

The Toxicology of Benzene

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Benzene is metabolized, primarily in the liver, to a series of phenolic and ring-opened products and their conjugates. The mechanism of benzene-induced aplastic anemia appears to involve the concerted action of several metabolites acting together on early stem and progenitor cells, as well as on early blast cells, such as pronormoblasts and normoblasts to inhibit maturation and amplification. Benzene metabolites also inhibit the function of microenvironmental stromal cells necessary to support the growth of differentiating and maturing marrow cells. The mechanism of benzene-induced leukemogenesis is less well understood. Benzene and its metabolites do not function well as mutagens but are highly clastogenic, producing chromosome aberrations, sister chromatid exchange, and micronuclei. Benzene has been shown to be a multi-organ carcinogen in animals. Epidemiological studies demonstrate that benzene is a human leukemogen. There is need to better define the lower end of the dose-response curve for benzene as a human leukemogen. The application of emerging methods in biologically based risk assessment employing pharmacokinetic and mechanistic data may help to clarify the uncertainties in low-dose risk assessment.

Introduction

The biological impacts of benzene have been studied in humans and in animal models for most of this century. This review concentrates on several aspects of the biology of benzene. We discuss its metabolic fate, our current understanding of the mechanism by which it produces its effects, attempts to develop pharmacokinetic models for its disposition, and, finally, we discuss the impact of benzene on humans. Our aim is not only to present a synopsis of the literature on benzene toxicity, but also to challenge investigators to initiate new studies that will lead to a thorough understanding of benzene as a hazardous agent in our environment.

Metabolism

Benzene is the smallest and most stable aromatic hydrocarbon. In order to be toxic, benzene must be metabolized to reactive intermediates (1-3). Available studies suggest that benzene toxicity is mediated by multiple metabolites acting on multiple cellular targets (4). This discussion briefly reviews benzene metabolism, mechanism of metabolite formation and the chemical reactivity of benzene metabolites in relation to cellular targets. For recent detailed reviews, the reader is referred to Kalf (5), Snyder and Chatterjee (6), and Yardley-Jones et al. (7).

Metabolic Pathways

The major metabolism of benzene *in vivo* takes place in the liver and consists of the formation of ring-hydroxylated compounds (Fig. 1). Early studies conducted in rabbits (8-10) showed that benzene is hydroxylated to phenol, catechol, hydroquinone, and 1,2,4-benzenetriol, which are excreted as ethereal sulfates and glucuronides in the urine (Fig. 2). Work by Jaffe (11) and other investigators (12) showed that benzene metabolism also involves ring opening as indicated by the urinary excretion of *trans,trans*-muconic acid, a ring-opened, six carbon diene dicarboxylic acid. The formation of *trans,trans*-muconic acid (muconic acid) was definitively established by Parke and Williams (9), who demonstrated the excretion of [¹⁴C]muconic acid in the urine of rabbits dosed with [¹⁴C]benzene.

The metabolic pathways of benzene originally determined in rabbits were subsequently established in rats and mice (13-16). Two metabolic pathways appear to be involved, one leading to ring-hydroxylated compounds and one that involves ring opening of benzene. The hydroxylated compounds are further metabolized to glucuronide or sulfate conjugates, which are excreted in the urine. These conjugates are detoxification products, because conjugation leads to their elimination and prevents the formation of toxic intermediates derived from hydroxylated benzene metabolites. Conjugation with glutathione and urinary excretion of the mercapturic acid is an additional pathway that detoxifies benzene oxide, the initial reactive intermediate formed during metabolism of benzene to phenol (discussed below). The urinary metabolite muconic acid is at present the only known ring-opened metabolite formed from benzene *in vivo*. This metabolite is a detoxification product of *trans,trans*-muconaldehyde, a reactive intermediate formed during microsomal metabolism of benzene via ring opening (17).

The initial step in the metabolism of benzene results in the for-

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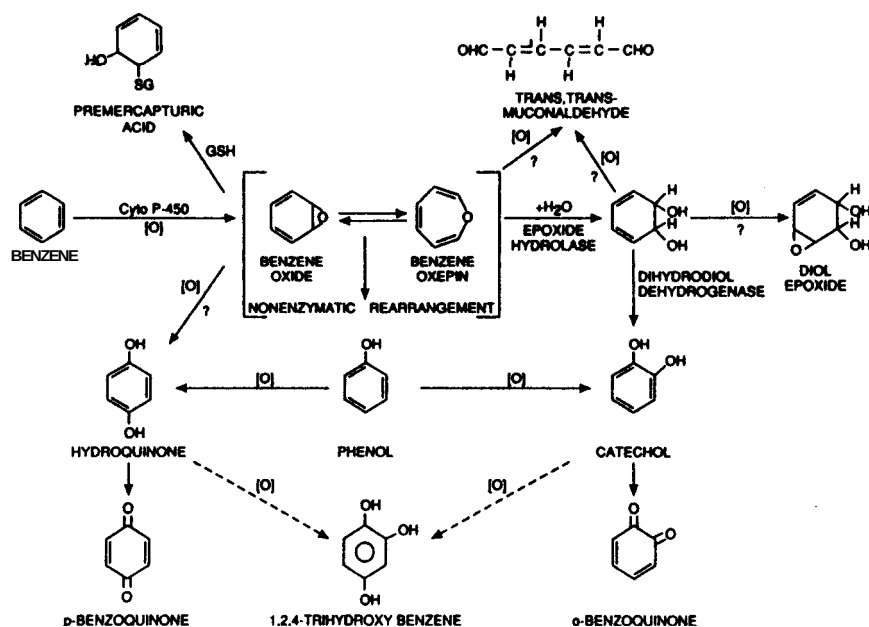
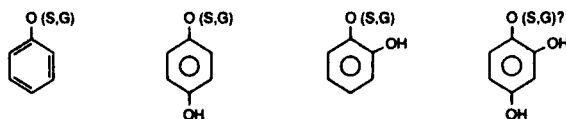
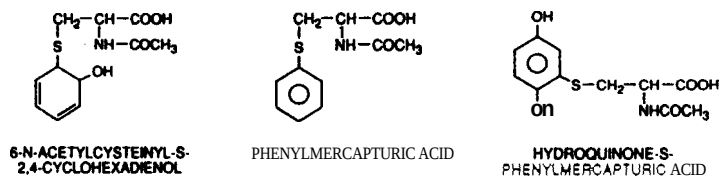


FIGURE 1. Intermediary metabolism of benzene. [O] indicates oxidation without specifying mechanism; **GSH**, glutathione. Question marks indicated suspected but as yet unproven pathways. Dotted lines leading to 1,2,4-trihydroxybenzene suggest that it may be formed by either pathway.

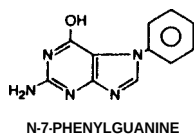
Glucuronide and Sulfate Conjugates



Mercapturic Acids



DNA-based Adduct(s)



Ring-Opened Metabolite(s)

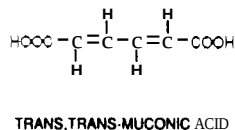


FIGURE 2. Urinary metabolites of benzene. The sulfate and glucuronide conjugates of phenol, catechol, and hydroquinone have been characterized. The location of the conjugate on 1,2,4-trihydroxybenzene has yet to be established. The glutathione derivatives, the DNA base derivative and the ring-opened product are those which have been identified in urine.

mation of phenol. Numerous *in vitro* studies utilizing cellular fractions or reconstituted purified enzyme systems indicate that this step involves metabolism of benzene by cytochrome P-450 monooxygenase (18). Benzene is a substrate for isozyme P-450 IIE1 (19), and P-450 monooxygenases are also active in the

metabolism of phenol to hydroquinone and catechol (20). The formation of phenol is believed to involve the intermediate formation of benzene oxide followed by rearrangement to phenol (21). Alternatively, phenol can also be formed from benzene oxide by acid-catalyzed opening of the epoxide ring, followed by

aromatization via loss of a proton.

Mechanisms of phenol formation that do not involve an intermediate epoxide but rather direct insertion of oxygen or aromatic hydroxylation (22) may also account for the formation of appreciable amounts of phenol. Phenol formation from benzene in a reconstituted system containing rabbit liver P-450 isozyme LM2 (P-450 IIE1) or microsomes was reported to be inhibited by the hydroxyl radical scavengers mannitol and dimethyl sulfoxide (DMSO), as well as by catalase, horseradish peroxidase, and superoxide dismutase (23). These findings suggest that the cytochrome P-450-dependent formation of phenol can be mediated by hydroxyl radicals most likely generated from hydrogen peroxide. The latter is thought to be formed by dismutation of superoxide anion radicals released from cytochrome P-450. Hydroxyl radical-mediated phenol formation involves addition of a hydroxyl radical to the benzene ring, resulting in the formation of a reactive hydroxy cyclohexadienyl radical (24). Phenol can subsequently be formed by a dismutation reaction as suggested by Cohen and Ofodile (25), who investigated phenol formation from benzene in a hydroxyl radical-generating Fenton system. A role for hydroxyl radicals in the metabolism of benzene to phenol has also been suggested by Gorsky and Coon (26), who concluded that the hydroxyl radical-mediated formation of phenol is the dominant pathway at micromolar concentrations, whereas at higher (millimolar) concentrations, the direct oxidation by P-450 is quantitatively of much greater importance.

Cytochrome P-450 mediated metabolism of phenol leads to the formation of hydroquinone and catechol and the subsequent formation of 1,2,4-benzenetriol. An alternative pathway for the formation of catechol involves metabolism of benzene oxide by epoxide hydrolase to benzene-*trans*-dihydrodiol, which is converted to catechol by the action of a dehydrogenase. Benzene oxide can also react with glutathione under the influence of a glutathione transferase to eventually yield a prephenylmercapturic acid (6-*N*-acetyl-cysteinyl-S-2,4-cyclohexadienol). Under acidic conditions this metabolite is aromatized by dehydration to *S*-phenylmercapturic acid, and both compounds have been observed in urine (27). The hydroxylated aromatic benzene metabolites are further metabolized to sulfate or glucuronic acid conjugates.

The metabolite 2-(*S*-glutathionyl) hydroquinone has been identified in microsomal metabolism mixtures incubated with benzene or phenol in the presence of added glutathione (28,29). It is presumably formed by the reaction of glutathione with benzoquinone, a reactive intermediate derived from the oxidation of hydroquinone. Hydroquinone-*S*-phenyl mercapturic acid derived from the metabolism of 2-(*S*-glutathionyl) hydroquinone has been identified as a urinary metabolite in rats dosed intraperitoneally with benzene, phenol, or hydroquinone (30). Thus, Figure 2 shows that the urinary metabolites of benzene may include the glucuronic and sulfate conjugates of the phenolic metabolites, the premercapturic and the mercapturic acids derived from benzene oxide, the mercapturic acid derived from *p*-benzoquinone, *N*-7-phenylguanine, derived from a DNA adduct of benzene, and muconic acid.

Microsomal metabolism of benzene leads to the formation of *trans,trans*-muconaldehyde (Fig. 3), a reactive ring-opened, six-carbon diene dialdehyde (27). Muconaldehyde was also shown to be formed in aqueous solutions of benzene irradiated with X-

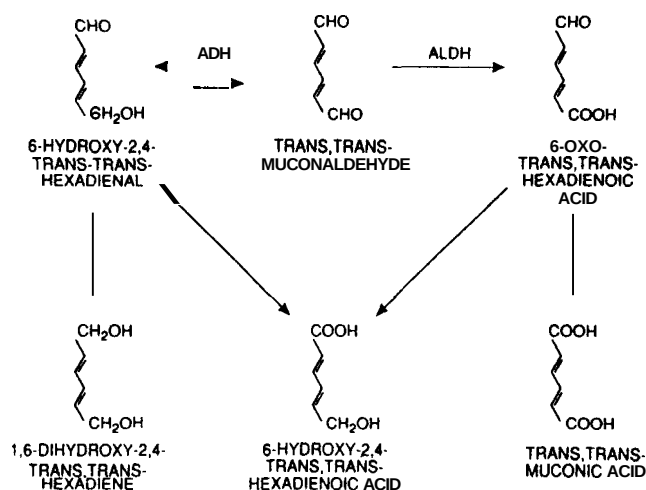


FIGURE 3. Intermediary metabolism of muconaldehyde. ALDH, aldehyde dehydrogenase; ADH, alcohol dehydrogenase. The pathway with the question mark indicates a possible route of metabolism.

rays (31,32) presumably via hydroxyl radical-mediated opening of the benzene ring. These latter studies and others on the hydroxyl radical-mediated formation of phenol suggest that free radicals may participate in benzene metabolism.

