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REPEATED EXPOSURE OF C57Bl MICE TO INHALED BENZENE AT I0 ppm MARKEDLY DEPRESSED ERYTHROPOIETIC COLONY FORMATION

(Benzene; depressed erythropoiesis)

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NMMARY

Exposure of C57Bl mice to 10 ppm benzene (the current occupational exposure limit) for 6 h/day, 5 days/week causes a progressive depression in the in vitro colony forming ability of one of the erythroid progenitor cells, the colony-lomini unit-erythroid (CN-E). Colony growth of cells from exposed mice was only 5% of control colony growth after I78 days of exposure. Burst-forming-cell growth was depressed to 55% of control growth after 66 days but returned to control growth values at 178 days. in addition. benzene-exposed mice exhibited depressions in the numbers of splenic nucleated red cells and in the numbers of circulating red cells and lymphocytes. These results suggest that low-level exposure xo benzene may be hematotoxic.

INTRODUCTION

Over 60 years of study have demonstrated that benzene is a potent hematotoxicant [I]. The most severe blood dyscrasias associated with benzene exposure are aplastic anemia and myeioblastic leukemia [2]. Several good reviews have been written detailing the hematotoxic responses of both human and animals exposed to benzene 12-41.

The current occupational exposure limit for benzene is 10 ppm or a time-weighed average [5]. Several years ago. the OSHA attempted to lower the exposure limit to

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Abbreviations: BFU-E, burst-forming unit-erythroid; CFU-E, colony-forming unit-erythroid; OSHA, Occupational Safety and Health Administration.

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counts were determined by resuspending aliquots of the respective single-cell suspensions into a hypotonic solution of 3:1 heat-inactivated fetal calf serum and water, incubating the suspensions for 10 min and preparing smears stained with 1% dimethoxybenzidine countered with Wright–Giemsa. 1000 cells per organ per animal were scored from these slide preparations.

The BFU-E and CFU-E were quantified by the methyl cellulose method as modified by Urabe and Murphy [17]. 6 aliquots of cells were cultured from each animal. Cells from age-matched, air-exposed animals were concurrently cultured each time the assays were performed on benzene-treated animals.

RESULTS

The mean daily benzene concentration ± SD during the course of the exposures was 10.1 ppm ± 0.3 ppm.

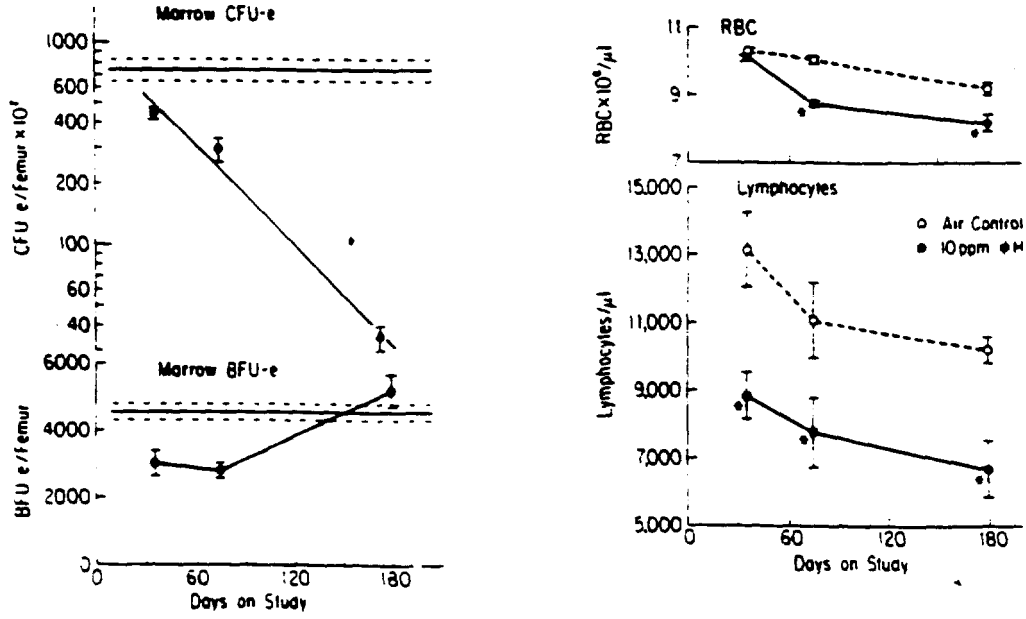

Fig. 1. Effect of repeated exposure to 10 ppm benzene on femoral committed erythroid progenitor cells. Closed circles and error bars are mean ± S.E. of determinations from 5 benzene-exposed animals. Dashed lines are ± S.E. of control culture growths. Mean CFU-E culture growths of cells from benzene-treated mice were statistically significant ($P < 0.01$) at all time points. Mean BFU-E culture growths of cells from benzene-treated mice were statistically significant ($P < 0.01$) only for assays performed on the 66th day of the study).

