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Mice Exposed *in* Utero to 20 **ppm** Benzene Exhibit Altered Numbers of Recognizable Hematopoietic Cells Up to Seven Weeks after Exposure

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Mice Exposed *in Utero* to 20 ppm Benzene Exhibit Altered Numbers of Recognizable Hematopoietic Cells Up to Seven Weeks after Exposure. KELLER, K. A., AND SNYDER, C. A. (1988). *Fundam. Appl. Toxicol* 10, 224-232. Pregnant Swiss Webster mice were exposed from Day 6 through Day 15 of gestation to either air, 5 ppm, 10 ppm, or 20 ppm benzene. On Day 16 of gestation, 2 days after birth, and 6 weeks after birth, progeny of the exposed dams were assayed for the amount and type of hemoglobin produced and for recognizable hematopoietic cells in the peripheral blood and hematopoietic organs. None of the benzene exposures induced significant changes in the indices assayed from the 16-day fetuses. In contrast, 2-day neonates exposed *in utero* to all concentrations of benzene exhibited reduced numbers of circulating erythroid precursor cells. In addition, those 2-day neonates exposed In utero to 20 ppm benzene exhibited increased numbers of hepatic hematopoietic blast cells and granulopoietic precursor cells accompanied by decreased numbers of erythropoietic precursor cells. Six-week adult mice exposed *in utero* to 20 ppm benzene exhibited a similar pattern of enhanced granulopoiesis. These animals exhibited elevated numbers of splenic hematopoietic blast cells and granulopoietic precursor cells accompanied by decreased numbers of marrow erythropoietic precursor cells. These results suggest that *in utero* exposures to low concentrations of benzene can induce persistent enhanced production of recognizable granulopoietic elements in the hematopoietic systems of mice.

Although benzene is a hematotoxicant, it is one of the most widely used industrial chemicals (Chem. and Eng. News. 1984). Estimates of U.S. worker populations exposed to benzene range from 600,000 to 2,000,000 (U.S. Department of HEW. 1974; U.S. OSHA, 1978; Luken and Miller. 1981). Occupational exposures to benzene are currently regulated at a threshold limit value (TLV) of 1 ppm. In addition to occupational exposures, it is estimated that U.S. urban dwellers are exposed to nearly 1 mg of benzene daily (National Research Council, 1980).

Several studies concerning the developmental toxicity of benzene have been performed using a variety of exposure routes (see Murray *et al.*, 1979, and Mehlman *et al.*, 1980, for reviews). The results of these studies indicate that benzene is not a teratogen although it may be embryotoxic. Surprisingly, although benzene is a potent hematotoxicant, there have been very few studies concerning the effects of benzene on the developing hematopoietic system (Pollini *et al.*, 1965; Koizumi and Suzuki. 1966; Murray *et al.*, 1979; Mizens. 1982). Benzene may be particularly toxic to fetal hematopoietic cells since the populations of these cells are actively expanding (Paul *et al.*, 1969) and since benzene is particularly toxic to actively replicating cells (Snyder. 1987).

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Recently we reported marked effects on the erythrocytic and granulocytic colony forming cells of mice exposed *in utero* to 5, 10, or 20 ppm benzene (Keller and Snyder, 1986). Moreover, when mice previously exposed *in utero* to 10 ppm benzene were reexposed to 10 ppm benzene as adults, marked depressions in the numbers of granulocytic and erythrocytic colony forming cells were observed. We now report our findings concerning the effects of *in utero* exposure of mice to 5, 10, or 20 ppm benzene on recognizable hematopoietic precursor cells, peripheral blood cells, and hemoglobin production. Exposures were conducted from Days 6 to 15 of gestation in order to coincide with the time of rapid expansion of the embryonic and fetal hematopoietic systems. Assays were performed on 16-day-old fetal, 2-day-old neonatal, and 6-week-old progeny of air or benzene-exposed dams. These protocols allowed for the assessment of both the immediate and long-term effects of the exposures.

METHODS

1. *Animals*

Male and female Swiss Webster mice (Crl: CFW(SW)Br, Charles River) were used throughout the experiments. Animals were purchased at 6 weeks of age and allowed to acclimate to a 12-hr light/dark cycle for 2 weeks in a climate-controlled room. Except during exposure periods animals were allowed food (Purina lab chow) and water ad *libitum*. Timed pregnancies were obtained by placing two normal cycling proestrus, virgin females (age 8–12 weeks) with one male during the last 2 hr of the dark cycle. Females were then checked for presence of a vaginal plug or sperm in a vaginal smear with the day of mating considered as Day 0 of pregnancy. Randomly selected pregnant females were then placed into individual control and test groups of 5–10 animals each. Female body weights, as well as general appearance and behavior, were monitored throughout pregnancy.

2. *Inhalation Exposures*

Whole body inhalation exposures were conducted in 1.0 m^3 stainless-steel and plexiglass dynamic chambers (Drew and Laskin, 1973). Animals were exposed in wire mesh cages and were not allowed food and water during exposures. Exposures were conducted for 6 hr/day over Days 6 through 15 of gestation. Air sham-treated pregnant mice were exposed in duplicate chambers to filtered, conditioned air using the same exposure and animal handling regimes.

Benzene atmospheres were generated by a flow–dilution system (Snyder *et al.*, 1978). The apparatus consisted of a 1000-ml flask and reservoir tank containing glass distilled benzene of chromatographic quality (Burdick and Jackson Laboratories, Inc., Muskegon, MI). Benzene vapor was produced by passing an airstream over the surface of liquid benzene at a flow rate controlled by an external rotometer. The benzene-ladened air was then mixed with filtered air leading into the exposure chamber.

