# Chemical Leukemogenesis: Benzene as a Model

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## BENZENE CARCINOGENESIS IN MAN

BENZENE has been accepted as an environmental carcinogen by US regulatory agencies. It is ubiquitous. being produced by natural processes in nature and in huge amounts in industry. For example. in the US. 1.6 x $10^9$ gallons were produced in 1980 in the petroleum industry alone.[1] In 1897, LeNoir[43] described a case of purpura in man. and in 1928. acute leukemia in a person exposed to benzene was reported by Delore and Borgomano.[17] The evidence of benzene being a human carcinogen is based to a large extent upon the clinical studies of Aksoy et al[1-4] and Vigliani and Saita.[70] The clinical evidence incriminating benzene is described by Aksoy et al.[2] Their study is summarized. In Turkey, chronic benzene poisoning occurred in workers involved in the manufacture of shoes and slippers where benzene is used as a solvent. Of 34 individuals with pancytopenia, six subsequently developed acute leukemia. Aksoy stated, "from 1967 to September 1973. 26 patients with acute leukemia or preleukemia were seen in Istanbul among 28,500 shoe workers exposed chronically to benzene. The incidence of leukemia among these workers was 13/100.000. which is a marked and statistically significant increase over that for leukemia in the general population and provides cogent evidence for the clinical impression of a leukemogenic effect of benzene in man." Aksoy,' in his most recent publication, reported that leukemia in benzene-exposed workers decreased after the phase out of benzene in Istanbul. and there was no leukemia observed in workers during the last 3 years of his last study. implicating benzene as a probable causative agent in the earlier intervals. That benzene perturbed hematopoiesis has long been known. Selling[60] and Weiskotten et al[71] in 1916 clearly demonstrated that injection of benzene produced pancytopenia in rabbits and other mammals. Evidence that benzene might be leukemogenic was first deduced from the sequence of events in human beings. It appears that after intermittent chronic exposure to inhaled benzene of the order of 200 to 300 ppm for unknown' periods of time. pancytopenia may develop after a variable latent period. In those individuals that survive after variable latent periods, acute leukemia may develop. The leukemias described have been acute myelogenous leukemia, lymphoblastic leukemia. and erythroblastic leukemia. Whereas the incidence of pancytopenia in workers exposed chronically to benzene is unquestioned. whether the incidence of leukemia is significantly increased has been questioned and will be considered next. Ott et al[51] studied 594 occupationally exposed workers. Three cases of leukemia were observed when 0.8 was expected. Their statistical analysis showed $P < .047$. They concluded. "work histories and lack of medical history made a retrospective assessment of the possible relationship to benzene exposure very judgmental."

Further incrimination of benzene as a human leukemogen has been suggested by the detailed epidemiological studies of Infante et al.[34] Rinsky et al,[56] White et al,[72] and Infante and White.[33] In the first place. 'there are a large number of uncertainties in the estimation of human cancer risk because many of the important conditions with which one is concerned lie outside the range of scientific observation. Gaffey,[21] in criticism of White et al,[72] points out a potential defect in all risk assessments. namely, the age-specific incidence of cancer varies with age and is increasing in most instances. The assumption that relative risk is constant for all age groups is questionable and usually false. In response. Infante et al[34] point out that in reality, they used two cumulative mortalities for leukemia: one for people exposed to benzene and one for the white male population aged 20 to 84 years. The ratio of these two x 100 is the standardized mortality rate (SMR). It appears that the Gaffey criticism is