The metabolism of muconaldehyde (MUC; Fig. 3) by mouse liver cytosol or a mouse liver soluble fraction results in a variety of metabolites that are formed by oxidation and/or reduction of the aldehyde functional group(s) (33-35). Initial oxidation or reduction leads to the aldehyde acid and aldehyde alcohol analog of MUC, respectively. Subsequent reduction of the aldehyde group of the mixed-aldehyde acid MUC analog or oxidation of the mixed-aldehyde alcohol analog of MUC leads to the formation of the mixed-hydroxy acid MUC analog, a metabolite in which one aldehydic functional group of MUC is reduced to the alcohol and one is oxidized to the carboxylic acid. Interestingly, the initial reduction to the aldehyde alcohol MUC analog is reversible (G Witz, unpublished data). The mixed aldehyde carboxylic acid analog of MUC is further metabolized to muconic acid, a urinary metabolite of benzene (9,36,37).

Reactive Metabolites in Benzene Metabolism

The major metabolism of benzene takes place in the liver where it is mediated by cytochrome P-450 monooxygenase. Metabolism of benzene by cytochrome P-450 results in the formation of benzene oxide, an electrophilic reactive intermediate. Evidence that benzene is indeed metabolized *in vivo* to benzene oxide comes from studies by Mueller et al. (38) who identified *N*-7-phenylguanine in the urine of rats exposed to 500 ppm benzene for 8 hr. This adduct is most likely formed by reaction of benzene oxide with the *N*-7 of guanine, followed by aromatization of the benzene ring via loss of a molecule of water and subsequent depurination of the adduct from DNA. Although benzene is also metabolized to phenol and other hydroxylated metabolites in the bone marrow (39), P-450 mediated metabolism in this tissue occurs to a relatively limited extent. This is due to low levels of this enzyme system in the bone marrow compared with liver.

Indirect evidence indicates that muconaldehyde may be another reactive metabolite formed in the liver (17). In addition, the reactive intermediates *p*-benzoquinone and *o*-benzoquinone may be formed in this tissue by oxidation of hydroquinone and catechol, respectively. These reactive quinone metabolites and muconaldehyde are direct-acting alkylating agents capable of reacting with cellular nucleophiles, including DNA (40,41). Numerous *in vitro* and *in vivo* studies indicate that these metabolites are genotoxic (42–45). Once formed in the liver, it is unlikely that they survive long enough to reach the bone marrow. It is possible, however, that reactive benzene metabolites, similar to other reactive intermediates, may be bound to carriers that release them after transport to the target tissue. Reactive intermediates may also be conjugated to form less reactive intermediates or “storage forms” which, after transport, release the reactive metabolite through deconjugation. A third possibility germane perhaps to muconaldehyde may consist of metabolism to a less reactive compound, *i.e.*, a mixed aldehyde alcohol MUC analog (Fig. 3), which itself may be hematotoxic or is subsequently reactivated in the target tissue by metabolism to the original reactive metabolite, *i.e.*, muconaldehyde.

The bone marrow has been shown to accumulate hydroquinone and catechol (46,47), two phenolic metabolites of benzene. As discussed above, bone marrow, unlike the liver, contains low levels of cytochrome **P-450** and high levels of peroxidases. Phenolic compounds are excellent electron donors for the peroxidase-mediated metabolism of hydrogen peroxide. Studies by Eastmond et al. (48,49) and Sadler et al. (50) have shown that phenol, catechol, and hydroquinone are excellent substrates for myeloperoxidase, a peroxidase present in high concentrations in the bone marrow. During myeloperoxidase metabolism, these phenolic metabolites undergo one-electron oxidation and covalent binding to protein. The reactive species involved in the binding are presumably radical intermediates, such as the semiquinone free radical of hydroquinone, which are formed during one-electron oxidation.

Para-benzoquinone is a reactive α,β -unsaturated diketone that has been implicated in bone marrow toxicity (51,52). Phenol and catechol have been shown to stimulate the myeloperoxidase dependent conversion of hydroquinone to *p*-benzoquinone (52,53). Based on these studies, Smith et al. (54) proposed a two-step mechanism of benzene toxicity that involves metabolism to phenolic compounds in the liver, transport and accumulation of these compounds in the bone marrow, followed by myeloperoxidase-mediated conversion of hydroquinone to *p*-benzoquinone in a process stimulated by phenol and possibly catechol. This mechanism of *p*-benzoquinone formation does not exclude a similar involvement by prostaglandin (H) synthetase and eosinophil peroxidase in benzene toxicity (55).

Mechanisms of Benzene Toxicity

The most frequently observed toxic effect of benzene in humans and in animal models used for the study of benzene toxicity has been bone marrow depression leading to aplastic anemia (14). The correlation between the effects of benzene exposure in humans and the effects produced in animal models was demonstrated by Santesson in 1897 (56), Selling in 1916 (57) and Weiskotten et al. in 1916 and 1920 (58,59). As a result many studies in animals have been aimed at uncovering the mechanism

of benzene toxicity for the purpose of applying these observations toward an understanding of the human disease.

During the latter half of this century, it has become apparent that benzene also causes chromosomal abnormalities indicative of genetic damage (60–64). Attempts to demonstrate mutagenic activity of benzene have been largely unsuccessful (42), but benzene is a human leukemogen (65) and causes solid tumors in animals (66–68). Unfortunately, there is no convenient model for studying the mechanisms by which benzene produces neoplastic effects in animals. Although each of these effects, *i.e.*, aplastic anemia, chromosome damage, and carcinogenesis, are indicative of the ultimate impact of benzene on bone marrow, the relationship between these phenomena and the mechanisms by which they are initiated remain to be fully understood. This section describes the effects of benzene in model systems, discusses the mechanisms by which these effects occur as we currently understand them, and points out data gaps, which when filled may help us to a better mechanistic appreciation of the biological impact of benzene.

Hematopoiesis

The mechanism of benzene toxicity has been examined on the basis of its effects on stem and progenitor cells in the bone marrow as well as its effects on the hematopoietic microenvironment. Hematopoietic stem cells in the bone marrow give rise to the major cell types in the circulation, *i.e.*, erythrocytes, thrombocytes, and the several major subdivisions which comprise the leukocytes (69). The self-renewing stem cells which reproduce themselves, also yield a cell committed to eventual maturation to a circulating cell. Growth factors direct committed cells to enter the maturational process, *e.g.*, in the red cell line the committed erythroid cell is sensitive to the initiating action of erythropoietin. To assure that the level of mature cells is constant despite the regular destruction of aged erythrocytes requires that the marrow provide a mechanism for both maturation from the morphology of the primitive stem cell to that of the mature circulating erythrocyte, plus a mechanism for amplification to sustain the requisite number of circulating erythrocytes, *i.e.*, approximately 5 million cells/ μ L in humans with a survival time of 120 days. Thus, a complex series of biosynthetic events results in the passage of these cells through several defined morphological stages, during many of which the cells undergo mitosis to increase total numbers. A related, but unique process leads to megakaryocyte production and eventual release of platelets in large numbers (approximately 250,000/ μ L in humans with a survival time of 10 days). Fewer leukocytes, *e.g.*, between 5 and 12,000 cells/ μ L in most species, are required. However, the process is complicated by the need for several different forms including granulocytes, lymphocytes, monocytes, *etc.*, each divided into several functional and morphological subtypes and with differing lifetimes. These also undergo a series of steps directed by cytokines or other growth factors involving both maturation and amplification.

The hematopoietic microenvironment is composed of a variety of cells and structures including endosteal cells, fibroblasts, reticuloendothelial cells (monocytes and macrophages), fat cells, and bone and sinusoids of the marrow and is responsible for sustaining stem and progenitor cells. Proteins such as erythropoietin, colony-stimulating factors, the interleukins and related cytokine mediators are products of microenvironmental cells and

play key roles in directing these events (69). In the absence of the microenvironment, primitive blood cells cannot mature.

Effects of Benzene

Many studies have shown that treatment of whole animals with benzene leads to decrements in the levels of circulating blood cells (14). Mechanistic studies have benefited from the development of a variety of *in vitro* techniques. Thus, the bone marrow of animals exposed to benzene using various exposure protocols can be removed and grown in culture to explore the effects of benzene on specific cell lines at more or less specific stages of maturation. Several laboratories have reported on decreases in the multipotential hematopoietic stem cell (spleen colony forming unit or CFU-S) in mice after a variety of exposure protocols (70–73). Similar effects have been observed using the early granulocyte-macrophage CFU-C assay (72,74). Decreases have also been observed using the erythroid colony-forming unit (CFU-E) and the burst-forming colony unit (BFU-E) assay, each of which defines a committed stem cell early in erythrocyte development (75,76). Exposure of mice *in utero* to benzene at relatively low concentrations resulted in decrements in bone marrow colony forming units postnatally (77,78). These studies indicate that benzene exerts a significant impact on both primitive and early committed stem cells.

In addition to the effects of benzene on stem cells and committed stem cells, both morphological and functional evidence has linked benzene to impairment of the processes of maturation and amplification. Pronormoblasts, normoblasts, and reticulocytes appear successively at 24, 48, and 72 hr after reinitiation of red cell development by erythropoietin (79). Synthesis of hemoglobin, including the incorporation of iron, occurs only between the pronormoblast and reticulocyte stage of cellular differentiation (80,81). One method to observe the effects of chemicals on various stage of erythropoiesis *in vivo* involves administration of ^{59}Fe intravenously followed by sampling 1 day later of the red cells for incorporation of the iron into hemoglobin (82,83). This provides a functional measurement of the reticulocyte pool. Treatment with chemicals at various times before iron administration resulted in a decrease in iron uptake. These data indicate an impairment in red cell development, provided it is known that the chemical did not interrupt reticulocyte hemoglobin synthesis. In the case of benzene (82,83) hemoglobin synthesis proceeds normally. The maximum decrease in iron uptake following administration of benzene occurred 48 hr before iron administration, a time at which the reticulocytes are at the earlier pronormoblast stage of development. Normoblast development was also inhibited but no effect was observed in these studies on committed stem cells nor on reticulocytes. Taken together, these studies suggest effects of benzene at both the stem cell and the progenitor cell stages of erythropoiesis.

Studies by Dexter et al. (84) established a method for the evaluation of the hematopoietic microenvironment. The system allows for the growth of an adherent cell layer of stromal cells in liquid culture. Stem cells added to this preparation begin the process of maturation and proliferation supported by the stromal cells. Using this method, Garnett et al. (85) exposed mice to benzene at various doses, removed the bone marrow, and grew adherent layers that supported the growth, but not the differentiation, of colony-forming units from control mice. Inhibition of

differentiation may have been due to the failure of these preparations to grow hydrocortisone-induced fat cells essential to stromal-supported differentiation. Frash et al. (86) exposed mice to both radiation and benzene and injected normal mouse marrow cells. Using the CFU-S technique, they reported failure to restore marrow function (86). Incubation of bone marrow cells with benzene before injection into irradiated mice had no effect on spleen colony formation. Frash et al. (86) concluded that the hematopoietic microenvironment was damaged as a result of benzene treatment.

