BENZENE TOXICITY:

REVIEW OF RECENT LITERATURE

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by

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INTRODUCTION

This review of the literature concerning the toxicity of benzene focuses almost entirely on work published from 1978 to the present. It is intended as a followup of the information in "Benzene Toxicity: A Critical Evaluation" edited by Laskin and Goldstein (1977). That was a multi-authored volume containing over 1,000 references which described in depth the known benzene literature to that point. This present single-authored review will not attempt to be as comprehensive in its coverage. Rather, the focus will be on reviewing and interpreting the recent literature particularly pertinent to scientific questions underlying the regulation of benzene exposure.

The following brief overview of the literature until 1977 and of the hematologic basis of benzene toxicity is intended as background for the present review. For those interested, a more detailed description is provided in Laskin and Goldstein (1977) and other scholarly reviews (Haley, 1977; White et al., 1980; Goldstein and Snyder 1982; Snyder et al., 1982).

Benzene has been known to produce hematological toxicity in man since the nineteenth century. The earliest reports of benzene toxicity focussed on its ability to profoundly decrease all formed elements in the blood (red blood cells, white blood cells, and platelets). This disease, known as aplastic anemia, is characterized by loss of bone marrow cells which are the precursors of mature circulating blood cells. Investigation of workplaces with significant benzene exposure revealed a wide spectrum of response ranging from severe aplastic anemia to mild decreases in red blood cells (anemia), white blood cells (leukopenia), and platelets (thrombocytopenia); the milder form of this disorder often being known as pancytopenia. An association of benzene exposure with acute myelogenous leukemia was first noted over five decades ago. The evidence confirming a causal relationship between benzene exposure and this disease comes from a variety of different ap-

diverse disorders as acute and chronic lymphatic leukemia, chronic myelogenous leukemia, multiple myeloma and various types of lymphoma including Hodgkin's Disease, may also be related to benzene exposure. Reports of such disorders linked to benzene exposure are tabulated by Goldstein (1977).

Information available in 1977 about the mechanism of benzene toxicity pointed strongly to the action of a metabolite of benzene rather than benzene itself in producing bone marrow toxicity. Other studies in both animals and man suggested that host factors, such as age, sex, obesity, and genetic factors, might modulate the extent of benzene toxicity in terms of its aplastic effects. Although dose-responsive pancytopenia was readily produced evidence of leukemia in laboratory animals exposed to benzene was not observed. Also of note was the consistent failure to observe mutagenesis due to benzene in bacterial systems such as the Ames test despite ample evidence that chromosomal damage was produced in animals and man.

STUDIES IN MAN

Among the more important studies published in the past five years has been that of Rinsky et al (1981) which extended and elaborated on the prior study by Infante et al (1977). These investigators from NIOSH originally reported seven deaths due to leukemia in a cohort of 748 white male rubber workers employed at any time during the period of 1940-1949 who died between January 1, 1950 and June 30, 1975 (Infante et al, 1977). Of the seven cases, 4 were reported as acute myelogenous leukemia, 2 as monocytic leukemia, and 1 as chronic myelogenous leukemia. The expected number of deaths in the cohort was 1.38 based on the general population of US white males and 1.38 based upon a control group of white males employed in the same state and at the same time in manufacture of a fibrous glass product. These findings, and the assertion that the workers had been exposed to benzene levels within allowable workplace standards, led to an attempt by OSHA to promulgate a more rigorous standard of 1 ppm TWA as compared to the existing 10 ppm TWA. Although the standard remains at 10 ppm

recently to exceedingly high levels of benzene comes from industrial hygiene surveys conducted by the University of North Carolina in 1973-74 in which levels of 193-355 ppm benzene were noted inside a dryer and it was reported that respirators were often not worn. It is further stated that men could stay up to 30 minutes at this location but it is not clear whether that occurred without use of a respirator. Similarly, during a 1972 shutdown operation, it was noted by the safety manager that during a short-term check an employee was not wearing respiratory protection in an area in which benzene levels were estimated to be over 200 ppm.

Using this monitoring data, Rinsky et al (1981) have attempted to estimate the benzene exposure levels for the individuals who developed leukemia. They present short case reports including the type of leukemia, interval between exposure and onset of disease, and time period of employment. For the 7 individuals originally reported by Infante et al (1977), TWA exposure levels while at work are estimated to be 35 ppm (one worker), 40 ppm (three workers), "less than 100 ppm" (one worker), unable to estimate due to exposure occurring between 1940-42 (one worker), and, in a worker employed for a very short period of time, a possible single exposure episode to 1,000 ppm benzene.