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not pertinent to the Infante et al[24] study, but is an important consideration in epidemiologic studies. Van Raalte et al[69] observed that there is inadequate information on exposure of groups in which leukemia was present and he also questioned linearity of response with exposure. The permissible exposure or time-weighted average (TWA) imposed by the Occupational Safety and Health Administration is 10 ppm benzene averaged over an eight-hour work day. All epidemiologic estimates of the incidence of any disease in a population chronically exposed to benzene must be correlated with the exposure level. Infante and White[70] have shown that in the period 1937 to 1940, the TWA was of 150 ppm in inspired air, and from 1941 to 1946, it was 100 ppm; in the interval, 1948 to 1956, the TWA was 35 ppm, from 1957 to 1962, it was 25 ppm, from 1963 to 1968, it was 25 ppm, and from 1969 to 1975, it was 10 ppm. Certainly the exposure of workers has been steadily decreasing and it is well-documented in the study of Scott.[70] Of 35,000 data points, 87% represented exposure to 1.0 ppm or less and 98.4% were less than 10 ppm. It is of interest, and perhaps explains why there have been no reports of alleged benzene-induced leukemia among workers commencing initial exposure since 1977. On the other hand, further prospective and retrospective epidemiologic studies on the relationship of benzene exposure in the workplace and a possible incidence of leukemia and other cancers are clearly needed. It appears to this author that the evidence for benzene leukemogenesis in man is nearly incontrovertible. The crucial questions are as follows: (1) Is there a threshold exposure of no effect? (2) What is the dose-effect relationship? Infante and White[22-29] and White et al[72] have addressed these issues. Considering the interval exposure of 1952 to 1974, Infante and White[33] concluded that 42 to 185 ppm years is associated with a 3.75-fold relative risk of leukemia. These investigators and the International Agency for Research in Cancer[7] adopted the one-hit linear model, which assumes no threshold and that every increment in dose of benzene is accompanied by an increment in the excess cancer risk. Inherent in the one-hit hypothesis is the assumption that there is a single, unique chemical event that is irreversible or that if this event takes place on DNA, there is no repair of the injury. Covalent binding of benzene and its metabolites to DNA in vitro has been demonstrated by Rushmore et al.[59] Other adducts are repaired by known biochemical mechanisms and there is no a priori reason why the adducts formed by benzene metabolites cannot, in principle at least, be partly repaired. Chandler[7] has addressed in more detail the undesirable nature of the one-hit model. The problem of chemical carcinogenesis, relationship to exposure dose, and concentration of DNA adducts has been carefully analyzed by Hoel et al.[31] They point out that the relation between the applied dose and incidence of cancer is frequently nonlinear, but the tumor response is in some instances linear with the resulting concentration of DNA adducts in the target organ. The most likely explanation lies in the fact that many chemicals are noncarcinogenic in themselves, but are converted to carcinogenic metabolites. The pharmacokinetics of conversion, degradation, covalent binding of active metabolites, excision of adducts, and DNA repair will probably result in nonlinear processes producing the ultimate DNA adducts presumed to be part of the initiation of the carcinogenic process. These authors quite convincingly demonstrate that mathematical models used for low-dose extrapolation may overestimate the risk by several orders of magnitude in the presence of nonlinear pharmacokinetics. Because benzene is converted to active metabolities by oxidative enzymes, the determination of the levels of benzene that saturate DNA repair, detoxification of active metabolites, and measurement of DNA adducts in DNA of appropriate tissues are really needed before one can adopt the appropriate mathematical model for relationship between exposure to benzene and incidence of leukemia or any cancer. Irvine[37] clearly pointed out the need for understanding the mechanism of benzene carcinogenesis and low-dose data points or the debate will not be resolved. The assertion of Infante et al[33,35] and White et al[72] that exposure to benzene at 1 ppm is leukemogenic should be questioned for the reasons discussed above.

Leukemia is not the only hemopoietic disease observed in benzene-exposed workers. Tsongas[67] has reviewed occupational factors in the epidemiology of chemically induced lymphoid and hemopoietic cancers. In her discussion of benzene, she pointed out that multiple myeloma, reticulum

cell sarcoma. lymphosarcoma, and othcr lymphoid ncoplasnis havc bccn obscrved in higher than expected rates. One can conclude from the accumulated human experience that workcrs exposed to bcnzene have a higher incidencc of hematopoietic neoplasms. In the opinion of the author. one cannot construct a dose-erect curve from human exposures. Furthermore, one cannot exclude the possibility of a threshold of benzene exposure that is not carcinogenic. For the reasons discussed earlier. it is highly unlikely that the "one hit" modcl is appropriate for estimating the risk of cxposurc to bcnzcnc.