Effects of Benzene Metabolites

It appears that benzene toxicity occurs only under conditions that permit the production of benzene metabolites. Thus, animals were protected from benzene toxicity after partial hepatectomy, which reduced total benzene metabolism *in vivo* and resulted in a decrease in covalent binding of metabolites in bone marrow (2). This was not the first suggestion that benzene metabolites play a critical role in toxicity. Parke and Williams (9), in their pathfinding report suggested that the phenolic metabolites of benzene might be responsible for benzene toxicity. A number of investigators subsequently attempted to identify the toxic metabolites of benzene. Both Bolscak and Nerland (3) and Snyder and co-workers (87–89) have studied the effects of benzene metabolites on ^{59}Fe uptake in mice and found that hydroquinone and catechol (50–100 mg/kg) are more potent in decreasing iron uptake than phenol. Pam-benzoquinone, the metabolic product of hydroquinone, is more potent than hydroquinone. Muconaldehyde and p-benzoquinone were effective in inhibiting iron uptake in the range of 1–4 mg/kg.

Several studies have focused on the possibility that benzene toxicity is the result of synergistic interactions among benzene metabolites. Eastmond et al. (49) studied the effect of administering a combination of phenol and hydroquinone and reported that the combination enhanced the ability of each to inhibit bone marrow function. Possible explanations for the interaction between hydroquinone and phenol include the preferential conjugation of phenol by sulfate and glucuronide leading to a greater effective concentration of free hydroquinone, the stimulation of the production of reactive metabolites from hydroquinone by phenol, or both. The same interaction was also observed in Snyder's laboratory (87,88) using the ^{59}Fe uptake technique.

Guy et al. (89) studied the effects of combining muconaldehyde plus either hydroquinone or p-benzoquinone on iron uptake into erythrocytes. They reported that when the binary mixtures were administered together at doses of either which were ineffective in reducing iron uptake, the combined effect was to severely inhibit red cell production. Higher doses also indicated interaction. Multiple regression analysis was used to study the contributions of the components of the binary mixtures. The results suggested that in inhibiting red cell production, muconaldehyde and hydroquinone react at the same site. Muconaldehyde and p-benzoquinone appear to act at two sites. The nature of these sites are not yet known, but these studies suggest that benzene toxicity is the result of the interaction between benzene metabolites rather than the effect of a single metabolite acting independently.

The effects of benzene metabolites on the microenvironment have also been studied. Gaido and Wierda (90–92) evaluated the

effects of benzene metabolites on the ability of adherent cells from mouse bone marrow to support **the** growth of GM-CFU-C cells. They reported that phenol was not effective below millimolar concentrations, whereas hydroquinone and benzoquinone were the most toxic. Catechol and 1,2,4-benzenetriol were less potent than hydroquinone and p-benzoquinone. Previous studies that showed that DBA/2n mice were more sensitive to benzene toxicity than C57/B16 mice (93) led Gaido and Wierda (90-92) to examine the relative ability of adherent cells from B6C3F₁ and DBARJ mice to support hematopoiesis. Although no strain differences were observed related to this activity, phenol, but not benzene, reduced the ability of stromal cells to support the development of granulocyte/macrophage precursor cells.

Stromal macrophages appear to be a significant target in benzene toxicity. For example, Post et al. (94) found peroxidase-mediated metabolism of phenol to covalently binding species along with **the** inhibition of RNA synthesis by hydroquinone and p-benzoquinone. Renz and Kalf (95) suggested that in these cells hydroquinone inhibits the activation of interleukin-1 and the administration of recombinant interleukin-1a before benzene treatment prevented benzene toxicity in mice. These data suggest that **not** only are several benzene metabolites involved in benzene toxicity, but there may **also** be several targets. It has been demonstrated that stem cells, progenitor cells, and some stromal cells are sensitive to benzene metabolites.

Genotoxicity

Mutagenicity

Benzene has not been found to produce mutagenicity in short-term tests **in** vitro (42). The Ames Salmonella test, yeast mutagenicity test, unscheduled DNA synthesis, mouse lymphoma test, 6-thioguanine resistance test in Chinese hamster V79 cells, the ouabain resistance test in Chinese hamster ovary (CHO) cells, and human lymphoblast HGPRT and TK tests did not indicate that benzene was a mutagen. Tests aimed at detecting neoplastic cell transformation such as the BALB/c 3T3 mouse fibroblast test, C3H 10T1/2 mouse cell test, CHO test, and enhancement of viral (SA79) transformation test were also negative.

There **was** a report that benzene yielded a positive Ames test using strain TA100 and Aroclor-induced rat liver microsomes, taking advantage of a highly sensitive microflocculation assay (43,96). Another report suggested a positive cell transformation result in Syrian hamster embryo cells (97). The results suggest that except for these **two** systems, either those cellular **targets** that would have to be affected to yield a positive result were not sensitive to benzene, or the negative results were caused **by** the inability of those systems to effectively metabolize benzene to reactive intermediates capable of producing a positive **response**. The hepatic stage of benzene metabolism **in vivo** is mediated primarily **by** cytochrome P-450 IIE1, although under some circumstances cytochrome-P-450 IIB1 or other cytochromes P-450 **may** metabolize benzene. Further metabolic activation in bone marrow cells requires peroxidatic activity. In the absence of both types of activating enzymes, mutagenic metabolites will not be formed and the results would suggest that exposure to benzene would not yield a mutagenic effect.

Mutagenicity of benzene metabolites, however, has been well demonstrated. Thus, Glatt et al. (43) studied the mutagenic activity of 13 benzene metabolites using strain TA1535. In the absence of S9, **trans**-1,2-benzenedihydrodiol and benzene diol epoxides were mutagenic. Dihydrodiol dehydrogenase, which converts the dihydrodiol to catechol, protected against the mutagenic effect. It is the postulated, but not demonstrated, that the dihydrodiol can be converted to a series of isomeric diol epoxides via a further epoxidation. The anti-diol epoxide was a mutagen in strains TA97, TA98, TA100, TA102, TA104, and TA1535. In V79 cells, the anti-diol epoxide also induced sister chromatid exchange, acquisition of 6-thioguanine resistance and ouabain resistance, and micronuclei. Although the anti-diol epoxide has not been demonstrated to be a metabolic product of benzene as yet, the potential for its formation remains, and it might be one of the most mutagenic products derived from benzene.

Other metabolites effective in the V79 cells were 1,2,4-trihydroxybenzene, hydroquinone, catechol, and the **trans**-1,2-dihydrodiol which elevated resistance to 6-thioguanine. The most striking effect in these studies, however, was both the potency and great effectiveness of p-benzoquinone in increasing the frequency of 6-thioguanine resistance while having no effect on ouabain resistance, **and** producing no chromosome damage in V79 cells. Furthermore, p-benzoquinone was relatively ineffective in Salmonella.

The potential genotoxic activity of **trans**,**trans**-muconaldehyde, a chemically reactive ring opening product of benzene in microsomal preparations, was investigated in studies by Witz and co-workers (44,45). **Trans**,**trans**-muconaldehyde is generally highly toxic to cells **in vitro**. It is an active mutagen in V79 cells but is weakly mutagenic in Salmonella (44). In CHO cells **trans**,**trans**-muconaldehyde is a potent clastogen (37,45). Thus, it appears that if benzene **can** undergo metabolic activation, some of its metabolites, both ring closed and ring opened, can mediate mutagenic events.

Chromosome Damage

Since the first reports of Pollini and co-workers (98,99), benzene has been known to produce chromosome damage. They cultured both bone marrow cells and peripheral lymphocytes from patients displaying severe benzene hemopathy **and** reported a high **rate** of aneuploid cell production. Subsequently, there were many **reported** observations of chromosome aberrations in blood from workers exposed to benzene. Notable among these were the reports of Forni and her collaborators (60,61), who observed chromosome aberrations in a group of **workers** exposed to benzene at a greater frequency **than** workers exposed to toluene (60). Follow-up studies of these workers revealed that both stable and unstable benzene-induced chromosome aberrations occurred. The unstable variety eventually disappear, whereas the stable form persist and may relate to the eventual development of leukemia.

Erexson et al. (200) reported that low-dose exposure to benzene (10-28 ppm) over 4-6 hr produced sister chromatid exchanges (**SCE**) in peripheral mouse B-lymphocytes. Tice et al. (63) demonstrated the production of sister chromatid exchanges in mice after short-term exposure to high concentrations of benzene. The effect **was** exacerbated when the mice were pre-

treated with phenobarbital, presumably because at these high doses phenobarbital induces an enzyme that can efficiently bioactivate benzene. Although partial hepatectomy was not protective, co-administration of toluene reduced SCE by 90%. Witz et al. (45) reported that administration of *trans,trans*-muconaldehyde caused SCE in B6C3F₁ mice. The data suggested that benzene metabolites play a significant role in producing SCE.

In cultured human lymphocytes *in vitro*, it is clear that benzene metabolites induce SCE (101). Phenol, catechol, and hydroquinone produced SCE in a dose-dependent manner in the absence of added metabolic activation, and the effect was prevented by glutathione. Sister chromatid exchanges were induced with the following order of potencies: catechol > 1,4-benzoquinone > hydroquinone, 1,2,4-benzenetriol > phenol > benzene.

The report by Hite et al. (62) that benzene produced micronuclei in bone marrow polychromatic erythrocytes in mice led to the development of an assay featuring the use of micronuclei in peripheral red cells as a rapid screen for chromosomal damage (102) caused by benzene. Since then micronuclei have been observed after benzene administration via various doses, routes, and treatment regimens (5). Harper et al. (103) suggested that benzene metabolism was necessary to yield micronuclei. Pirozzi et al. (104) demonstrated that indomethacin, which inhibits the metabolic activation of phenolic metabolites in bone marrow by prostaglandin H synthetase (105), also protects against micronucleus formation.

Covalent Binding to Macromolecules

The observations that in the course of its metabolism some benzene is converted to reactive metabolites that covalently bind to protein (106,107) RNA and DNA (108) have led to a series of studies on the mechanism of benzene toxicity.

Irons and Nepton (109) demonstrated that hydroquinone, but not phenol or catechol, inhibited polymerization of tubulin. Tubulin possesses nucleophilic sulfhydryl groups that bind guanosine triphosphate (GTP), which in turn stabilizes tubulin for further polymerization to microtubules necessary for spindle formation during cell division (72). Hydroquinone and *p*-benzoquinone interfere with GTP-tubulin binding by alkylating SH groups (109,110). Another protein that is inhibited by benzene metabolites, hydroquinone, and *p*-benzoquinone, but not phenol or catechol, is mitochondrial DNA polymerase- γ , which also possesses a sulfhydryl group at the active site (111). The binding of benzene metabolites to these critical proteins may play a role in benzene toxicity.

The observations of chromosome damage described above suggest that the binding of benzene metabolites to DNA may also represent a significant event in benzene toxicity in view of the chromosome damage discussed above. Lutz and Schlatter (108) exposed rats to either [¹⁴C]- or [³H]benzene by inhalation and found that liver nuclear DNA contained covalently bound benzene residues, although Lutz (112) noted that when compared with other carcinogens, relatively little radioactivity derived from benzene was bound to DNA. Rushmore et al. (113) took advantage of the properties of mitochondrial DNA to study DNA binding by metabolites of benzene. The mitochondrion contains no mechanism for DNA repair and contains a small circular DNA. Furthermore, the presence of a cytochrome P-450 mono-

oxygenase in mitochondria capable of metabolizing benzene facilitated production of reactive intermediates (114). Seven potential DNA adducts on deoxyguanosine and one on deoxyadenosine were observed, and structures have been proposed for one of the adducts on deoxyguanosine (40) and for the deoxyadenosine adduct (115). Neither thymine nor cytosine adducts were investigated.