The study of Rinsky et al.(1981) has been interpreted as indicating that benzene levels at or near the present standard are leukemogenic (Rosenstock, 1982; White et al., 1981). This of course is a possible interpretation of the relation of benzene to acute myelogenous leukemia in man. However, in my judgment the Rinsky et al. data, and the original findings of Infante et al., do not provide significant direct support for leukemogenesis occurring at levels of benzene at or near the present OSHA standard. This interpretation is due both to the numerically low incidence of leukemia and to the marked heterogeneity of benzene exposure in the work force studied. Even when benzene exposure leads to an increase in leukemia incidence sufficient to be statistically appreciable, as in the Infante et al study, the total incidence of leukemia has fortunately always been a very small fraction of

1978).

Aksoy has continued to review and summarize his experience with shoe workers and others occupationally exposed to benzene in Turkey (Askoy 1980 a,b). During the 1960s shoe workers in Istanbul replaced gasoline with benzene as their solvent of choice for working with adhesives. Cases of aplastic anemia followed by cases of acute leukemia were observed in this work population. Exposure levels were well over 100 ppm benzene. The recent review articles contain further substantiation of a decrease in the incidence of leukemia among shoe workers in recent years which could be attributed to the gradual prohibition and discontinuation of the use of benzene in Istanbul beginning 1969. The substitutes now in place contain little or no benzene (Aksoy and Erdem, 1978). After reaching a high of seven cases in the year 1973, leukemia incidence in shoe workers in 1974 was four cases, in 1975 three cases, and in 1976-78 no cases. Aksoy does report four cases of leukemia in benzene exposed individuals in 1979 however only one was a shoe worker. Also of interest is that 11 of the 44 individuals with leukemia had a documented period of pancytopenia preceding the onset of leukemia. No evidence is presented which would allow one to conclusively exclude the possibility of a pancytopenic period preceding the onset of leukemia in the other 33 patients. In studying those patients who originally presented with pancytopenia, Aksoy noted complete remission in 23, death due to aplastic anemia or its complications in 14, and leukemia in six of a total of 44 patients. In a tabulation of 42 individuals with leukemia and benzene exposure Aksoy noted 16 with acute myelogenous leukemia, eight with erythroleukemia which can be considered a variant of acute myelogenous leukemia; one with acute promyelocytic leukemia, also a variant of acute myelogenous leukemia, and seven individuals with preleukemia which usually also evolves into acute myeloblastic leukemia. In addition he reports four individuals with acute lymphoblastic leukemia, three with acute monocytic leukemia, two with chronic myelogenous leukemia, and one with acute undifferentiated leukemia. No individuals with chronic lymphocytic leu-

forming their risk estimate, and as the mean and median age of his patients with myelogenous leukemia is well below that at which peak incidence occurs, this tends to underestimate the relative risk. Aksoy's observation that many of the individuals with benzene induced pancytopenia progress through preleukemia into acute myelogenous leukemia is in keeping with that reported by many authors in individual case reports following exposure to benzene around the world. These observations had led hematologists to accept the causal relationship of benzene to acute myelogenous leukemia decades ago. Finally, if more proof were needed, the virtual cessation of new cases of both pancytopenia and leukemia among these workers following a decrease in benzene usage fits a temporal pattern that is conclusive. There thus remains no question that an epidemic of aplastic anemia and acute myelogenous leukemia occurred due to exposure to high levels of benzene in the Turkish shoe industry.

Pagnotto et al. (1979) have presented an interesting follow-up on 38 workers previously exposed to benzene levels generally between 5-50 ppm until 1964. This study is notable for the relatively extensive industrial hygiene and hematological studies that were performed between 1960 to 1964 which resulted in reasonably detailed characterization of the workforce exposed to benzene. The observed ambient levels of benzene were confirmed by measurement of urinary phenol excretion. Mild hematological abnormalities were present in a number of the workers when studied in 1961 and 1963. Since cessation of benzene exposure in 1964, retesting performed in 1973 and 1977 revealed what appeared to be complete recovery. No hematological malignancies were observed during this 13 year follow-up period. Although this lack of leukemia in these well-studied workers is reassuring, the cohort is far too small to be meaningful.