## BENZENE METABOLISM

Busch et al[9] havc extensively rcvicwcd thi: metabolism of bcnzcnc. Bcnzcne. except at very high concentrations. has no known biological effects. At concentrations of many thousand ppm, it causes cardiotoxic and neurologic effects. With the present exposure limits, thcsc are of no consequence.

About 40% of benzenc is exhaled unchanged in the expired air. Thus 60% is converted by a series of enzymes. The first step is conversion to benzene cpoxidc by arylhydrocarbon hydroxylase (AHH). Spontaneously, about 23% to 50% is convcrtcd to phcnol. This in turn is convcrtcd to phenyl glucuronide and excreted in the urine along with othcr Icsscr conjugates. A small fraction of phcnol is convcrtcd to hydroquinol. Another small fraction is convcrtcd to bcnzcne glycol by epoxidc hydrase that in turn is convcrtcd to catcchol by dchydrogcnasc. The major candidates for the active mctabolitcs arc benzcnc epoxidc. hydroquinol. and catcchol. Because major effects of bcnzcne arc upon the hematopoictic progcnitors. the question has bccn asked whether bone marrow cclls havc the enzymes for converting bcnzcnc to the active nictabolitcs. probably catcchol and hydroxyquinone. This has bcen answered in the affirmative by Andrews et al[5] and Irons et al.[36] Microsomal cytochrome P-450 produced phcnol. catcchol. hydroquinone. and P-benzoquinone. Rushmorc et al[59] have rcportcd that rabbit bonc marrow mitochondria. stripped of thcir outer mcmbranc. have bccn shown to carry out the NADPH-dependent bioaciivation of radiolabeled benzene in vitro to metabolites that covalently bind to mitochondrial DNA. thus inhibiting transcription. Thus it is probable that these mctabolitcs also can covalently bind to chromosomal DNA. Kalf et al[38] extcndcd the earlier studics of Rushmorc et al[59] and identified several deoxyguanosinc-adducts suggesting that P-benzoquinone, hydroquinone. phenol. and 1,2,4-benzenetriol form adducts with guanine.

Heidelberger[39] and Miller and Miller[48,49] discuss in detail the enzymatic activation of inactive procarcinogens to chemically reactive electrophilic species. This activation is generally carried out by the nonspecific drug-metabolizing microsomal enzymes using cytochrome P-450. Epoxides are the activated metabolites of several polycyclic hydrocarbons.

Lawley[42] has studied alkylation of DNA by several known carcinogens. Alkylation at 0 to 6 guanine is correlated with the induction of thymomata in C57B1/6 mice. Alkylation of DNA is most likely randoni and the probability. thcrcfore. for mutation would depend on the extent of alkylation at various sites. Lawley believes that carcinogenesis involves specific modification of DNA of target organs and the changes associatcd with tumorigenesis are those associated with mutagenesis through miscoding rather than deletion of genetic material. Although one can correlate incidence of tumors with degree of adduct formation. othcr factors not clearly dcfincd arc required for a tumor to be expressed.

It would appear that an intensive study for presence of DNA adducts in hcniopoictic tissues of mice is in order.

## EFFECTS OF BENZENE UPON HEMOPOIESIS

Early studies conccntratcd on the effect of bcnzcnc on the peripheral blood and histology of hcmopoictic tissues. For example, in 1916 Selling[60] and Weisskotten et al[71] observed lymphopenia, marrow hyperplasia, and severe anemia after feeding or injecting benzene. There is an extcnsivc literature on the effects of benzene on the pcriphcral blood of several species after different routes of administration and amounts of bcnzcnc. These studics are well summarized by Leong[44] and Goldstein.[25] Lymphopenia and its duration is a direct function. of the amount of bcnicnc administered. as is anemia and thrombopenia. Granulocytes are relatively resistant to benzene. showing a diminution only after long

cxposurcs to large amounts of bcnzcnc. The degree of hypoplasia and its duration arc also dose dependent. The prcccding arc effects occurring late in the hemopoietic pathway and may not be obscrved when subtle damage is induced in stcm cells.