Schlosser et al. (105) investigated the oxidation of hydroquinone by prostaglandin H synthetase and found that whereas 1,4-benzoquinone, the oxidation product of hydroquinone, was bound to cysteine or DNA, inhibition of the enzyme by indomethacin inhibited both types of binding as well as micronucleus formation (104) and benzene-induced myelotoxicity (116).

The low level of binding has made it difficult to detect DNA adducts *in vivo*. However, use has been made of the ³²P-post-labeling technique to measure benzene related adducts because of its great sensitivity. Bauer et al. (117) reported on DNA adduct formation in rabbit liver. Snyder et al. (87) suggested that DNA adducts are formed in the bone marrow of benzene-treated rats. Using the same method, Reddy et al. (118) was unable to detect DNA adducts from benzene in the Zymbal gland, a target for the carcinogenic activity of benzene in the rat, or in bone marrow, presumably the target for leukemogenic activity. Further research is needed to determine the role of DNA binding of benzene metabolites in benzene toxicity and carcinogenesis.

Carcinogenesis

From the mechanistic point of view, benzene-induced carcinogenic events have been difficult to study because of the lack of a convenient animal model. Chronic exposure of rats and mice to benzene by inhalation (119) suggests that benzene might induce acute and chronic myelogenous leukemia and thymic lymphoma in these species. Large scale, chronic exposure studies by Maltoni et al. (66,67) in rats and mice using inhalation or oral administration revealed the appearance of Zymbal gland tumors as well as a variety of other cancers. These results were confirmed and extended in a National Toxicology Program study (68). However, these studies have not led to the development of models of use to study the mechanism of leukemogenesis. A more promising approach came from Cronkite et al. (120), who reasoned that the difficulty in producing leukemia during chronic exposure is because benzene is an inhibitor of cell replication, a process essential to the development of leukemia. Therefore, these workers treated mice for 16 weeks with 300 ppm of benzene and then ceased exposure. Subsequently, none of 88 control mice demonstrated neoplasms, but 8 of 90 benzene-exposed mice displayed lymphoma/leukemias (120). This method has yet to be fully exploited in the study of the mechanism of benzene-induced leukemia.

Prevailing theories suggest that initiating events in carcinogenesis may be covalent binding of reactive intermediates to DNA or oxidative damage of DNA. Subsequent attempts to either repair or replicate the DNA propel the cascade of events termed carcinogenesis. Furtherance of this process is thought to require the intervention of one or more promotional steps that stimulate proliferation of the genetically altered cells. Alternatively, so-called epigenetic events may lead to carcinogenesis. The mechanism of benzene toxicity has yet to be defined within these frameworks.

If we assume that a genetic alteration underlies the mechanism of benzene-induced carcinogenesis, we can look toward the results of the genotoxicity studies cited above as indicators of the pathway toward neoplasia. Thus, metabolism to reactive intermediates would have to be postulated as an important early step in the mechanism. It would be expected that benzene should then stimulate DNA repair, which would be detectable by unscheduled DNA synthesis. However, when measured in hepatocytes and HeLa cells, it did not appear likely that benzene caused DNA damage subject to repair in mammalian cells in culture (42). Subsequent to a genetic change, it should be possible to measure cell transformation. The positive Syrian hamster embryo test suggested that given the proper metabolic activating system, benzene metabolites may be capable of inducing cell transformation.

The second step would require promotion. The possibility that unmetabolized benzene might play a promotional role arose from the observations by Da Silva et al. (121) that benzene activates membrane protein kinase C, which is also activated by tumor promoters such as phorbol esters. This activity is shared with toluene, which produces neither aplastic anemia nor leukemia. However, whereas toluene might activate protein kinase C, its metabolites are neither hematotoxic nor carcinogenic.

Some attempts have been made to investigate the mechanism of benzene carcinogenesis in other organs. Reddy et al. (122) and Low et al. (123) examined the formation of DNA adducts in Zymbal gland cells in culture and detected DNA binding. However, adducts were not detected in this organ when the animals were treated *in vivo*. Of perhaps greater significance, however, were the studies of Busby et al. (124), who treated newborn mice with benzene, benzene oxide, racemates of benzene dihydrodiol, and benzene diol epoxide-1 and benzene diol epoxide-2. Although benzene and benzene diol epoxide-1 were inactive in this assay, benzene oxide, the racemates of benzene dihydrodiol, and benzene diol epoxide-2 induced lung tumors in the mice. It is likely that benzene oxide and the dihydrodiol were metabolized to the diol epoxide-2 to initiate these tumors. It will be important to evaluate the likelihood that the diol epoxide can be formed in bone marrow *in vivo* to determine whether it can be important in leukemogenesis.

One mechanism that might explain the initiation of leukemogenesis by benzene derives from recent considerations relating chromosome aberrations with cancer (125). Although chromosome breaks and sister chromatid exchanges have been identified as outcomes of benzene exposure, specific studies of the possibilities of chromosome translocations have not been performed. Metcalf et al. (126) discussed the relationship between translocations and leukemogenesis and recalled that about two dozen nonrandom chromosome translocations have been reported in both B-cell and T-cell tumors. Often these translocations have resulted in the close association of a proto-oncogene with a gene that codes for an immunoglobulin in a B-lymphocyte or a T-cell receptor in a T-lymphocyte. The proto-oncogene is then deregulated and can play an important role leading to the expansion of a neoplastic clone. A recent example of this phenomenon was reported by de The et al. (127), who demonstrated an association of a specific translocation, designated as t(15;17), with acute promyelocytic leukemia, in which the net effect is the fusion of the retinoic acid receptor, RAR α , with a gene called *PML*. These authors suggest that the translocation results in the

formation of an RAR mutant, the activity of which is to interfere with promyelocytic differentiation and thereby facilitate the process of leukemogenesis. Thus, at least three types of chromosome changes caused by benzene metabolites, i.e., gross chromosome aberrations, micronuclei, and sister chromatid exchange, have been reported. If benzene metabolites can produce translocations as well, this might be a mechanism for leukemogenesis that is parallel to the example cited above.

Pharmacokinetics of Benzene

There have been a number of attempts to develop pharmacokinetic models of the fate of benzene with the intention of applying the results to risk assessments for benzene. The first attempt to model the fate of benzene was made by Sato et al. (128) who exposed three men to a single exposure of 25 or 100 ppm of benzene for 2 hr and then observed a triexponential decay of benzene from their blood. Sato et al. constructed a three-compartment model made up of richly perfused tissues, poorly perfused tissues, and fat, which acted as a major sink for benzene. Snyder et al. (18) repetitively exposed mice or rats to 100 or 300 ppm of benzene for 20 days and sampled the blood for benzene on days 1, 6, and 20. At higher doses mice achieved greater increases in blood levels of benzene than the rats. The mice also displayed a greater elimination rate constant than the rats. The rate of blood benzene disappearance from mice at the high dose shifted from monoexponential to biexponential between the 6th and 20th day, which may have been due to enzyme induction of benzene hydroxylase, i.e., cytochrome P-450 1IE1.

More recent approaches have attempted to factor metabolism into physiologically based pharmacokinetic models of the fate of benzene and to develop a method for extrapolating from animal models to humans. Beliles and Totman (129) constructed a model using Zymbal gland tumors and blood related cancers as end points and, recognizing the possibilities of synergy and multiple mechanisms, assumed that the combined metabolites represented the "reactive agent." Using largely the data of Sabourin et al. (13) and applying an allometric technique to estimate scaling between species, they suggested that their model, which predicts that a working lifetime exposure (40–45 years) to benzene at 10 ppm would result in 6–14 cases of leukemia/1000, is in good agreement with federal regulatory risk assessments based on epidemiological data.

Physiologically based pharmacokinetic models aimed at relating benzene metabolism and interspecies differences, using both experimental data and simulations, have been developed by Medinsky et al. (130), Travis et al. (131,132), Boiset et al. (133), and Spear et al. (134). Each described a multicompartment system and attempted to relate end points to the generation of metabolites with emphasis on specific metabolites assumed to be responsible for generating the end points under consideration. Thus, Medinsky et al. (130) considered the role of hydroquinone and muconaldehyde generated in the liver and Bois et al. (133) added the metabolic activity of the bone marrow in the further metabolism of metabolites generated in liver. While each represents an important advance in interpreting the metabolic fate of benzene in relation to the development of benzene-related disease, they all suffer from lack of sufficient data, as well as from variability of the data between experimental designs. There generally is difficulty in fitting empirical data to theoretically

generated curves, which suggests that the models need to be improved.

Whereas the disappearance of benzene from the blood, following administration via any route, can be measured readily, the requirement for metabolism before benzene can produce serious chronic effects, makes modeling difficult because of the uncertainties pertaining to which metabolites are responsible, the relative impact of each, and the potential for multiple target effects.

A large number of metabolites have been described above. Because of their demonstrated, direct effects on bone marrow, pharmacokinetic models have concentrated on hydroquinone, alone or in combination with phenol, and muconaldehyde, and for the most part have assumed that they are formed in liver and transported to the bone marrow. The possibility that some of the critical metabolites are ultimately formed in bone marrow serves to complicate the modeling process. For example, although the metabolites transferred to the bone marrow may be innocuous in themselves, further metabolic activation in bone marrow, perhaps mediated by peroxidases, may generate the ultimate toxic metabolites (135). Thus, hydroquinone may be released from the liver, free or conjugated, reach the bone marrow by some mechanism, and in its free form may be oxidized to *p*-benzoquinone by a peroxidase. Muconaldehyde might be formed in bone marrow, and the two working in concert would then produce a toxic effect. The models would have to reflect these factors, as well as take into account the activity of other potential toxic metabolites, if they are to accurately picture the process and be used in risk assessment with accuracy.

Human Impact

Benzene is a ubiquitous pollutant in both the workplace and in the general environment. Ambient concentrations can be detected worldwide, including pristine areas, although at levels much lower than in modern urban societies. Major sources of benzene include petroleum and petroleum products. Levels of benzene in gasoline range from approximately 1 to 5%. Gasoline serves as a major source of benzene exposure in a wide variety of situations including groundwater contamination through leaky underground storage tanks; air contamination through the evaporation of gasoline in workplaces and in homes; and transdermal exposure when gasoline gets on the skin. The major cause of high-level exposure remains inappropriate use in an unregulated workplace; although control measures are leading to a decreased use of benzene as a solvent and organic synthetic starting block. A major source of personal benzene exposure comes from cigarette smoking (136), and benzene is also present in sidestream smoke leading to exposure through passive smoking. Although there are measurable amounts of benzene in certain foods, these are no longer believed to be a significant source of benzene exposure to the general public, particularly in comparison to indoor air pollution from gasoline and benzene-containing solvents or from cigarette smoke within the home or the office (137).

Nonhematological Effects

The odor threshold for benzene is in the range of 4–5 ppm (138). The acute nervous system toxicity of benzene appears

similar to the general anesthetic effects of lipophilic solvents and is assumed to be a direct effect of benzene unrelated to its metabolites. Acute symptoms include drowsiness, lightheadedness, headache, delirium, vertigo, and narcosis. Levels at which acute central nervous system effects become apparent are at least above 100 ppm, an earlier occupational health standard for benzene, although milder effects could conceivably occur at lower levels. Based on structure–activity relationships, benzene might be expected to have acute central nervous system effects similar to alkyl benzenes at a slightly lower dose for benzene. Chronic nervous system effects of benzene have not been clearly demonstrated.

Nonhematological tumors have also been demonstrated in long-term animal studies (139,140). However, at present there is no reasonably convincing evidence of nonhematological solid tumors occurring in humans exposed to benzene. A meta-analysis of benzene-exposed populations may be of value in this regard.

Studies of the productive and developmental effects of benzene have shown little aside from hematological effects in the fetus (77,78). A more thorough evaluation has been called for by Davis and Pope (141).

Immune dysfunction as a result of high-level exposure to benzene in laboratory animals has been clearly demonstrated (142). Lymphocytopenia is an early effect of benzene exposure in animals and in man (143), and there is also evidence suggesting that the immune system can be affected by benzene independent of lymphocytopenia (144). However, there is currently no evidence to demonstrate an effect on the immune system of humans exposed to allowable levels of benzene.