Fig. 2. Effect of repeated exposure to 10 ppm benzene on the numbers of circulating red cells (RBC) and lymphocytes. Bars denote ± S.E. Asterisks denote statistically different from controls ($P < 0.05$).

TABLE I

SPLENIC ERYTHROID CELLS

	32 Days	66 Days	178 Days
	CFU-E/spleen ± S.E. . 10^{-3}		
Treated	6.5 ± 2.1	—[a]	2.5 ± 0.5[b]
Control	9.3 ± 2.3	—[a]	23.6 ± 7.3
	BFU-E/spleen ± S.E.		
Treated	281 ± 150	235 ± 35	438 ± 164
Control	70 ± 32	1s2 ± 46	166 ± 55
	Cellularity/spleen ± S.E. $.10^{-6}$		
Treated	108 ± 62	123 ± 5.9	76 ± 1.9[b]
Control	93 ± 3.4	110 ± 7.9	90 ± 4.4
	Nucleated red cells/spleen ± S.E. $.10^{-6}$		
Treated	1.8 ± 0.7	7.9 ± 1.2	0.6 ± 0.2[b]
Control	0.8 ± 0.3	36 ± 1.6	4.0 ± 1.0

[a]Cells from both treated and control mice exhibited a 20-fold increase in growth with large variances of growth from culture to culture. These growths were likely artifacts.
[b]Indicates significantly depressed relative to control values. $P<0.05$.

Progenitor cells from benzene-exposed mice showed markedly reduced abilities to form colonies compared to cells from control mice. The numbers of marrow CN-E colonies from benzene-exposed mice showed a progressive decline during the exposure period reaching 5% of control values after 178 days (Fig. 1). Colony numbers declined exponentially during the exposures with a half-life of about 2 months (Fig. 1). Marrow BN-E colonies from benzene-exposed mice were significantly depressed to 55% of control values at 66 days but returned to control values at 178 days (Fig. 1).

Splenic CN-E colony formation was depressed to 10% of control values in benzene-exposed mice at 178 days (Table I). The exposures had no effect on splenic BFU-E colony growth at any monitoring period (Table I).

Benzene-exposed mice also exhibited changes in the numbers of differentiated hematopoieric ceils. Significant depressions in the numbers of circulating red cells and lymphocytes were observed in exposed mice. Red cells were depressed at 66 and 178 days while lymphocytes were depressed at all three monitoring periods (Fig. 2). Levels of circulating neutrophils were unaffected by the exposures (nor shown). At 178 days. benzene-exposed mice exhibited depressions in splenic nucleated cellularity and in splenic nucleated red cell numbers. The latter were depressed to about 15% of control values (Table I). Marrow cellularity and marrow-nucleated red cell numbers were unaffected by the exposures (not shown).

DISCUSSION

Exposure to 10 ppm benzene clearly inhibited the ability of the mouse CN-E to form colonies in vitro. By the end of the exposures, 95% of the CN-E were unable to form colonies. It is unlikely that 95% of the CN-E were destroyed since a loss of that magnitude could not maintain the nucleated red cell numbers found in the marrow. A more likely explanation would be a progressive inability of the CFU-E to replicate and differentiate in culture under the stimulus of erythropoietin. The inability of CFU-E to differentiate in culture may not reflect an inability of these cells to differentiate in vivo. However, an inability to differentiate in culture indicates that at least one functional characteristic of these cells has been changed. Alternatively, damage to the ability of the preceding cell types to replace CN-E could have a role in this effect. CN-E have limited proliferative potential and are progeny of BFU-E which have a large proliferation potential [18, 19]. Even a minor amount of benzene-induced damage to BN-E could result in significant depressions in CN-E numbers. Evidence for damage to the BN-E population was observed at 66 days when the in vitro colony forming ability of these cells was reduced to 55% of control values.

Under conditions of erythropoietic stress, the spleen can become a site of extradmedullary erythropoiesis in the rodent [20, 21]. Splenic CN-E and the numbers of more differentiated nucleated red cells in the spleen were reduced at 178 days in mice exposed to benzene. Thus, erythropoiesis was also disrupted in this compensatory erythropoietic organ.

The failure of the erythrons (marrow and spleen erythropoiesis) of benzene-exposed mice to support normal red cell mass is illustrated by the significant reduction in peripheral red cell numbers in these animals during the last two monitoring periods. It is clear from these data that exposures to levels of benzene considered occupationally safe markedly disrupt normal erythropoiesis in these mice.

Since peripheral lymphocyte numbers were also depressed, 10 ppm benzene exposure may also be affecting other cell types. We have acquired evidence for this in preliminary work in which we found that femoral pluripotent stem cells (CFU-S) were reduced to 50% of control values after 178 days exposure to 10 ppm (not shown). Further work concerning the hematotoxic effects of inhaled benzene at or below 10 ppm seems to be warranted.

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