Bone marrow cell cycle kinetics of short-term benzene exposure have been studied by Irons et al[36] after injection of benzene. Flow cytometry showed a relative incrcasc in cclls in $G_1$ or M phase of cell cycle. In addition. thcrc was an early increase in uptake of $^3$HTdR into DNA. This was followed by a decrease suggesting a defect in maturation of precursor cclls. Snydcr ct al[62] gave lifctimc exposure of 100 and 300 ppm bcnzcne in air six hours a day, five days a week to AKR and C57B1/6 mice. AKR mice developed anemia and lymphocytopenia. Marrow hypoplasia occurred in 20% of the benzene-exposed compared to 2% in the control mice. Thirty-three percent of C57B1/6 mice dcvclopcd granulocytic marrow hyperplasia coniparcd to none in the controls. The incidence of neoplasia will be covered later in this review. Snyder et al[63] also exposed rats to 100 ppm benzene in air for a lifctimc. Anemia and lymphopenia were significant compared to the control sham-exposed rats. In addition, benzene-exposed rats showed marked accumulation of splcnic hemosiderin compared to sham-exposed rats. suggesting hemolysis.

The prcccding studies clearly indicate that benzene metabolites arc damaging to bonc marrow but do not provide insight into cellular sites of action in bonc marrow.

Phenotypic changes in hematopoietic cclls. such as biochemical and morphologic abnormalitics. irrcspcctivc of cause. result from genotypic alterations in the pluripotent stem cell that perpetuates hematopoiesis or in early progcnitors that havc little or no self-renewal capability. In the latter casc. the phenotypic changes will bc fleeting and disappear as the precursor cell and its progeny dividc. niaturc. and arc removed. In studies on hematotoxicity of pollutants. one concentrates on the pluripotent hcniopoictic stem cell. its rate of proliferation and differentiation into identifiable cell lines. and the mitotic potential of the immediate descendants of tlic pluripotent stem cell.

It is well known that the pluripotent stem ccll can only be assayed in the mouse by counting the nunibcr of gross spleen colonies formed in the fatally irradiated mouse following injection of a known nunibcr of bonc marrow cclls. This technique was dcvclopcd by Till and McCulloch.[66] The cell colony-forming unit in the spleen is called the CFU-S. The colonies are clones arising from a single ccll. After several self-renewing mitoses. the colonies commence to differentiate among the red. white, megakaryocytic. or mixed cell lines. After seven days, the colonics are easily scen by the eye on the surface of the splccn. It has bcen shown that although the total number of colonics sccn in the spleen remains constant. colonics arc disappearing apparently by differentiation after metastasis into the blood at the same rate as colonies are reappearing. These observations. made originally by Magli et al[45] and later confirmsd by Priestly and Wolf,[11] in an clcgant tcchniquc of transparent chambers in which the splccn is visualized, introduced new notions into the interpretation of splccn colonies.

The hematologic phenotypic alterations induccd by bcnrcnc have bcen reviewed by Laskin and Goldstein[41] and Snydcr and Kocsis.[61] They indicate injury to the sclf-renewing CFU-S that is transmitted to the identifiable progeny. The injury may be manifested by a permanent change in genotype. in which case the phenotypic expression will be permanent. Disappearance of the phenotypic expression is indicative of cithcr repair of the genotypic injury by proccsscs yet to be identified or dying out of the affected clone of stem cclls.