The Occupational Health Studies Group of the University of North Carolina School of Public Health has conducted a number of studies of mortality of workers in the US rubber industry. They noted an excess of deaths attributed to neoplasms of

chosen to separate this from the acute myelogenous leukemia group. Also rather unusual is the finding that of the 37 cases of leukemia in Company A only one individual had chronic myelogenous leukemia. A more expected incidence for this age group would be the five of 27 cases of leukemia reported in Company B. The use of death certificates to sub-divide the type of hematological malignancy is clearly a perilous undertaking, particularly in the differentiation of leukemias. This probably accounts for the variable findings between companies, although the possibility that some highly unusual process was occurring in Company A cannot be excluded. Even if this were the case, it seems most unfortunate that the investigators undertook this major effort without attempting to confirm the hematological diagnosis of these individuals through careful review of the clinical findings. In view of these problems it is not surprising that no association was found between myelogenous leukemia and solvent use. The authors conclude that these findings support their previously reported association of lymphatic leukemia with work history of possible solvent exposure but that the association was weaker than had been noted. However, it is also stated that a recently detailed environmental evaluation reported as an abstract is said to support the initial findings and that further studies are therefore required to resolve the issue. Perhaps of note is that of the job categories evaluated lymphatic leukemia and monocytic leukemia were particularly observed in general service workers.

Monson and his colleagues at the Harvard School of Public Health have also continued their series of studies evaluating the health of rubber workers. Recent studies include an assessment of cancer morbidity and mortality among 13,570 white male rubber workers who had worked for at least five years at an Akron, Ohio ruber plant (Monson and Fine, 1978). Occurrence of cancer was measured by death certificates and by a survey of local hospital tumor registries. Comparison of mortality rates with those of US white males was performed as was analysis of cancer morbidity rates among different subgroups of workers. Excesses in a number of dif-

SMR reported is that for central nervous system neoplasms with an SMR of 69. This is said to be the only statistically significant effect. Among male tire workers SMRs for leukemia were 129 and stomach cancer 145. Kilpikari (1982) has also published a study evaluating mortality among male rubber workers in Finland showing a decrease in risk of cardiovascular disease and an apparent increase in cancer incidence. However, the cohort size is too small for further evaluation.

Another paper compiling a series of studies is that by Burrows (1980) commenting on health care of workers in research laboratories. In three studies in different countries, that of the American Chemical Society membership, male graduates of the School of Chemical Engineering, Stockholm, and graduates and fellows of the Royal Institute of Chemistry in Great Britain, there is an increase in the number of neoplasms of the hematological system.

Two studies from Sweden suggest an association between lymphoma and solvent exposure. Hardell et al. (1980) reported on a case control study of 169 cases of all types of lymphoma and for each case there are two controls. A statistically significant relative risk of 2.8 was observed for those with a history of substantial exposure to solvents, while those with a lesser exposure had a relative risk of 1.2. Increased relative risks were also observed in individuals exposed to phenoxy acids and to chlorophenols and there seems to be an enhanced risk with combined exposure to the various chemicals. It should be noted that only one individual gave a history of substantial exposure to benzene. In another study, Olsson and Brandt (1981) used a standardized interview in 61 consecutive male patients with a diagnosis of non-Hodgkin's lymphoma to evaluate a possible exposure to organic solvents. They report that in those who had a history of organic solvent exposure there was a much higher incidence of supradiaphragmatic presentation of disease as compared to those without such history of exposure. Again in this study an overt history of benzene exposure was found in only one patient. Benzene was apparently banned for occupational use in Sweden in 1972. However, the extent to which other solvents con-

risk 0.70 and 0.94 respectively). A relative risk of 3.34 was observed for exposure to benzene, but due to the small numbers this was not statistically significant. Of interest is that 3 of 4 patients with a history of benzene exposure were diagnosed as having chronic lymphocytic leukemia.

Timonen and Ilvonen (1978) suggested that contact with hospitals is an etiologic factor in leukemia (defined as acute leukemia and chronic myeloid leukemia). This was based on a study of 45 adults and a control group of patients from the same hospital in Finland. No significant difference in the use of chemicals between the two groups was observed, although there was apparently no history of the use of benzene by any of the study population.

