, D.V. Parke, J.J. Kocsis, D.J. Jollow, G.G. Gibson, and C.M. Witmer, Eds. Biological Reactive Intermediates. II: Chemical Mechanisms and Biological Effects. New York, N.Y.: Plenum Press.

Irons, R.D., and D.A. Neptun. 1980. Effects of the principal hydroxy-metabolites of benzene on microtubule polymerization. Arch. Toxicol. 45:297-305.

Jerina, D.M., and J.W. Daly. 1974. Arene oxides. A new aspect of drug metabolism. Science 185:573-582.

Jung, R., D. Beermann, H.R. Glatt, and F. Oesch. 1981. Mutagenicity of structurally related oxirane derivatives of benzene and its hydrogenated congeners. Mutat. Res. 81:11-19.

Kalf, G.F., T. Rushmore, and R. Snyder. 1982. Benzene inhibits RNA synthesis in mitochondria from liver and bone marrow. Chem. Biol. Interact. 42:353-370.

Kimura, E.T., D.M. Ebert, and P.W. Dodge. 1971. Acute toxicity and limits of solvent residue for sixteen organic solvents. Toxicol. Appl. Pharmacol. 19:699-704.

Kuna, R.A., and R.W. Kapp, Jr. 1981. The embryotoxic/teratogenic potential of benzene vapor in rats. Toxicol. Appl. Pharmacol. 57:1-7.

Lebowitz, H., D. Brusick, D. Matheson, D.R. Jagannath, M. Reed, S. Goode, and G. Roy. 1979. Commonly used fuels and solvents evaluated in a battery of short-term bioassays. Env. Mutagen. 1:172-173. [Abstract No. Eb-8]

Lee, E.W., J.J. Kocsis, and R. Snyder. 1981. The use of ferrokinetics in the study of experimental anemia. Environ. Health Perspect. 39:29-37.

Longacre, S.L., J.J. Kocsis, C.M. Witmer, E.W. Lee, D. Sammett, and R. Snyder. 1981a. Toxicological and biochemical effects of repeated administration of benzene in mice. J. Toxicol. Environ. Health 7:223-237.

Longacre, S.L., J.J. Kocsis, and R. Snyder. 1981b. Influence of strain differences in mice on the metabolism and toxicity of benzene. Toxicol. Appl. Pharmacol. 60:398-409.

Lutz, W.K., and C. Schlatter. 1977. Mechanism of the carcinogenic action of benzene. Irreversible binding to rat liver DNA. Chem. Biol. Interact. 18:241-245.

Lyon, J.P. 1976. Mutagenicity Studies with Benzene (Ph.D. Thesis, University of California, 1975). Dissert. Abstracts B36:5537.

Maltoni, C., B. Conti, and G. Cotti. 1983. Benzene. A multipotential carcinogen. Results of long-term bioassays performed at the Bologna Institute of Oncology. Am. J. Ind. Med. 4:589-630.

Maltoni, C., B. Conti, G. Cotti, and F. Belpoggi. 1985. Experimental studies on benzene carcinogenicity at the Bologna Institute of Oncology. Current results and ongoing research. Am. J. Ind. Med. 7:415-446.

Morimoto, K. 1983. Induction of sister chromatid exchanges and cell division delays in human lymphocytes by microsomal activation of benzene. Cancer Res. 43:1330-1334.

Morimoto, K., and S. Wolff. 1980. Increase of sister chromatid exchanges and pertubations of cell division kinetics in human lymphocytes by benzene metabolites. Cancer Res. 40:1189-1193.

Morimoto, K., S. Wolff, and A. Koizumi. 1983. Induction of sister-chromatid exchanges in human lymphocytes by microsomal activation of benzene metabolites. Mutat. Res. 119:355-360.

Murray, F.J., J.A. John, L.W. Rampy, R.A. Kuna, and B.A. Schwetz. 1979. Embryotoxicity of inhaled benzene in mice and rabbits. Am. Ind. Hyg. Assoc. J. 40:993-998.

National Institute for Occupational Safety and Health. 1974. Criteria for a Recommended Standard....Occupational Exposure to Benzene. Washington, D.C.: U.S. Department of Health, Education, and Welfare.