Uyeki et al[68] exposed mice eight hours a day to 4,680 ppni benzene for one-, three-, and eight-hour sessions and then measured the cellularity. CFU-S. and colony-forming units in culture (GM-CFU-C) of bonc marrow. GM-CFU-Cs are the progenitor cclls for granulocytes and macrophages. They arc detectable by their ability to form macroscopic colonics of granulocytes and/or macrophages in agar culture.[7] One eight-hour exposure rcducrd the GM-CFU-C content of marrow to 37% of the control value the day after the exposure without any change in cellularity. The GM-CFU-C progressively increased on days 3, 4, and 7. but was still 23% below controls. Three eight-hour exposures resulted in further decreases in GM-CFU-C content of tlic marrow. Three eight-hour expo-

sures dropped the CFU-S content of marrow by nearly a factor of three, measured 24 hours after the last session. The concentration of benzene was much greater than in other studies and observations were made for only a few days. Gill et al[23] exposed mice to 4,000 ppm benzene six hours a day, five days a week for 6 weeks, reducing the ratio of CFU-S to marrow cells by a factor of four. The development of eight-day splenic colonies after rescue marrow transfusions of the fatally irradiated mouse was inhibited by continuous exposure to 100 ppm benzene in air, clearly demonstrating that benzene metabolites inhibit stem cell self renewal. Green et al[26] studied the effect of graded exposure to benzene in air upon stem cells by the spleen colony technique and upon early granulocytic-macrophage precursors by in vitro culture. Their exposure regimen used concentrations of 1.1; 9.9; 103; 306; 603; 1,276; 2,416; or 4,862 ppm six hours per day for five days. In another study, mice were given 9.6 ppm for 50 days. In a third study, mice were exposed to 302 ppm for 26 weeks. Five days' exposure to 103 ppm or more induced decreases in marrow and splenic cellularity, in addition to decreases in the marrow and splenic CFU-S and GM-CFU-C. Whereas exposure to 103 ppm for five days reduced CFU-S and GM-CFU-C, exposure to 9.6 ppm for 50 days (nearly the same integral dose) had no effect.

The previous studies on stem cells and hemopoietic precursors were of relatively short duration. The ability to recover and the fraction in DNA synthesis were not examined. Cronkite et al[13,15,16] performed studies with two-, four-, eight-, and 16-week exposures and post exposure up to 15 weeks. Benzene concentrations were 10, 25, 100, 300, and 400 ppm for 6 hours per day, five days per week. Exposure to 100 ppm or higher for 2 weeks (ten exposures) reduced marrow cellularity and the number of CFU-S in the bone marrow. The fraction of CFU-S in DNA synthesis was significantly increased; thus a smaller number of CFU-Ss were producing nearly a normal number of blood cells. The reduction in CFU-S was observed as early as two days after commencing exposure to 400 ppm and was maximal at five days, at which time a significant increase in the fraction of CFU-S in DNA synthesis was seen. A lymphopenia was observed after ten exposures to 25 ppm benzene, a concentration only 2.5 times the permissible exposure level. When mice were exposed to 300 ppm for 2 or 4 weeks, the CFU-S had returned to normal levels by 2 weeks after exposure. When exposed for 8 weeks, there was a slow linear increase in the number of CFU-S in the marrow reaching the level of the sham-exposed 16 weeks after termination of exposure. When exposed for 16 weeks, it took 25 weeks for the CFU-S to attain the level of the sham-exposed mice. The peripheral lymphocyte count returned to the level of sham-exposed by 8 weeks after termination of exposure, irrespective of the duration of exposure. Clearly, stem cells are seriously perturbed for a long interval. Research is needed on the gene rearrangement and protooncogene activation. Does exposure to benzene produce the same genetic rearrangement and activation in all mice? If so, why don't all mice become leukemic? This will be considered later under Leukemogenesis.