The study of Vianna and Polan led Smith and Lickiss (1980) to evaluate the extent to which Hodgkin's Disease and non-Hodgkin's lymphoma is associated with benzene exposure in Tasmania. Their study includes all new cases diagnosed between 1972 and 1979. Using the same criteria for occupational benzene exposure used by Vianna and Polan, they were unable to confirm an association with lymphoma. Of note, however, is that a statistically significant increase in deaths due to acute myelogenous leukemia was observed suggesting that benzene exposure had in fact occurred.

Cytogenetic abnormalities have been demonstrated to result from hematotoxic levels of benzene in previous studies in animals and man (see below). Another recent, and somewhat controversial, study reporting cytogenetic abnormalities in workers exposed to relatively low levels of benzene is that of Picciano (1979). The author reported an increase in chromosome aberration rates in the peripheral lymphocytes of 52 workers in a chemical company as opposed to a 44 person control group observed for pre-employment examination. The age of the exposed group was significantly greater than that of the control group but was said by the author not to affect the results. The workers were reported to have been exposed to less than 10

cohort studies of entire industries. The finding of fewer than expected leukemia deaths in the entire cohort of this industry in which benzene exposure is known to occur apparently conceals a true benzene effect which can only be observed with more careful evaluation of the workforce assessing the degree and duration of exposure. It should be noted that in this study, as in many others, the observed leukemia incidence, although less than that expected based upon a control group derived from the general population, is actually more than that observed for all neoplasms or all causes of death. This "healthy worker effect" is well known and is almost uniformly demonstrated in studies of this type. Such results argue in favor of the use of proportional mortality ratios as opposed to standard mortality ratios.

Another approach to evaluate mortality among workers at petroleum refineries and petrochemical plants was taken by Thomas et al (1980). They studied the mortality experience of 3,105 members of the Oil, Chemical and Atomic Workers International Union (OCAW) reported by locals in Texas to have died between 1947-77 and for whom death certificates were available. The proportional mortality ratio was calculated based on expected mortality for the general population adjusted for age, race, and cause of death. The workers were divided into four categories depending upon the type of work performed. A statistically significant increase in the PMR for all cancers in white males (1.17) was observed. This was particularly notable for skin (1.76), brain and CNS (1.46) and respiratory system (1.25); all of which were statistically significant. 63 hematologic neoplasms were observed as compared to 54.4 expected resulting in a PMR of 1.16 which was not statistically significant. The subgroup with the highest PMR for hematologic neoplasms was that of "petroleum refining and production of petrochemicals" (44 observed, 36.3 expected; PMR 1.21). In this work category, there was a tendency to an increase in the PMR for leukemia with increasing length of membership in the Union. This was also observed when the expected tumor frequency was calculated based upon data for Texas males. However, in no case were the findings for hematologic neoplasms statistically significant.

below is that benzene may be capable of producing non- hematological neoplasms in man. Furthermore, the lack of myelogenous leukemia in Maltoni's studies could even be interpreted as indicating that benzene causes these other tumors at a higher incidence than it does leukemia. Obviously, one can not readily extrapolate relative cancer incidence from animals to man; there being many instances of species differences in organ selectivity of a carcinogen. The findings by Maltoni et al., if one accepts their validity, do however serve to raise the hypothesis that benzene will cause non-hematological neoplasms in man. This has also been suggested by Aksoy (1980a,b) who noted five cases of lung cancer associated with chronic benzene exposure (see below). Of note is that it is at least possible to begin to evaluate this hypothesis. There are numerous cohort studies of work groups who have been exposed to benzene and for whom cancer mortality has been evaluated. As discussed above, an increased incidence of hematological neoplasms has been observed for many of these groups. Inasmuch as the causal link between benzene exposure and at least certain hematological neoplasia is now beyond reasonable doubt, one can as a first approximation utilize an observed increase in hematological cancers in these work groups as a marker for significant benzene exposure. Therefore, in cohorts selected in this way it should be possible to evaluate the relative increase in risk of non-hematological cancers from this same benzene exposure. There are of course a number of problems with an approach of this type. These include the fact that all hematological neoplasia have not been proven to be associated with benzene; that exposure in none of the cohorts is to benzene alone but to a mixture of workplace chemicals and factors; and that for many of these cohorts the increases in hematological neoplasia are too small to reach statistical significance. Two other considerations which are inherent in an approach of this type must be kept in mind . An actual causative role for benzene in a specific non-hematologic cancer may not be observed in a cohort with benzene-induced hematological neoplasia simply because the relative risk for these hematological cancers is greater than that for the other neoplasm. Stated another way, one may have a cohort of a sufficient size to observe a

these caveats, it should be pointed out that a simple sign test or other non-parametric statistical approach which evaluated simply whether or not each finding was above or below the expected incidence, might well lead to a statistically significant association with hematologic cancers, depending upon how the studies were chosen.