Hematological Effects

In humans, as in animal models, the bone marrow is the target of benzene toxicity. A major unanswered question about benzene toxicity concerns its primary localization to the bone marrow, a finding that is almost unique in solvent toxicology. As with many other solvents, benzene is primarily metabolized in the liver, yet despite evidence that metabolism is necessary for bone marrow toxicity, hepatotoxicity is not observed. In essence, there are two different explanations given for bone marrow localization: specificity in the local metabolism of benzene within the bone marrow or particularities in the susceptibility of the bone marrow, as compared to the liver or other organ, to a benzene metabolite or metabolites. For example, Subrahmanyam et al. (135) recently emphasized the potential role of free radicals in benzene toxicity, pointing out that the relatively high level of myeloperoxidase and of other peroxidases may be responsible for localization of benzene toxicity to the bone marrow. In contrast, Goldstein and Witz (145), noting the relative sensitivity of the bone marrow to chemotherapeutic alkylating agents, have focused on a potential hematologic role for *trans,trans*-muconaldehyde, an α,β -unsaturated aldehyde alkylating agent which, although highly reactive, has a sufficient half-life to theoretically travel from liver to bone marrow.

Benzene was initially identified as being a human hematological toxin in the 19th century. Since that time the literature is replete with case studies and series in which fatal aplastic anemia has been reported in individuals exposed to significant levels of benzene (146,147). As described above, aplastic anemia is a

serious, often fatal, disorder in which the formed elements of the bone marrow are replaced by fat and there is a significant decrement in the formed elements of the blood. Humans may display a decrease in white blood cells (leukopenia), potentially resulting in death due to infection; a decrease in platelet count (thrombocytopenia), potentially resulting in death due to hemorrhage; and a decrease in red blood cell count (anemia). A lesser extent of benzene exposure produces lesser degrees of damage to the bone marrow. The normal human bone marrow has sufficient reserve capacity to be able to produce approximately six times more red blood cells under stress than it does under normal conditions. There is also a wide range of statistically normal values in peripheral blood counts. Thus, earliest forms of bone marrow damage due to a compound such as benzene may not be notable in terms of a decrease in circulating blood counts. As described above, studies in laboratory animals have shown that a decline in colony forming cells indicative of the function and number of bone marrow precursors can be seen with relatively low levels of benzene exposure. In humans, individuals with benzene exposure may initially have a count lower than statistically normal in any one of the formed elements, but with significant exposure, pancytopenia will be observed (146).

There is a wide range between those blood count levels which are statistically abnormally low and those which produce functional abnormalities. Thus, the range of normal in a laboratory platelet count is often 150,000–350,000/ μ L; yet evidence of abnormal hemostasis is usually not observed in humans until the platelet count is less than 50,000/ μ L. There is, however, some evidence that in significant benzene toxicity not only is there a quantitative decrease in formed blood cell elements, but there is also a qualitative alteration. Such qualitative changes might be expected to interfere with function of platelets or white blood cells. A hallmark of benzene effect in red cells is an increase in average cell size, known as the mean corpuscular volume (calculated by dividing the hematocrit by the red blood cell count). An increase in the mean corpuscular volume also occurs with folic acid deficiency and vitamin B₁₂ deficiency, two disorders which have in common an interference with DNA synthesis in the bone marrow. An increase in the mean corpuscular volume is observed in almost any form of aplastic anemia. As an increased mean corpuscular volume also occurs relatively early in alcoholism, as well as other conditions, it cannot be taken as a definitive test for benzene exposure at the workplace, although it can be a useful confirmatory clue (143).

As with any toxicant, there appears to be a variation in the extent to which individuals differ in their sensitivity to benzene, and some suggestion of a familial tendency (148). However, there is no evidence of an idiosyncratic low-level sensitivity to benzene-induced aplastic anemia similar to that observed with chloramphenicol. Interaction with other agents may affect benzene toxicity as described above with respect to ethanol and toluene. Metabolic interaction between toluene and benzene would be expected when exposure occurs to a mixture containing a high ratio of toluene/benzene because toluene is a competitive inhibitor of benzene metabolism (1). However, Sato and Nakajima (128) reported that there is no significant interaction between benzene and toluene with respect to their fate when human experimental exposure was near the threshold limit value.

Neoplastic Effects

LeNoir and Claude reported the first case of benzene-associated leukemia in humans in 1897 (149) but the diagnosis was not well documented. In 1965 Browning (149) recorded 61 cases, and by 1977 Goldstein (146) had assembled 121 cases of benzene-associated leukemia. The studies of Vigliani et al. (150), Aksoy et al. (151), and Infante et al. (152), each of which examined large populations, presented convincing evidence of the leukemogenic activity of benzene.

There is unequivocal evidence that benzene is a cause of acute myelogenous leukemia (AML), the adult form of acute leukemia. Individual cases in benzene-exposed individuals began to be reported in the 1920s, but the causal relation was not fully accepted until less than two decades ago. This relationship was first accepted by hematologists who have long recognized that anyone with aplastic anemia from apparently any cause has an increased risk of AML. For example, AML as a second tumor is, unfortunately, common in cancer patients whose bone marrow is affected by radiation and alkylating agents used in chemotherapy (153). Benzene exposure also leads to cytogenetic abnormalities in bone marrow cells and in circulating lymphocytes (60,61).

Epidemiological evidence strongly supportive of the causal relationship between benzene exposure in the workplace and acute myelogenous leukemia includes studies of shoe and other leather workers in Italy and in Turkey (150,154). In Turkey, a glue used in the fashioning of leather goods which had relatively low levels of benzene as a solvent was replaced in the 1960s with one containing much higher levels. Soon thereafter, Aksoy and his colleagues reported a wave of patients with aplastic anemia followed by identification of numerous individuals with AML, many of whom had previously been identified as having aplastic anemia (154,155). Studies of large cohorts of benzene-exposed workers in the chemical and petrochemical industries have tended to show an increase in hematological neoplasms (156–160). The most thoroughly studied cohort has come from Goodyear Rubber facilities in Ohio. Repetitive follow-up of a cohort of workers by the National Institute of Occupational Safety and Health (NIOSH) has identified at least 10 cases of acute and chronic myelogenous leukemia, with only two expected (152,161,262). To obtain a better understanding of the level and pattern of benzene exposure associated with this increased risk of AML, NIOSH has performed one of the most thorough retrospective exposure assessments ever done on a cohort of workers (162). This exposure analysis has been used as a basis for the current risk assessment of benzene by the U.S. Environmental Protection Agency (EPA). However, it does appear that NIOSH has significantly underestimated the extent of exposure in the Goodyear plants during World War II (163,164), thus leading to some overestimation of the risk of benzene derived from this cohort. However, it should be emphasized that the current EPA risk assessment of benzene has more uncertainty associated with the dose response part of this equation than it does with the exposure. Despite the many arguments concerning the extent to which workers who developed AML were exposed to benzene, the differences all lie within an order of magnitude. In contrast, the dose-response relationship between daily environmental benzene exposure in the range of parts per billion and leukemia has a much larger degree of uncertainty.

This uncertainty can only be resolved by a better mechanistic understanding of benzene leukemogenesis.

More problematic is the potential causal relationship between benzene and lymphopoietic cancers in humans. While in no case is the evidence incontrovertible, there does appear to be more than sufficient grounds to link benzene exposure with multiple myeloma (165) and perhaps with certain forms of non-Hodgkin's lymphoma and acute lymphoblastic leukemia. In the case of multiple myeloma the observations include individual case reports and a statistically significantly increased number of myeloma cases in the Pliofilm cohort that is under study by NIOSH (162, 166). In the latter cohort, the association has been suggested to be problematic in view of the lack of relationship with the extent of exposure. However, as there is a small number of cases, it is difficult to expect an obvious dose relationship. There is a strong element of biological plausibility that supports a causal relationship between benzene exposure and multiple myeloma, as well as other lymphatic neoplasms. Lymphocytes are definitely a target of benzene, with lymphocyte depletion occurring early in the course of benzene exposure (146, 167). The readily detected cytogenetic abnormalities in lymphocytes following significant benzene exposure clearly indicate that lymphocyte DNA is affected as a result of benzene exposure, and there is no question that hematopoietic tissue is subjected to a carcinogenic metabolite of benzene in view of the clear causal relationship with acute myelogenous leukemia.

Non-Hodgkin's lymphomas represent a grab bag of different diseases. A certain portion of these disorders reflects severe immune depletion as occurs with AIDS or secondary to treatment related to organ transplantation, in which the tumors occur very rapidly, sometimes in less than 1 year. However, there is no clear evidence that benzene produces this form of immune depletion. Non-Hodgkin's lymphoma is observed in certain work groups that have been exposed to solvents or other chemicals, but the evidence that benzene is causally related remains suggestive but uncertain. Similar arguments as to biomedical plausibility could be used to link benzene exposure to acute lymphoblastic leukemia, the leukemia most commonly observed in children. However, in view of the fortunate absence of well-defined populations of children that have been exposed to benzene, it will be particularly difficult to develop the epidemiological data necessary to evaluate the potential causal relation. Individual cases in relationship to household benzene exposure are being observed medically and legally but have not yet been reported in the clinical literature.