Recent studies by Baarson et al[6] show a very significant decrease in the number of late erythrocytic precursors (CFU-Es) that can be cultured in mice after exposure of C57B1/6 to 10 ppm benzene in air six hours a day, five days a week for 178 days. The CFU-Es were 5% of the CFU-E in the sham-exposed mice. The burst-forming units (BFU-Es) fell to 55% of the level in the sham-exposed mice by 66 days, but were normal at 178 days. Lymphocytes were about 70% of normal at 178 days. The anemia was very modest, only about 8.2 x $10^6$ $\mu$L compared to 9.3 x $10^6$ $\mu$L or 89% of the sham exposed. If the CFU-E is an obligatory pathway to the circulating red cell and amplification is unchanged, one would expect a severe anemia of less than 1.0 x $10^6$ red blood cells (RBCs)/$\mu$L. One must search for an explanation for this discrepancy. The few CFU-Es may have a greater amplification through additional mitoses. If so in vivo, this might have been expressed in vitro by very large colonies that were not described. Irrespective of the explanation of the discrepancy between decreased CFU-E and peripheral red count, one cannot ignore the fact that exposure to 10 ppm, the present TWA, has a significant effect upon hemopoiesis. Baarson et al (unpublished research) have shown that consumption of 5% ethanol in drinking water enhances the effect of 10 ppm in inspired air.

Other approaches to the study of hematotoxicity in iiiicc have been rcportcd. Harigaya et al[28] have comparcd long-term culturc of bone marrow from iiiicc csposcd to bcnzcnc compared to cultures of bonc marrow from sham-expose3 mice. C57B1/6 female mice were exposed to 400 ppm bcnzcnc in air for six hours a day. five days a week for nine or 11 days. We observed that cultures derived from bone marrow of bcnzcnc-exposed micc showed a progressive reduction in the nuiiibcr of CFU-S compared to cultures dcrivcd froni bone marrow of sham-exposed mice. Different combinations of adherent cclls and rcinosulatcd cclls showcd that cultures containing marrow cells from benzene-exposed mice had a lower capacity to maintain stcm cell proliferation than normal combinations. irrcspcctive of whether benzene-exposed marrow was in the adherent or reinoculated cclls. Thcse data show that benzene inhalation produces stem cell injury leading to diminished self replication, and injury to cells producing adherent population. leading to poor support of normal stem cell self renewal.

Garnett et al[22] studicd the effect of bcnzenc exposure upon long-term bonc marrow cultures with and without addition of hydrocortisone. Adherent cell layers from mice exposed to 100 or 400 ppni for nine days were devoid of fat cclls regardless of whcthcr hydrocortisone was in the culture. These data indicate that bcnzcnc influences the behavior of marrow stromal cclls in long-term bonc marrow culturc.

Bcnzcnc also affects B and T lymphocyte populations. Rozcn et al[57] csposcd niicc for six hours a day for six days to 300. 100, 30. and 10 ppni bcnzcnc in air. Exposurc to 10 ppni rcduccd the capacity of B cclls to produce colonies when stimulated by lipopolysaccharidc. Phytohemagglutinin (PHA)-induced blastogenesis was dcprcsscd by 31 ppm benzcnc in air. Thus. another study has show an cffcct when mice wcrc exposed to 10 ppm benzene in air, the current permissible TWA. Pfeifer and Irons"" studicd the effect of bcnzcnc mctabolitcs upon PHA stimulation of rat bonc marrow. Hydroquinone inhibitcd mitogenesis. The prcscncc of micromolar amounts of metabolite (p-benzoquinone and 1,2,4-benzenetriol) partially inhibited PHA stimulation. In earlier studies. Greenlee et al[27] and Rickert et al[55] showed that benzene toxicity of hemopoietic tissues is associated with the concentration of hydroquinone and catechol. but not phenol. Whereas these studies suggest that the above bcnzcnc metabolites may be the active agents, the studics do not eliminate bcnzcnc epoxide, a short-lived precursor of the above metabolites, as being an important reactive metabolite.