To summarize, epidemiological studies since 1978 have provided further confirmatory evidence that occupational exposure to benzene results in hematological neoplasms, particularly acute myelogenous leukemia and its variants. There has been marginal improvement in the ability to make a dose response estimation of the levels of benzene responsible for adverse hematological effects, including leukemia. This is in part due to many additional thorough studies of benzene exposed cohorts, confirmatory evidence concerning the relation of benzene exposure to aplastic anemia and acute myelogenous leukemia in Turkish leather workers, and the follow-up information obtained by Rinsky et al. (1981). concerning exposure in a workforce of rubber workers previously shown to have a high incidence of leukemia. However, the human data do not appear to provide any evidence concerning the shape of the benzene-leukemia dose response relationship, and particularly, the relationship to leukemia of benzene levels at or below the current occupational standard.

STUDIES IN ANIMALS AND IN VITRO

There has been an explosive increase in the number of publications detailing laboratory investigation into the metabolism and toxicity of benzene. The following discussion is aimed at highlighting certain of the directions in the research. No attempt will be made to provide a detailed description of the literature or to reference every publication.

Perhaps the most notable finding in the past five years has been the development of animal models of benzene carcinogenesis. The previous inability to demonstrate that benzene produced tumors in animals had been intriguing; particularly

cidence of other pathological lesions, described as nodular hyperplasia and dysplasia, were reported in the benzene-exposed rats, with again a zero incidence in the control animals. Subsequently, an increased incidence of mammary tumors has also been observed in this experimental group (unpublished data). In the third experiment, 7 week old Sprague-Dawley rats were given 500 mg/kg benzene, toluene, xylene, or ethyl benzene by stomach tube once daily, 4-5 times weekly, for 104 weeks. Observations at 84 weeks again revealed an increased incidence of zymbal gland tumors in the benzene-exposed rats. No hepatomas had been observed although the mean latency period for this tumor based on their previous study had not yet been reached. The incidence of hematologic neoplasms also did not differ from the control group. Of note, however, was the observation of a statistically significant increase in tumors of the oral cavity. A few tumors of this type were observed in the first study, also performed by stomach tube. It is interesting to speculate that the different patterns of neoplasms observed in these studies might reflect variation in the site of action of benzene depending upon the mode of administration. Obviously, more information is needed concerning the results of these ongoing experiments. The presence of hepatic pathology and tumors is particularly intriguing and deserves further evaluation, including restudy of the pathological findings in the NYU Institute of Environmental Medicine studies which also exposed Sprague-Dawley rats to similar benzene concentrations. As discussed above, these findings should lead to a closer examination of the non-hematological cancer mortality experience of workers with known exposure to benzene.

Inhalation toxicology studies at the New York University Institute of Environmental Medicine by C. Snyder and his colleagues have provided clear-cut evidence of benzene induced lymphoma and the first suggestion of myelogenous leukemia in laboratory animals. The inhalation regimen used in this laboratory consists of exposures six hours a day, five days a week, for up to lifetime. In C57/BL mice exposed to 300 ppm benzene a higher incidence of an inducible thymic lymphoma was observed

duced myelogenous leukemia. Such a model would allow evaluation of whether pancytopenia is a necessary prerequisite for the development of acute myelogenous leukemia. Studies by this group exposing various mouse strains and Sprague-Dawley rats to 100 ppm or 300 ppm benzene up to lifetime are summarized by Goldstein and Snyder (1982). The hematological findings in these animals document strain differences, as well as the marked relative resistance of rats as compared to mice which is reflected in benzene pharmacokinetics (Snyder et al, 1981a). Lymphocytopenia is the earliest finding and the most sensitive indicator of benzene effects in these animals among standard hematologic tests (Snyder et al, 1978;1980;1982; Green et al, 1981a) A proliferative effect on myeloblasts and/or promyelocytes was observed in CD-1 mice, the strain which appears to be susceptible to benzene-induced leukemia (Snyder et al, 1981). Other studies by this group evaluating the interaction of benzene and ethanol ingestion, and assessment of bone marrow cells in culture are described below.