National Research Council, Committee on Toxicology. 1976. Health Effects of Benzene: A Review. Washington, D.C.: National Academy of Sciences. 23 pp.

National Toxicology Program. 1985. Toxicology and Carcinogenesis Studies of Benzene (CAS NO. 71-43-2) in F344/N Rats and B6C3F_1 Mice (Gavage Studies). Technical Report Series No. 289. Research Triangle Park, North Carolina: National Toxicology Program. (unpublished)

Nawrot, P.S., and R.E. Staples. 1979. Embryo-fetal toxicity and teratogenicity of benzene and toluene in the mouse. Teratology 19:41A. (abstract)

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

Ott, M.G., J.C. Townsend, W.A. Fishbeck, and R.A. Langner. 1978. Mortality among individuals occupationally exposed to benzene. Arch. Environ. Health 33:3-10.

Pagnotto, L.D., H.B. Elkins, H.G. Brugsch, and J.E. Walkley. 1961. Industrial benzene exposure from petroleum naphtha. I. Rubber coating industry. Am. Ind. Hyg. Assoc. J. 22:417-421.

Parke, D.V., and R.T. Williams. 1953. Studies in detoxication. 49. The metabolism of benzene containing [^{14}C]benzene. Biochem. J. 54:231-238.

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

Post, G.B., and R. Snyder. 1983. Effects of enzyme induction on microsomal benzene metabolism. J. Toxicol. Environ. Health 11:811-825.

Rickert, D.E., T.S. Baker, J.S. Bus, C.S. Barrow, and R.D. Irons. 1979. Benzene disposition in the rat after exposure by inhalation. Toxicol. Appl. Pharmacol. 49:417-423.

Rinsky, R.A., R.J. Young, and A.B. Smith. 1981. Leukemia in benzene workers. Am. J. Ind. Med. 2:217-245.

Rozen, M.G., C.A. Snyder, and R.E. Albert. 1984. Depressions in B- and T-lymphocyte mitogen-induced blastogenesis in mice exposed to low concentrations of benzene. Toxicol. Lett. 20:343-349.

Saita, G., and E.C. Vigliani. 1962. The action of benzol in inducing leukemia. Med. Lavoro 53:581-586.

Santesson, C.G. 1897. Chronic poisoning with coal tar benzene; four deaths. Arch. Hyg. 31:336-376.

Sarto, F.S., I. Cominato, A.M. Pinton, P.G. Brovedani, E. Merler, M. Peruzzi, V. Bianchi, and A.G. Levis. 1984. A cytogenetic study on workers exposed to low concentrations of benzene. Carcinogenesis 5:827-832.

Savilahti, H. 1956. More than 100 cases of benzol poisoning in a shoe factory. Arch. Gewerbepathol. Gewerbehyg. 15:147-157.

VVV 000009128

Sawahata, T., and R.A. Neal. 1982. Horseradish peroxidase-mediated oxidation of phenol. Biochem. Biophys. Res. Commun. 109:988-994.

Schrenk, H.H., W.P. Yant, S.J. Pearce, F.A. Patty, R.R. Sayers. 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. Reports 17:83-142.

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

Snyder, C.A., B.D. Goldstein, A.R. Sellakumar, S.R. Wolman, I. Bromberg, M.N. Erlichman, and S. Laskin. 1978. Hematotoxicity of inhaled benzene to Sprague-Dawley rats and AKR mice at 300 ppm. J. Toxicol. Environ. Health 4:605-618.

Snyder, R., and J.J. Kocsis. 1975. Current concepts of chronic benzene toxicity. CRC Crit. Rev. Toxicol. 3:265-288.

Snyder, R., E. W. Lee, J.J. Kocsis, and C.M. Witmer. 1977. Bone marrow depressant and leukemogenic actions of benzene. Life Sci. 21:1709-1721.

Snyder, R., E.W. Lee, and J.J. Kocsis. 1978. Binding of labeled benzene metabolites to mouse liver and bone marrow. Res. Commun. Chem. Pathol. Pharmacol. 20:191-94.