Cytogenetic effects of bcnzcnc have been studied. Tice et al[58] exposed mice to 3,100 ppm bcnzcnc in air for four hours and induced a significant increasc in sister chromatid exchanges in bonc marrow and inhibited cell proliferation. Pretreatment with phenobarbitol, an inducer of P-450 cytochrome oxidases, increased the effect on sistcr chromatid exchanges and ccll proliferation and induced chromatid-type chromosomal aberration. Gad-El-Karim et al[20] found that benzcnc induces an increasc in micronuclei of polychromatic normoblasts. The cffcct was increased by inducers of P-448 monoxydase but not by phenobarbitol, an inducer of P-450.

## LEUKEMOGENESIS

Cronkite[12] in 1961 bcforc discovery of protooncogenes believed that any agent that produced marrow aplasia would be leukemogenic. With morc sophisticated knowledge on carcinogenesis, thcrc is a growing body of cvidcncc that indicates leukemogenesis is a multistep, complex process. There are several types of leukemia that in turn suggest that there are different molecular mechanisms involved or that the same molecular mechanisms are induced in different cell lines or at different stages of differentiation in a given cell line. There is extensive evidence for the role of key gcncs in carcinogenesis by Bishop,[7] Heldin and Westermark,[30] Cooper and Lane,[11] Klein and Klein." and Land et al.[40] The notion of carcinogenesis being a multistep process is not new. Burch[8] dcduccd this on the basis of a mathematical analysis of radiation-induced cancer.

Whereas thcrc is overwhelming evidence implicating the activation of protooncogenes to active oncogenes with production of the oncogenic proteins that in some instances are homologous with growth factors, tlic multiplicity of the genes, their products (protein kinases), receptors. and interactions, present a very complicated picture that is not as yet unraveled. Leukemogenesis is clearly a multistep process involving.

amcng othcr factors, a random production of DNA adducts. The heterogenity of abnormalities is well-reviewed by Francis.'" She presents a hypothetical mechanism for induction of myeloid leukemia in man involving activation of the tyrosine kinase group of oncogenes. impaired differentiation. and imparts a role to **DNA** topoisomerase, the DNA untwisting enzyme. Inhibition of **DNA** topoisomerase freezes cclls in the ccll cycle. but **docs** not kill them and appears to be involvcd in induction of sister chromatid exchanges. This enzyme is involved in DNA rcpair and dcfcctivc function **may** result in defectivc repair. Whcrcas one cannot **yet** piccc together all of the sequential steps rcquircd **to** initiate and produce **a** Icukcmia. it is clear that animal models for induction of leukemia to be described next arc available for intensive **study** of molecular changes in **DNA** during leukemogenesis. Farbcr'' dcscribcs the cellular scquencc of events in carcinogenesis that may be helpful in designing experiments on the murine models **to** be described.

Exposurc of mice to inhaled bcnzcne has contributed substantially to our practical knowledge **on** exposure conditions **that are** leukemogenic in different **strains** of micc. Stoner et al[64] exposcd hairless micc to bcnzcne throughout thcir whole life. The incidcncc of Icukcmia **was** not increased. **In** fact, **an** interpretation of their data **suggests that** exposure **to** bcnzcnc by inhalation may havc supprcsscd the dcvclopnicnt of Icukcmia. Snydcr ct al[62] exposcd C57B1/6 and AKR niicc to 300 ppni 67 hours a day. **five** days a **wcck** for **a** lifctimc. Thcrc **was** a significant incrcasc in the incidcncc of leukemia/lymphoma. In studies **on** rats exposed to 100 ppni for **a** lifctimc. **a** single case of chronic myelogeneous Icukcmia, **a** rarity in the rat. was obscrvcd by Goldstein et **al.**[24] Furthcr studies by Snydcr et **al**[63] showed scvcrc hematotoxicity of inhaling 100 ppni bcnzcnc in air. but no significant increase in hcmopoictic neoplasms. Again, rare neoplasms of the rat wcrc sccn. suggesting a neoplastic effect.