The mechanism by which benzene might cause leukemia or other cancers is still unknown. Benzene has not been found to be mutagenic in standard bacterial tests. Negative studies include that of Florin et al. (1980) who tested benzene concentrations of 0.03, 0.3, 3, and 30 micromoles/plate using Salmonella typhimurium strains TA98 and TA100 with and without an activated liver preparation from 3-methyl cholanthrene-induced rats. Despite these negative findings in vitro there is no question that benzene produces cytogenetic abnormalities in vivo in both animals and man. Recent observations include demonstration of a positive micronucleus test in animals administered benzene (Hita et al., 1980; Diaz et al.,1980; Tunek et al.,1982). However, one does wonder about the validity of the micronucleus test as a marker for genetic toxicity due to hematological poisons. Perhaps the most interesting of the short-term in vivo tests for genetic toxicology recently used with benzene is that of sister chromatid exchange (SCE). Tice et al (1980) demonstrated an increase in SCE in bone marrow cells of DBA/2 mice inhaling 3100 ppm of benzene for

ture techniques which allow the growth and identification of different stages of maturing hematopoietic precursor cells. Exposure to benzene has been demonstrated to inhibit growth of bone marrow cells in culture by a number of investigators using different techniques and different animal species. Findings include a decrease in bone marrow colony forming cells following exposure of BDF1 mice to 4680 ppm benzene for 8 hours (Uyeki et al, 1977); a decrease in stem cells measured as spleen colony forming cells in C57BL/6J female mice exposed to 400 ppm benzene 6 hours daily for 9-11 days (Harigaya et al, 1981); a decrease in stem cells observed during exposure of C57BL/6 male mice to 4,000 ppm benzene 6 hours daily, five days weekly, for up to six weeks (Gill et al, 1980), and the prevention of the development of spleen colonies in C57BL/6 mice transplanted with normal marrow cells prior to exposure to 100 ppm benzene for 8 days (Gill et al,1980). In this latter study, the authors report a dose-dependent decrease in white blood cell count in which there is an approximately 80% decrease in mice continuously exposed to 100 ppm benzene for only 4 days. Haak and Speck (1982) have recently reported that rabbits treated subcutaneously with 0.2 ml/kg benzene, 5-7 days weekly, for 2 months develop not only an inhibition of in vitro colony formation of their own bone marrow, but also of normal bone marrow cocultivated together with the marrow from the benzene treated rabbits. This interesting and surprising finding, which suggests that benzene is not only directly myelotoxic but also results in bone marrow cells with growth inhibitory potential, requires replication and extension.

Green et al (1981b), at the New York University Institute of Environmental Medicine, performed an extensive dose response study evaluating splenic colony forming cells and granulocyte/macrophage progenitor cells in CD-1 mice. Effects on splenic granulocyte/ macrophage progenitor cell levels and on femoral bone marrow and splenic colony forming units were observed following exposure to 103 ppm benzene or higher levels 6 hours daily for five days. In a second study, mice were exposed to 9.6 ppm benzene, 6 hours daily, five days weekly, for a total of 50 exposure days (e-

catechol, hydroquinone, and benzoquinone in rat tissue following inhalation of benzene. Free catechol and hydroquinone were found to persist in the bone marrow longer than benzene or phenol (Richert et al, 1979). Greenlee et al (1981) have suggested that the mechanism by which polyphenol metabolites produce hematotoxicity is through the autoxidation of hydroquinone and 1,2,4-benzenetriol resulting in the formation of semiquinone and quinone oxidation products as well as superoxide anion radical. Irons and Neptun (1980) have advanced the interesting hypothesis that the polyhydroxy metabolites or their autoxidation products interfere with microtubule function perhaps resulting in cytoskeletal abnormalities central to benzene toxicity. The findings in these and other studies provide relatively convincing evidence of a role for polyhydroxy metabolites in benzene toxicity. There is however still some question concerning why short and long-term studies of the toxicity of phenol or other non-benzene precursors of these polyphenol metabolites do not demonstrate pancytopenia. It is possible that the polyphenol metabolites participate in benzene toxicity, particularly the lymphotoxic effects, but do not account for the totality of the observed effects.