Snyder, R., S.L. Longacre, C.M. Witmer, J.J. Kocsis, L.S. Andrews, and E.W. Lee. 1981. Biochemical toxicology of benzene. Rev. Biochem. Toxicol. 3:123-153.

Srbova, J., J. Teisinger, and S. Skramovsky. 1950. Absorption and elimination of inhaled benzene in man. Arch. Ind. Hyg. 2:1-8.

Styles, J.A., and C.R. Richardson. 1984. Cytogenetic effects of benzene. Dosimetric studies on rats exposed to benzene vapour. Mutat. Res. 135:203-209.

Svirbely, J.L., R.C. Dunn, and W.F. von Oettingen. 1943. The acute toxicity of vapors of certain solvents containing appreciable amounts of benzene and toluene. J. Ind. Hyg. Toxicol. 25:366-373.

Teisinger, J., V. Bergerova-Fiserova, and J. Kudrna. 1952. Metabolismus benzenu u cloveka. Prac. Lek. 4:175-188.

Tice, R.R., T.F. Vogt, and D.L. Costa. 1982. Cytogenetic effects of inhaled benzene on murine bone marrow, pp. 257-275. In R.R. Tice, D.L. Costa, and K.M. Schaich, Eds. Genotoxic Effects of Airborne Agents. New York, N.Y.: Plenum Press.

Toft, K., T. Olotsson, A. Tunek, and M. Berlin. 1982. Toxic effects on mouse bone marrow caused by inhalation of benzene. Arch. Toxicol. 51:295-302.

Tunek, A., K.L. Platt, P. Bentley, and F. Oesch. 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., K.L. Platt, M. Przybylski, and F. Oesch. 1980. Multi-step metabolic activation of benzene. Effect of superoxide dismutase on covalent binding to microsomal macromolecules, and identification of glutathione conjugates using high pressure liquid chromatography and field desorption mass spectrometry. Chem. Biol. Interact. 33:1-17.

Tunek, A., B. Hogstedt, and T. Olofsson. 1982. Mechanism of benzene toxicity. Effects of benzene and benzene metabolites on bone marrow cellularity, number of granulopoietic stem cells and frequency of micronuclei in mice. Chem. Biol. Interact. 39:129-138.

U.S. Department of Labor. 1978. Occupational Exposure to Benzene. Final rule. Fed. Regist. 43:5918-5970.

U.S. Department of Labor. 1985. Toxic and Hazardous Substances. 29 Code of Federal Regulations 1910.1000.

U.S. Environmental Protection Agency. 1978. Assessment of Health Effects of Benzene Germane to Low-Level Exposure. EPA-600/1-78-061. Prepared by the Office of Health and Ecological Effects. Washington, D.C. Government Printing Office.

U.S. 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, N.C.: US EPA, Office of Air Quality Planning and Standards.

U.S. International Trade Commission. 1985. Preliminary Report on U.S. Production of Selected Synthetic Organic Chemicals (Including Synthetic Plastics and Resin Materials). Preliminary Totals. 1984 S.O.C. Series C/P-85-1. Washington, D.C.: U.S. International Trade Commission.

Uyeki, E.M., A.E. Ashkar, D.W. Shoeman, and T.U. Bisel. 1977. Acute toxicity of benzene inhalation to hemopoietic precursor cells. Toxicol. Appl. Pharmacol. 40:49-57.

Vigliani, E.C., and A. Forni. 1976. Benzene and leukemia. Environ. Res. 11:122-127.

Vigliani, E.C., and G. Saita. 1964. Benzene and leukemia. N. Eng. J. Med. 271:872-876.

Watanabe, G.I., and S. Yoshida. 1970. The teratogenic effect of benzene in pregnant mice. Acta Med. Biol. 17:285-291.

Wilson, R.H. 1942. Benzene poisoning in industry. J. Lab. Clin. Med. 27:1517-1521.

Wolf, M.A., V.K. Rowe, D.D. McCollister, R.L. Hollingsworth, and F. Oyen. 1956. Toxicological studies of certain alkylated benzenes and benzene. AMA Arch. Ind. Health 14:387-398.