Maltoni et **al.**[46] summarizing many years of investigation. havc produced strong cvidcncc **that** bcnzcnc by injection and ingestion produces **an** excess number of different tumors in the rat. They emphasize the nccd for dose-effect studics to establish the relationship between **csposurc** and neoplastic incidcncc.

Cronkitc et **al**[13-15,15] approached the problem of **benzene** leukemogenesis using C57B1/6 male **mice known to** harbor the radiation leukemia virus **that is** activated by exposure to ionizing radiation **and the CBA/Ca** male **mousc.** which **has** a very low spontaneous incidcncc of acute myeloblastic leukemia (AML) and **has** been shown by Major and Mole[50] to **develop** a high incidencc of AML after csposiire **to** ionizing radiation. The exposure regimen **was** based **on** the apparent exposure conditions of **human** beings described by Aksoy et **al**[1-4] and Vigiiani and Saita.[70] Human beings do not commence work in industry until 18 years or older. **Thus.** exposure of micc **was** comnicnccd at 12 io 14 weeks of age, when they are sexually mature. It was assumed that human beings were cxposcd for about **15%** of thcir lives and, accordingly, micc were exposed for 16 **weeks,** approximately 15% of thcir lifespan. The work week **was** mimickcd **by** exposing mice six hours a **day. tivc** days **a** week. When **C57B1/6** female mice were cxposed to 300 ppm bcnzene and hold for lifetime study. a highly significant earlier onset of thymic lymphomata. nonthymic lymphoma. **and** leukemia was observed. Male **C57B1/6** had a **more** rapid **onset** of hemopoietic ncoplasms. In both sexes, the ultimate lifetime incidence was nearly the same (unpublished data). **When** CBA/Ca male micc wcrc cxposcd lo 300 ppm for 16 wccks. a striking difference between the benzene-exposed and the sham-cxposed micc was sccn. **A M** L commenced appearance at about 210 days of age or 2 weeks after termination of exposure. At 600 days of age, the incidence of **AML was** 28% and increasing with no AML in the sham-exposed micc. When **female** CBA/Ca mice wcre cxposed to 300 ppm or 100 ppm for 16 wccks. a highly significant incrcasc in ncoplasnis **was** observed, **particularly** mammary adenocarcinoma. Thcsc studics **are** not **as** yet complete. The **shape** of **the** incidence curves suggests that the ultimate incidcncc of acute leukemia **may** approach 50% coniparcd to less **than** 5% in the sham-exposed niicc. The time scquencc for appearance of **acute** Icukcmia is established. It appears **that** the **CBA/Ca** male mouse **is an** appropriate animal in which one could dctcrmine **the** abundance **of** DNA adducts in hcmopoictic tissues with time nnd identify the **activation** of protooncogenes to provide **better** insight into the

mechanism of benzene leukemogenesis. In parallel. otic could determine if the iticidcncc versus bcnzcnc cxposurc has a similar dose-effect relationship to DNA adducts. If both have the same mathematical relationship. one would have a reasonable experimental basis for intcrprctation of low-dose cxposurc of man.

## CONCLUSIONS

Our conclusions arc as follows: (1) Bcnzcne is leukemogenic in man and mice. (2) tlic dosc-effect relationships have not bcen cstablishcd in man or mousc. (3) thcrc arc adequate rcnsons to question the "one hit" model for prediction of low-dose effects of inhalation of bcnzcnc. (4) bcnzcnc mctabolitcs producc many toxic clfccts upon hcmopoicsis that arc dose dcpendcnt. (5) bcnzcne inhalation of 10 ppm has bcen shown to producc effects on early erythrocytic prccursors. The magnitude of the effect is not compatible with the slight anemia, and (6) the presence of hemopoietic clfccts after cxposurc to 10 ppm bcnzcnc in air rcquircs further investigation and consideration of whether the present TWA of 10 ppm is justified in the workplace.

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