Other studies by Irons and his colleagues include an assessment of bone marrow cell cycle kinetics in rats given benzene by subcutaneous injection in which the findings suggest that benzene toxicity is relatively specific for the cell differentiation and cell cycle phase of bone marrow precursor cells (Irons et al, 1979). A series of immunotoxicological studies by this group have included evidence that the lymphocytopenic effect of benzene in rabbits primarily affected B lymphocytes, although a decrease in T lymphocytes was also observed at higher doses (Irons and Moore, 1980). The effects of benzene on T cell and B cell function have also been studied by Karaulov and Frasch (1978). Wierda et al (1981) found a variety of alterations in lymphocyte responses, including inhibition of B and T cell mitogenesis, in C57Bl/6 mice injected intraperitoneally with 44-660 mg/kg benzene for three days. Of interest is that pretreatment with Aroclor 1254 altered some but

be present in other benzene metabolizing systems (Andrews et al, 1979; Snyder et al, 1982). Of particular interest is the recent observation by Kalf et al (1982) that benzene inhibits RNA synthesis in mitochondria from liver and bone marrow. Gill and Ahmed (1981) noted a significant extent of binding of label derived from radioactive benzene to bone marrow mitochondria in a study which also observed that 17% of the label in the marrow was bound to nucleic acids. This evidence of a mitochondrial effect opens a new line of investigation into the biochemical basis of benzene toxicity.

Goldstein et al (1981) have suggested that a ring-opened form of benzene may be responsible in part for its hematotoxicity. Evaluation of trans,trans-muconaldehyde, which is formed in the reaction of hydroxyl radicals with benzene, has found this compound to be hematotoxic but there is as yet no direct evidence that it is formed from benzene in vivo.

Studies of interactive effects have included the observation that 5% or 15% ethanol in drinking water markedly potentiates the hematotoxicity of 300 ppm benzene inhaled by C57Bl/6 mice, six hours daily, five days weekly (Snyder et al, 1981; Baarson et al, 1982). Of interest is that some facets of the hematotoxicity of the two agents together differ from those observed with benzene alone despite the fact that alcohol by itself had no overt hematological effect. The interaction of toluene and benzene continues to be evaluated. Combined exposure usually leads to a decrease in benzene-induced hematotoxicity, apparently due to inhibition of benzene metabolism (Andrews et al, 1977). However, the importance of this finding to man has been questioned in a study by Sato and Nakajima (1979) who found no significant interaction between benzene and toluene when disappearance rates in blood and in expired air were measured. Exposure was to 25 ppm benzene and 100 ppm toluene for two hours in three subjects. Tice et al (1982) also observed that the inhibition of SCE by toluene only occurred at higher benzene levels. An additional study suggesting that lead, another common component of gasoline, might interact with ben-

models of dose-response effects in animals potentially extrapolatable to man. A few of the more important approaches include the use of bone marrow cell culture techniques to obtain information about potential target cells of benzene toxicity. This is a major improvement over approaches measuring peripheral blood counts and bone marrow cellularity as an endpoint of benzene toxicity. Based on the data thus far, it is conceivable that careful dose-response studies evaluating laboratory animals, particularly mice, exposed to benzene inhalation regimens approximating the current TLV will demonstrate an effect on bone marrow hematopoietic precursor cells. The implications of such findings would bear careful evaluation and would certainly be of concern to regulators and other decision makers.

The ascertainment of a number of candidate hematotoxic metabolites of benzene is also potentially of importance. Evidence is beginning to accumulate that in fact it may be more than one metabolite that is responsible for the effects of benzene. It should be emphasized that studies of the toxicity of benzene metabolites have thus far focussed predominately on endpoints related to aplasia rather than leukemia.

Evidence that benzene does in fact cause cancer in laboratory animals has begun to accumulate. A model of benzene induced leukemia would be of particular value in answering questions concerning the shape and nature of the dose-response curve. The suggestion that benzene may cause non-hematological neoplasia in animals, other than that of zymbal gland tumors, warrants a thorough reevaluation of the cancer mortality experience of human cohorts in which benzene exposure is identifiable through the presence of a high incidence of hematological neoplasia.

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