REFERENCES

- Andrews, L. S., Lee, E. W., Witmer, C. M., Kocsis, J. J., and Snyder, R. Effects of toluene on the metabolism, disposition, and hemopoietic toxicity of ^3H benzene. *Biochem. Pharmacol.* 26: 293-300 (1977).
- Sammett, D., Lee, E. W., Kocsis, J. J., and Snyder, R. Partial hepatectomy reduces both metabolism and toxicity of benzene. *J. Toxicol. Environ. Health.* 5: 785-792 (1979).
- Bolcsak, L. E., and Nerland, D. E. Inhibition of erythropoiesis by benzene and benzene metabolites. *Toxicol. Appl. Pharmacol.* 69: 363-368 (1983).
- Goldstein, B. D. Introduction: Occam's razor is dull. *Environ. Health Perspect.* 82: 3-6 (1989).
- Kalf, G. F. Recent advances in the metabolism and toxicity of benzene. *CRC Crit. Rev. Toxicol.* 18: 141-159 (1987).
- Snyder, R., and Pirozzi Chatterjee, S. Benzene metabolism. In: *Molecular Aspects of Monooxygenases and Bioactivation of Toxic Compounds* (E. Arinc, J. B. Schenckman and E. Hodgson, Eds.). Plenum Press, New York 1991, pp. 375-386.
- Yardley-Jones, A., Anderson, D., and Parke, D. V. The toxicity of benzene and its metabolism and molecular pathology in human risk assessment. *Br. J. Ind. Med.* 48: 437-444 (1991).
- Porteous, J. W., and Williams, R. T. Studies in detoxication. The isolation of phenol, catechol, quinol and hydroxyquinol from the ethereal sulfate fraction of the urine of rabbits receiving benzene orally. *Biochem. J.* 44: 56-61 (1949).
- Parke, D. V., and Williams, R. T. Studies in detoxication 49. The metabolism of benzene containing (^{14}C) benzene. *Biochem. J.* 54: 231-238 (1953).
- Williams, R. T. *Detoxication Mechanisms*. Chapman and Hall, London, 1959, pp. 188-194.
- Jaffe, M. Über die Aufspaltung des Benzolrings im Organismus. I. Mitteilung. Das Auftreten von Muconsäure im Ham nach Darreichung von Benzol. *Hoppe-Seyl. Z. Physiol. Chem.* 62: 58-67 (1909).
- Drummond, J. C., and Finar, I. L. Muconic acid as a metabolic product of benzene. *Biochemistry* 32: 79-84 (1938).
- Sabourin, P. J., Bechtold, W. E., Birnbaum, L. S., Lucier, G., and Henderson, R. F. Differences in the metabolism and disposition of inhaled [^3H] benzene by F344/N rats and B6C3F₁ mice. *Toxicol. Appl. Pharmacol.* 94: 128-140 (1988).
- Snyder, R., and Kocsis, J. J. Current concepts of chronic benzene toxicity. *CRC Crit. Rev. Toxicol.* 3: 265-288 (1975).
- Rusch, G. M., Leong, B. K., and Laskin, S. Benzene metabolism. *J. Toxicol. Environ. Health* 2(suppl.): 23-36 (1977).
- Snyder, C. A. Benzene. In: *Ethel Browning's Toxicity and Metabolism of Industrial Solvents*, 2nd ed., Vol. 1, Hydrocarbons (R. Snyder, Ed.). Elsevier, Amsterdam, 1987, pp. 3-27.
- Latriano, L., Goldstein, B. D., and Witz, G. Formation of muconaldehyde, an open-ring metabolite of benzene in mouse liver microsomes: a novel pathway for toxic metabolites. *Proc. Natl. Acad. Sci. U.S.A.* 83: 8356-8360 (1986).
- Snyder, R., Longacre, S. L., Witmer, C. M., and Kocsis, J. J. Biochemical toxicity of benzene. In: *Reviews in Biochemical Toxicology*, Vol. 3 (E. Hodgson, J. R. Bend, and R. M. Philpot, Eds.), Elsevier, New York, 1981, pp. 123-153.
- Koop, D. R., Laethem, C. L., and Schnier, G. G. Identification of ethanol-inducible P450 isozyme 3a (P450_{11E1}) as benzene and phenol hydroxylase. *Toxicol. Appl. Pharmacol.* 98: 278-288 (1989).
- Sawahata, T., and Neal, R. Biotransformation of phenol to hydroquinone and catechol by rat liver microsomes. *Mol. Pharmacol.* 23: 453-460 (1983).
- Jerina, D. M., and Daly, J. W. Arene oxides: a new aspect of drug metabolism. *Science* 185: 573-582 (1974).
- Hanzlik, R. P., Hogberg, P. K., and Judson, C. M. Microsomal hydroxylation of specifically deuterated monosubstituted benzene. Evidence for direct aromatic hydroxylation. *Biochemistry* 23: 3048-3055 (1984).
- Johansson, I., and Ingelman-Sundberg, M. Hydroxyl radical-mediated, cytochrome P-450-dependent metabolic activation of benzene in microsomes and reconstituted enzyme systems from rabbit liver. *J. Biol. Chem.* 258: 7311-7316 (1983).
- Walling, C., and Johnson, R. A. Fenton's reagent. V. Hydroxylation and side chain cleavage of aromatics. *J. Am. Chem. Soc.* 97: 363-367 (1975).
- Cohen, G., and Ofodile, S. E. Activation of molecular oxygen during a Fenton reaction: a study with $^{18}\text{O}_2$. In: *Oxy Radicals and their Scavenger Systems*, Vol. 1. Molecular Aspects (G. Cohen and R. A. Greenwald, Eds.), Elsevier, New York, 1983, pp. 74-83.
- Gorsky, L. D., and Coon, M. J. Evaluation of the role of free hydroxyl radicals in the cytochrome P-450 catalyzed oxidation of benzene and cyclohexanol. *Drug Metab. Disp.* 13: 169-174 (1985).
- Sabourin, P. J., Bechtold, W. E., and Henderson, R. F. A high pressure liquid chromatographic method for the separation and quantitation of water-soluble radiolabeled benzene metabolites. *Anal. Biochem.* 170: 316-327 (1988b).
- Tunek, A., Platt, K. L., Przybylski, M., and Oesch, F. Multistep 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 (1980).
- Lunte, S. M., and Kissinger, P. T. Detection and identification of sulfhydryl conjugates of p-benzoquinone in microsomal incubations of benzene and phenol. *Chem.-Biol. Interact.* 47: 195-212 (1983).
- Nerland, D. E., and Pierce, W. M. Identification of N-acetyl-S-(2,5-di-

- hydroxyphenyl)-L-cysteine as a urinary metabolite of benzene, phenol, and hydroquinone. *Drug Metab. Disp.* **18**: 958-961 (1990).
31. Daniels, M., Scholes, G., and Weiss, J. Chemical action of ionizing radiations in solution. Part XV. Effect of molecular oxygen in the irradiation of aqueous benzene solutions with X-rays. *Biochem. J.* **832-834** (1955).
 32. Loeff, I., and Stein, G. Aromatic ring-opening in the presence of oxygen in irradiated solutions. *Nature* **184**: 901 (1959).
 33. Kirley, T. A., Goldstein, B. D., Maniara, W. M., and Witz, G. Metabolism of trans,trans-muconaldehyde, a microsomal hematotoxic metabolite of benzene by purified yeast aldehyde dehydrogenase and a mouse liver soluble fraction. *Toxicol. Appl. Pharmacol.* **100**: 360-367 (1989).
 34. Ross, D., Goon, D., Colagiovanni, D., Ruth, J., and Petersen, D. R. In vitro metabolism of trans,trans-muconaldehyde identification of a novel metabolite. *Toxicologist* **11**: 181 (1991).
 35. Zhang, Z., Rosen, R. T., Fukuda, E. K., Goldstein, B. D. and Witz, G. In vitro metabolism of trans,trans-muconaldehyde, a microsomal hematotoxic metabolite of benzene. *Toxicologist* **12**: 387 (1992).
 36. Gad-El Karim, M. M., Sadagopa Ramanujam, V. M., and Legator, M. S. Trans,trans-muconic acid, an open-chain urinary metabolite of benzene in mice. Quantification by high pressure liquid chromatography. *Xenobiotica* **15**: 211-220 (1985).
 37. Witz, G., Maniara, W. M., Mylavarapu, V. J., and Goldstein, B. D. Comparative metabolism of benzene and trans,trans-muconaldehyde to trans,trans-muconic acid in DBA/2N and C57BL/6 mice. *Biochem. Pharmacol.* **40**: 1275-1280 (1990).
 38. Mueller, G., Koelbel, M., Heger, M., and Norpoth, K. Urinary S-phenylmercapturic acid and phenylguanine as indicators of benzene exposure. In: *Biological Monitoring of Exposure to Chemicals*. Organic Compounds (M. H. Ho and H. K. Dillon, Eds.), John Wiley and Sons, New York, 1987, pp. 91-98.
 39. Irons, R., Dent, J., Baker, T., and Richert, D. Benzene is metabolized and covalently bound in bone marrow in situ. *Chem.-Biol. Interact.* **30**: 241-245 (1980).
 40. Jowa, L., Witz, G., Snyder, R., Winkle, S., and Kalf, G. F. Synthesis and characterization of deoxyguanosine-benzoquinone adducts. *J. Appl. Toxicol.* **10**: 47-54 (1990).
 41. Schatz-Kornbrust, E., Goldstein, B. D., and Witz, G. The binding of trans,trans-muconaldehyde to deoxyguanosine and calf thymus DNA. *Toxicologist* **11**: 253 (1991).
 42. Dean, B. J. Recent findings on the genetic toxicology of benzene, toluene, xylenes and phenols. *Mutat. Res.* **154**: 153-181 (1985).
 43. Glatt, H., Padykula, R., Berchtold, G. A., Ludewig, G., Platt, K. L., Klein, J., and Oesch, F. Multiple activation pathways of benzene leading to products with varying genotoxic characteristics. *Environ. Health Perspect.* **82**: 81-90 (1989).
 44. Glatt, H., and Witz, G. Studies on the induction of gene mutations in bacterial and mammalian cells by the ring-opened benzene metabolites trans,trans-muconaldehyde and trans,trans-muconic acid. *Mutagenesis* **5**: 263-266 (1990).
 45. Witz, G., Gad, S. C., Tice, R. R., Oshiro, Y., Piper, C. E., and Goldstein, B. D. Genetic toxicity of the benzene metabolite trans,trans-muconaldehyde in mammalian and bacterial cells. *Mutat. Res.* **240**: 295-306 (1990).
 46. Rickert, D. E., Baker, T. S., Bus, J. S., Barrow, C. S., and Irons, R. D. Benzene disposition in the rat after exposure by inhalation. *Toxicol. Appl. Pharmacol.* **49**: 417-423 (1979).
 47. Greenlee, W. F., Sun, J. D., and Bus, J. S. A proposed mechanism of benzene toxicity: Formation of reactive intermediates from polyphenol metabolites. *Toxicol. Appl. Pharmacol.* **59**: 187-195 (1981).
 48. Eastmond, D. A., Smith, M. T., Ruzo, L. O., and Ross, D. Metabolic activation of phenol by human myeloperoxidase and horse-radish peroxidase. *Mol. Pharmacol.* **30**: 674-679 (1986).
 49. Eastmond, D. A., Smith, M. T., and Irons, R. D. An interaction of benzene metabolites reproduces the myelotoxicity observed with benzene exposure. *Toxicol. Appl. Pharmacol.* **91**: 85-95 (1987).
 50. Sadler, A., Subrahmanyam, V. V., and Ross, D. Oxidation of catechol by horseradish peroxidase and human leukocyte peroxidase: Reactions of o-benzoquinone and o-benzosemiquinone. *Toxicol. Appl. Pharmacol.* **93**: 62-71 (1988).
 51. Irons, R. D. Quinones as toxic metabolites of benzene. *J. Toxicol. Environ. Health* **16**: 673-678 (1985).
 52. Smith, M. T., Yager, J. W., Steinmetz, K. M., and Eastmond, D. A. Peroxidase dependent metabolism of benzene's phenolic metabolites and its potential role in benzene toxicity and carcinogenicity. *Environ. Health Perspect.* **82**: 23-29 (1989).
 53. Steinmetz, K. L., Eastmond, D. A., and Smith, M. T. Hydroquinone metabolism by human myeloperoxidase is stimulated by phenol and several other compounds. *Toxicologist* **9**: 281 (1989).
 54. Smith, M. T., Robertson, M. T., Yager, J. W., and Eastmond, D. A. Role of metabolism in benzene-induced myelotoxicity and leukemogenesis. In: *Mutation and the Environment Part B* (M. L. Mendelsohn and R. J. Albertini, Eds.), Wiley-Liss, Inc., New York, 1990, pp. 125-136.
 55. Schlosser, M. J., Shurina, R. D., and Kalf, G. F. Metabolism of phenol and hydroquinone to reactive products by macrophage peroxidase or purified prostaglandin H synthase. *Environ. Health Perspect.* **82**: 229-237 (1989).
 56. Santesson, C. G. Über chronische Vergiftung mit Steinkohlenteerbenzin; vier Todesfälle. *Arch. Hyg. Berl.* **31**: 336-376 (1897).
 57. Selling, L. Benzol as a leukotoxin. Studies on degeneration regeneration of blood and hematopoietic organs. *Johns Hopkins Hospital Rep.* **17**: 83-142 (1916).
 58. Weiskotten, H. G., Schwartz, S. C., and Steinsland, H. S. The action of benzol on blood and blood forming tissues. *J. Med. Res.* **35**: 63-69 (1916).
 59. Weiskotten, H. G., Gibbs, G. B. F., Boggs, E. O., and Templeton, E. R. The action of benzol. VI. Benzol vapor leucopenia (rabbit). *J. Med. Res.* **41**: 425-438 (1920).
 60. Forni, A., Pacifico, E., and Limonta, A. Chromosome studies in workers exposed to benzene or toluene or both. *Arch. Environ. Health* **22**: 373-378 (1971a).
 61. Forni, A., Cappellini, A., Pacifico, E., and Vigliani, E. Chromosome changes and their evolution with past exposure to benzene. *Arch. Environ. Health* **23**: 385-391 (1971b).
 62. Hite, M., Pecharo, M., Smith, I., and Thornton, S. The effect of benzene in micronucleus test. *Mutat. Res.* **77**: 149-155 (1980).
 63. Tice, R. R., Costa, D. L., and Drew, R. T. Cytogenetic effects of inhaled benzene in murine bone marrow: Induction of sister chromatid exchanges, chromosomal aberrations, and cellular proliferation inhibition in DBA/2 mice. *Proc. Natl. Sci. U.S.A.* **77**: 2148-2152 (1980).
 64. Tice, R. R., Vogt, T. F., and Costa, D. L. Cytogenetic effects of inhaled benzene in murine bone marrow in genotoxic effect of airborne agents. *Environ. Sci. Res.* **25**: 257-275 (1982).
 65. IARC. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, Vol. 29. Benzene. International Agency for Research on Cancer, Lyon, 1982, 93-148.
 66. Maltoni, C., and Scarnato, C. First experimental demonstration of the carcinogenic effects of benzene. *Med. Lav.* **5**: 352-357 (1982b).
 67. Maltoni, C., Cotti, G., Valgimigli, L., and Mandrioli, A. Zymbal gland carcinomas in rats following exposure to benzene by inhalation. *Am. J. Ind. Med.* **3**: 11-16 (1982).
 68. Huff, J. E., Haseman, J. K., De Marini, D. M., Eustis, S., Maronpot, R. R., Peters, A. C., Persing, R. L., Chrisp, C. E., and Jacobs, A. C. Multiple-site carcinogenicity of benzene in Fischer 344 rats and B6C3F₁ mice. *Environ. Health Perspect.* **82**: 125-163 (1989).
 69. Pirozzi-Chatterjee, S., and Snyder, R. Mechanisms of benzene toxicity. In: *Molecular Mechanisms of Monooxygenases and Bioactivation of Toxic Compounds*. (E. Arinc, J. B. Schenkman, E. and Hodgson, Eds.), NATO ASI Series A: Life Sciences, Vol. 202. Plenum Press, New York, 1991, pp. 387-414.
 70. Uyeki, E. M., Askar, A. E., Shoeman, D. W., and Bisel, T. U. Acute toxicity of benzene inhalation to hemopoietic precursor cells. *Toxicol. Appl. Pharmacol.* **40**: 49-57 (1977).
 71. Gill, D., Jenkins, V., Kempen, R., and Ellis, S. The importance of pluripotent stem cells in benzene toxicity. *Toxicology* **16**: 163-171 (1980).
 72. Green, J. D., Snyder, C. A., Lobue, J., Goldstein, B. D., and Albert, R. E. Acute and chronic dose/response effects of inhaled benzene on multipotential hemopoietic stem (CFU-S) and granulocytelmacrophage progenitor (GM CFU-C) cells in CD-1 mice. *Toxicol. Appl. Pharmacol.* **58**: 492-503 (1981).
 73. Harigaya, K., Miller, M. E., Cronkite, E. P., and Drew, R. T. The detection of *in vivo* liquid bone marrow cultures. *Toxicol. Appl. Pharmacol.* **60**: 346-353 (1981).
 74. Toft, K., Olofsson, T., Tunek, A., and Berlin, M. Toxic effects on mouse bone marrow caused by inhalation of benzene. *Arch. Toxicol.* **51**: 295-302 (1982).
 75. Baarson, K., Snyder, C. A., and Albert, R. E. Repeated exposures of C57Bl/6 mice to 10 ppm inhaled benzene markedly depressed erythropoietic colony formation. *Toxicol. Lett.* **20**: 337-342 (1984).

76. Seidel, H. J., Barthel, E., and Zinser, D. The hematopoietic stem cell compartments in mice during and after long term inhalation of three doses of benzene. *Exp. Hematol.* 17: 300-303 (1989).
77. Keller, K. A., and Snyder, C. A. Mice exposed in utero to low concentrations of benzene exhibit enduring changes in their colony forming hematopoietic cells. *Toxicology* 42: 171-181 (1986).
78. Keller, K. A., and Snyder, C. A. Mice exposed in utero to 20 ppm benzene exhibit altered numbers of recognizable hematopoietic cells up to seven weeks after exposure. *Fundam. Appl. Toxicol.* 10: 224-232 (1988).
79. Filmanowicz, E., and Gurney, C. W. Studies on erythropoiesis. XVI. Response to a single dose of erythropoietin in polycythemic mice. *J. Lab. Clin. Med.* 57: 65-72 (1961).
80. Payne, B. J., Lewis, H. B., Murchinson, T. E., and Hart, E. A. Hematology of laboratory animals. In: *Handbook of Laboratory Science*, VIII, (E. C. Melby, Jr. and N. H. Altman, Eds.), CRC Press, Boca Raton, FL, 1976, pp. 383-463.
81. Bothwell, T., Charlton, R., Cook, J., and Finch, C. Iron Metabolism in Man. Blackwell Scientific Publications, Oxford, 1979.
82. Lee, E. W., Kocsis, J. J., and Snyder, R. Acute effect of benzene on [^{59}Fe] incorporation into circulating erythrocytes. *Toxicol. Appl. Pharmacol.* 27: 431-436 (1974).
83. Lee, E. W., Kocsis, J. J., and Snyder, R. The use of ferrokinetics in the study of experimental anemia. *Environ. Health Perspect.* 39: 29-37 (1981).
84. Dexter, T. M. Hemopoiesis in long-term bone marrow cultures. *Acta Haematol.* 63: 299-305 (1979).
85. Garnett, H. M., Cronkite, E. P., and Drew, R. T. Effect of in vivo exposure to benzene on the characteristics of bone marrow adherent cells. *Leukemia Res.* 7: 803-810 (1983).
86. Frash, V. N., Yushov, B. G., Karaulov, A. V., and Skuratov, V. L. Mechanism of action of benzene on hematopoiesis. Investigation of hematopoietic stem cells. *Bull. Exp. Biol. Med.* 82: 985-987 (1976).
87. Snyder, R., Dimitriadis, E., Guy, R., Hu, P., Cooper, K. R., Bauer, H., Witz, G., and Goldstein, B. D. Studies on the mechanism of benzene toxicity. *Environ. Health Perspect.* 82: 31-36 (1989).
88. Guy, R. L., Dimitriadis, E., Hu, P., Cooper, K. R., and Snyder, R. Interactive inhibition of erythroid [^{59}Fe] utilization by benzene metabolites in female mice. *Chem.-Biol. Interact.* 74: 55-62 (1990).
89. Guy, R. L., Hu, P., Witz, G., and Goldstein, B. D. Depression of iron uptake into erythrocytes in mice by treatment with the combined benzene metabolites p-benzoquinone, muconaldehyde, and hydroquinone. *J. Appl. Toxicol.* 11: 443-446 (1991).
90. Gaido, K. W., and Weir, D. *In vitro* effects of benzene metabolites on mouse bone marrow stromal cells. *Toxicol. Appl. Pharmacol.* 76: 45-55 (1984).
91. Gaido, K. W., and Weir, D. Modulation of stromal cell function in DBA/2J and B6C3F1 mice exposed to benzene or phenol. *Toxicol. Appl. Pharmacol.* 81: 469-475 (1985).
92. Gaido, K. W., and Weir, D. Suppression of bone marrow stromal cell function by benzene and hydroquinone is ameliorated by indomethacin. *Toxicol. Appl. Pharmacol.* 89: 378-390 (1987).
93. Longacre, S., Kocsis, J. J., and Snyder, R. Influence of strain differences in mice on the metabolism and toxicity of benzene. *Toxicol. Appl. Pharmacol.* 60: 398-409 (1981).
94. Post, G. B., Snyder, R., and Kalf, G. F. Metabolism of benzene and phenol in macrophages in vitro and the inhibition of RNA synthesis by benzene metabolites. *Cell Biol. Toxicol.* 2: 231-246 (1986).
95. Renz, J. F., and Kalf, G. F. A role for interleukin-1 in benzene induced hematotoxicity: inhibition of the conversion of pre-IL-1 α to mature cytokine in murine macrophages by hydroquinone and the prevention of benzene-induced hematotoxicity in mice by interleukin-1 α . *Blood* 78: 938-944 (1991).
96. Shimizu, M., Yasui, Y., and Matsumoto, N. Structural specificity of aromatic compounds with special reference to mutagenic activity in *Salmonella typhimurium*—a series of chloro- or fluoro-nitrobenzene derivatives. *Mutat. Res.* 116: 217-238 (1983).
97. Amacher, D. E., and Zelljadt, J. The morphological transformation of Syrian hamster embryo cells by chemicals reportedly non-mutagenic to *Salmonella typhimurium*. *Carcinogenesis* 4: 291-295 (1983).
98. Pollini, G., and Colombi, R. Il danno cromosomico midollare nell'anemia aplastica benzolica. *Med. Lav.* 55: 244-255 (1964).
99. Pollini, G., Biscaldi, G. P., and Robustelli Della Cuna, G. Chromosome changes in lymphocytes five years after benzene haemopathy. *Med. Lav.* 60: 743-758 (1969).
100. Erexson, G. L., Wilmer, J. L., and Klingerman, A. D. Induction of sister chromatid exchanges and micronuclei in male DBA/2 mice after inhalation of benzene. *Environ. Mutagen.* 6: 408 (1984).
101. Erexson, G. L., Wilmer, J. L., and Klingerman, A. D. Sister chromatid exchange induction in human lymphocytes exposed to benzene and its metabolites in vitro. *Cancer Res.* 45: 2471-2477 (1985).
102. Schlager, R., and MacGregor, J. T. The persistence of micronuclei in peripheral blood erythrocytes: detection of chronic chromosome breakage. *Mutat. Res.* 104: 367-369 (1982).
103. Harper, B. L., Ramanujam, V. M. S., Gad-El-Karim, M. M., and Legator, M. S. The influence of simple aromatics on benzene clastogenicity. *Mutat. Res.* 128: 105-114 (1984).
104. Pirozzi, S. J., Renz, J. F., and Kalf, G. F. The prevention of benzene-induced genotoxicity in mice by indomethacin. *Mutat. Res.* 222: 291-298 (1989a).
105. Schlosser, M. J., Shurina, R. D., and Kalf, G. F. Prostaglandin H synthetase catalyzed oxidation of hydroquinone to a sulfhydryl binding and DNA-damaging metabolite. *Chem. Res. Toxicol.* 3: 333-339 (1990).
106. Snyder, R., Lee, E. W., and Kocsis, J. J. Binding of labeled benzene metabolites to mouse liver and bone marrow. *Res. Commun. Chem. Pathol. Pharmacol.* 20: 191-194 (1978).
107. Tunek, A., Platt, K., Bentley, P., and Oesch, F. Microsomal metabolism of benzene to species irreversibly binding to microsomal protein and the effects of modification of this metabolism. *Mol. Pharmacol.* 14: 920-929 (1978).
108. Lutz, W. K., and Schlatter, C. H. Mechanism of the carcinogenic action of benzene: irreversible binding to rat liver DNA. *Chem.-Biol. Interact.* 18: 241-245 (1977).
109. Irons, R. D., and Neptun, D. A. Effects of principle hydroxy-metabolites of benzene on microtubule polymerization. *Arch. Toxicol.* 45: 297-305 (1980).
110. Pfeifer, R. W., and Irons, R. D. Alteration of lymphocyte function by quinones through sulfhydryl-dependent disruption of microtubule assembly. *Int. J. Immunopharmacol.* 5: 463-470 (1983).
111. Schwartz, C., Snyder, R., and Kalf, G. The inhibition of mitochondrial DNA replication *in vitro* by metabolites of benzene, hydroquinone and p-benzoquinone. *Chem.-Biol. Interact.* 53: 327-350 (1986).
112. Lutz, W. K. In vivo covalent binding of organic chemicals to DNA as a quantitative indicator in the process of chemical carcinogenesis. *Mutat. Res.* 65: 289-356 (1979).
113. Rushmore, T., Kalf, G., and Snyder, R. Covalent binding of benzene and its metabolites to DNA in rabbit bone marrow mitochondria *in vitro*. *Chem.-Biol. Interact.* 49: 133-154 (1984).
114. Karaszkievicz, J. W., Snyder, R., and Kalf, G. F. Partial purification of benzene hydroxylase activity from rat liver mitochondria. *Fed. Proc.* 45: 1748 (1986).
115. Pongracz, K., and Bodell, W. J. Detection of 3'-hydroxy-1,N6-benzthieno-2'-deoxyadenosine 3'-phosphate by 32P postlabeling of DNA reacted with p-benzoquinone. *Chem. Res. Toxicol.* 4: 199-202 (1991).
116. Pirozzi, S. J., Schlosser, M. J., and Kalf, G. F. Prevention of benzene-induced myelotoxicity and prostaglandin synthesis in bone marrow of mice by inhibitors of prostaglandin H synthetase. *Immunopharmacology* 18: 39-55 (1989b).
117. Bauer, H., Dimitriadis, E. A., and Snyder, R. An in vivo study of benzene metabolite DNA adduct formation in liver of male New Zealand rabbits. *Arch. Toxicol.* 63: 209-213 (1989).
118. Reddy, M. V., Blackburn, G. R., Schreiner, C. A., Mehlerman, M. A., and Mackerer, C. R. ^{32}P analysis of DNA adducts in tissues of benzene treated rats. *Environ. Health Perspect.* 82: 253-268 (1989).
119. Goldstein, B. D., Snyder, C. A., Laskin, S., Bromberg, I., Albert, R. E., and Nelson, N. Myelogenous leukemia in rodents inhaling benzene. *Toxicol. Lett.* 13: 169-173 (1982).
120. Cronkite, E., Bullis, J., Ince, T., and Drew, R. Benzene inhalation produces leukemia in mice. *Toxicol. Appl. Pharmacol.* 75: 358-361 (1984).
121. Da Silva, C., Fan, X., and Castagne, M. Benzene-mediated protein kinase C activation. *Environ. Health Perspect.* 82: 91-95 (1989).
122. Reddy, M. V., Blackburn, G. R., Irwin, S. E., Komminen, C., Mackerer, C. R., and Mehlerman, M. A. A method for in vitro culture of rat Zymbal gland: use in mechanistic studies of benzene carcinogenesis in combination with ^{32}P -postlabeling. *Environ. Health Perspect.* 82: 239-248 (1989).
123. Low, L. K., Meeks, J. R., Noms, K. J., Mehlerman, M. A., and Mackerer, C. R. Pharmacokinetics and metabolism of benzene in Zymbal gland and other key target tissues after oral administration in rats. *Environ. Health Perspect.* 82: 215-222 (1989).

124. Busby, W. F., Jr., Wang, J. S., Stevens, E. K., Padykula, R. E., Alekseychik, R. A., and Berchtold, G. A. Carcinogenesis 11: 1473-1478 (1990).
125. Solomon, E., Borrow, J., and Goddard, A. D. Chromosome aberrations and cancer. *Science* 254: 1153-1160 (1991).
126. Metacalf, D., Nowell, P. C., Rowley, J. D., and Sachs, L. Meeting Chromosomal and growth factor abnormalities in leukemia. *Cancer Res.* 51: 2494-2497 (1991).
127. de The, H., Lavau, C., Marchio, A., Chomienne, C., and Degos, J. The PML-RAR α fusion mRNA generated by the t(5;17) translocation in acute promyelocytic leukemia encodes a functionally altered RAR. *Cell* 66: 675-684 (1991).
128. Sato, A., and Nakajima, T. Dose-dependent metabolic interaction between benzene and toluene in vivo and in vitro. *Toxicol. Appl. Pharmacol.* 48: 249-256 (1979).
129. Beliles, R. P., and Totman, L. C. Pharmacokinetically based risk assessment of workplace exposure to benzene. *Regul. Toxicol. Pharmacol.* 9: 186-195 (1989).
130. Medinsky, M. A., Sabourin, P. J., Lucier, G., Birnbaum, L. S., and Henderson, R. F. A physiological model for simulation of benzene metabolism by rats and mice. *Toxicol. Appl. Pharmacol.* 99: 193-206 (1989).
131. Travis, C. C., Quillen, J. L., and Arms, A. D. Pharmacokinetics of benzene. *Toxicol. Appl. Pharmacol.* 102: 400-420 (1990a).
132. Travis, C. C., White, R. K., and Wards, R. C. Interspecies extrapolation of pharmacokinetics. *J. Theor. Biol.* 142: 285-304 (1990).
133. Bois, F. Y., Smith, M. T., and Spear, R. C. Mechanisms of benzene carcinogenesis: application of a physiological model of benzene pharmacokinetics and metabolism. *Toxicol. Lett.* 56: 283-298 (1991).
134. Spear, R. C., Bois, F. Y., Woodruff, T., Auslander, D., Parker, J., and Selvin, S. Modeling benzene pharmacokinetics across three sets of animal data: parametric sensitivity and risk implications. *Risk Anal.* 11: 641-654 (1991).
135. Subrdhmany, V. V., Ross, D., Eastmond, D. A., and Smith, M. T. Potential role of free radicals in benzene-induced myelotoxicity and leukemia. *Free Rad. Bio. Med.* 11: 495-515 (1991).
136. Wallace, L. A., and Pellizzari, E. D. Personal air exposures and breath concentrations of benzene and other volatile hydrocarbons for smokers and nonsmokers. *Toxicol. Lett.* 35: 113-116 (1986).
137. Wallace, L. A. The Total Exposure Assessment Methodology (TEAM) Study: Summary and Analysis: Vol 1. EPA-600/6-87-002a. U.S. Environmental Protection Agency, Washington, DC, 1987.
138. ATSDR. Toxicological Profile for Benzene. American Toxic Substances and Disease Registry. Atlanta, GA, 1989.
139. Maltoni, C., Conti, B., and Coni, G. Benzene: a multipotential carcinogen. Results of long-term bioassays performed at the Bologna Institute of Oncology. *Am. J. Ind. Med.* 4: 589-630 (1983).
140. NTP. Technical Report on the Toxicology and Carcinogenesis Studies of Benzene (CAS No. 71-43-2) in F344/N Rats and B6C3F₁ Mice (Gavage Studies). National Toxicology Program, Research Triangle Park, NC, 1983.
141. Davis, D. L., and Pope, A. M. Reproductive risks of benzene: Need for additional study. *Toxicol. Ind. Health* 2: 445-451 (1986).
142. Irons, R. D., Wierda, D., and Pfeifer, R. W. Immunotoxicity of benzene and its metabolites. *Adv. Mod. Environ. Toxicol.* 4: 37-50 (1983).
143. Goldstein, B. D. Biological and ambient monitoring of benzene in the workplace. *J. Occup. Med.* 28: 1051-1054 (1986).
144. Rosenthal, G. J., and Snyder, C. A. Inhaled benzene reduces aspects of cell-mediated tumor surveillance in mice. *Toxicol. Appl. Pharmacol.* 88: 35-43 (1987).
145. Goldstein, B. D., and Witz, G. Free radicals and carcinogenesis. *Free Rad. Res. Commun.* 11: 3-10 (1990).
146. Goldstein, B. D. Hematotoxicity in humans. In: *Benzene toxicity: a critical evaluation*. J. Toxicol. Environ. Health 2(suppl.): 69-105 (1977).
147. Goldstein, B. D. Clinical hematotoxicity of benzene. In: *Advances in Modern Environmental Toxicology*, Vol. 16. Benzene: Occupational and Environmental Hazards-Scientific Update. Princeton Scientific Publishing, Princeton, NJ, 1989, pp. 55-65.
148. Aksoy, M., Erdem, S., Erdogan, G., and Dincol, G. Combination of genetic factors and chronic exposure to benzene in the aetiology of leukaemia. *Hum. Hered.* 26: 149-153 (1976).
149. Browning, E. Toxicity and Metabolism of Industrial Solvents, Elsevier. Amsterdam, 1965.
150. Vigliani, E. C., and Saita, G. Benzene and leukemia. *N. Engl. J. Med.* 217: 872-876 (1964).
151. Aksoy, M., Erdem, S., and Dincol, S. Leukemia in shoe workers exposed chronically to benzene. *Blood* 44: 837-841 (1974).
152. Infante, P., Rinsky, R., Wagoner, J., and Young, R. Leukemia in benzene workers. *Lancet* ii: 76-78 (1977).
153. Goldstein, B. D., and Kipen, H. Lessons on the second cancers resulting from cancer chemotherapy. In: *Biological Reactive Intermediates IV* (C. M. Witmer, Ed.). Plenum Press, New York, 1990, pp. 619-625.
154. Aksoy, M., and Erdem, S. Follow-up study on the mortality and the development of leukemia in 44 pancytopenic patients with chronic benzene exposure. *Blood* 52: 285-292 (1978).
155. Aksoy, M., Dincol, K., Akgun, T., Erdem, S., and Dincol, G. Haematological effects of chronic benzene poisoning in 217 workers. *Br. J. Ind. Med.* 28: 296-302 (1971).
156. Wong, O. An industry wide mortality study of chemical workers occupational exposed to benzene. I. General results. *Br. J. Ind. Med.* 44: 365-381 (1987a).
157. Wong, O. An industry wide mortality study of chemical workers occupational exposed to benzene. II. Dose response analyses. *Br. J. Ind. Med.* 44: 382-395 (1987b).
158. Bond, G. G., McLaren, E. A., Baldwin, C. L., and Cook, R. R. An update of mortality among chemical workers exposed to benzene. *Br. J. Ind. Med.* 43: 685-691 (1986).
159. Yin, S. N., Li, G. L., Tain, F. D., Fu, N. I., Jin, C., Chen, Y. J., Luo, S. J., Ye, P. Z., Zhang, J. Z., Wang, G. C., Zhang, X. C., Wu, H. N., and Zhong, Q. C. Leukaemia in benzene workers: a retrospective cohort study. *Br. J. Ind. Med.* 44: 124-128 (1987).
160. Rushton, L., and Alderson, M. R. A case-control study to investigate the association between exposure to benzene and deaths from leukaemia in oil refinery workers. *Br. J. Cancer* 43: 77-84 (1981).
161. Rinsky, R. A., Young, R. J., and Smith, A. B. Leukemia in benzene workers. *Am. J. Ind. Med.* 2: 217-245 (1981).
162. Rinsky, R. A., Smith, A. B., Hornung, R., Filloon, T. G., Young, R. J., Okun, A. K., and Landrigan, P. J. Benzene and leukemia: an epidemiological risk assessment. *N. Engl. J. Med.* 316: 1044-1050 (1987).
163. Kipen, H. M., Cody, R. P., Crump, K. S., Allen, B., and Goldstein, B. D. Hematologic effects of benzene: a thirty-five year longitudinal study of rubber workers. *Toxicol. Ind. Health* 4: 411-430 (1988).
164. Kipen, H. M., Cody, R. P., and Goldstein, B. D. Use of longitudinal analysis of peripheral blood counts to validate historical reconstruction of benzene exposure. *Environ. Health Perspect.* 82: 199-206 (1989).
165. Goldstein, B. D. Is exposure to benzene a cause of human multiple myeloma? *Ann. N.Y. Acad. Sci.* 609: 225-334 (1990).
166. Aksoy, M., Erdem, S., Dincol, G., Kutlar, A., Bakioglu, I., and Hepyüksel, T. Clinical observations showing the role of some factors in the etiology of multiple myeloma. *Acta Haematol.* 71: 116-120 (1984).
167. Snyder, C. A., Goldstein, B. D., and Sellakumar, A. Hematotoxicity of inhaled benzene to Sprague-Dawley rats and AKR mice at 300 ppm. *J. Toxicol. Environ. Health* 4: 605-618 (1978).