The chamber concentrations of benzene were determined every 0.5 hr by uv spectrophotometry (5 and 10 ppm) or by an infrared gas analyzer (20 ppm). For uv spectrophotometry, chamber air was bubbled through a glass midget impinger. The resultant alcohol–benzene solution was read for absorbance at 254.5 nm and compared to a predetermined calibration curve, which had been corrected for bubbler efficiency. The infrared analyzer (Miran-1A CVF, Foxboro Analytical, Norwalk, CT) was precalibrated with known concentrations of benzene. A wavelength of 9.75 μm was used for optimum absorption of benzene, with an effective pathlength of 20.15 m.

3. *Exposure Protocols*

Experiment 1. The responses to in *utero* exposure to 5, 10, or 20 ppm benzene were determined in fetuses on the 16th day of gestation. Five benzene-exposed and five air-exposed pregnant mice were sacrificed by cervical dislocation, their uteri removed, and the number of live, dead, and resorbed fetuses recorded. Two male and two female fetuses were then randomly selected, weighed, and examined for any external gross morphological malformation. Peripheral blood samples were taken from trunk blood of decapitated fetuses for red and white cell counts and for hemoglobin analysis. Livers were removed aseptically for enumeration of recognizable cells in the hematopoietic differentiating, proliferating pool (DPP).

Experiment 2. The responses to *in* utero exposure to 5, 10, or 20 ppm benzene were determined in 2-day-old neonates. Five benzene-exposed and five air-exposed pregnant females were allowed to proceed through normal parturition. Two male and two female neonates were then randomly selected at 2 days of age and subjected to the same protocol as that described above with 16-day-old fetuses.

Experiment 3. Responses due to *in utero* exposure to 5, 10, or 20 ppm benzene were investigated in 6-week-old progeny. Five benzene-exposed and five air-exposed pregnant dams were again allowed to proceed through normal parturition. At 6 weeks of age, one male and one female were randomly selected from each litter. Peripheral blood samples were obtained from tail veins for red and white cell counts and for hemoglobin analysis. These animals were then terminated by cervical dislocation and their spleen and femurs removed aseptically for enumeration of recognizable cells in the DPP.

4. Blood Cell Parameters

Peripheral blood. Free flowing venous tail blood from 6-week-old adults and trunk blood from fetuses and new nates were used for red blood cell (RBC), white blood cell (WBC), and blood cell differential counts. Peripheral RBC and WBC counts were determined with a Coulter Counter Model ZB-1 (Coulter Electronics. Hileah. FL). Differential counts were determined by counting 100 nucleated cells in Wright/Giemsa stained blood smears.

Hemoglobin quantitation. Peripheral blood was obtained as described above. Mean corpuscular hemoglobin was determined using a standard spectrophotometric cyanmethemoglobin technique (Boehringer Mannheim Diagnostics, Inc.. Houston, TX). Briefly, 5–10 μl of blood was placed in Drabkin's reagent to lyse the red cells and release hemoglobin. The hemoglobin was convened to cyanmethemoglobin with $K_3Fe(CN)_6$ and KCN. Absorbance of the cyanmethemoglobin solutions were read at 540 nm. The average hemoglobin content per cell was then calculated by dividing total hemoglobin by the respective red cell count.

Hemoglobin typing. Peripheral blood obtained as described above was washed in isotonic saline and lysed with distilled water. After centrifugation. the supernate was removed and stored in glass vials at −20°C. Prior to separation on polyacrylamide gels. samples were thawed and assayed for protein content by the Pierce BCA Protein Assay (Pierce Chemical Co., Rockford. IL). All hemoglobin samples were diluted to 250 μg/ml with distilled water containing 0.01% potassium cyanide and stored at 4°C until the following day.

The hemoglobins were separated by isoelectric focusing on polyacrylamide tube gels. Solutions of 0.6% ethanolamine and 0.01 M phosphoric acid were used as catholyte and anolyte, respectively. Approximately 25 μg total protein in a volume of 100 μl was applied onto the top of the gels in a carrier solution of 8 M urea containing 5% ampholyte. A voltage of 400 V was maintained for 5 hr and thereafter increased to 800 V for 1 hr to increase the resolution of the bands. The pH gradient was assessed by sectioning a gel into 20 or more 5-mm pieces, eluting the ampholytes into 10 mM potassium chloride, and measuring the pH on a Beckman Model 3560 digital pH meter (Beckman Instruments, Inc., Irvine, CA). Additional control gels were stained for hemoglobin with benzidine to ensure correct identification of the hemoglobin bands.

Gels were fixed in 10% trichloroacetic acid and stained with Coomassie blue. Stained gels were scanned at 550 nm on a Gilford Model 250 spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, OH) and the relative amounts of Hemoglobin A major and the Hemoglobin A minor quantitated by measuring the areas under the curves.

DPP quantitation. Livers from 16-day-old fetuses and 2-day-old neonates were placed in ~5 ml ice-cold, sterile-supplemented α medium (SAM—Supplemented α media: 100× α media, 1% nonessential amino acid solution. 1% sodium pyruvate solution. 1% L-glutamine, 2% penicillin/streptomycin sulfate solution, sodium bicarbonate, pH 7.2). Single cell suspensions were prepared by repeated aspirations of the tissue through a siliconized fine-tipped Pasteur pipet. Suspensions of splenic cells were obtained by pressing the spleen tissue through a stainless steel wire mesh grid (150 divisions per square inch) and by repeated aspirations in SAM as described for liver cell suspensions. To obtain marrow cells, the marrow cavities of femurs were repeatedly flushed with cold SAM and separated into single cell suspensions by repeated aspirations. All three types of cell suspensions were then gently pelleted by centrifugation and resuspended in a solution of 3:1 fetal calf serum:H_2O. Smears of these cell suspensions were made and stained with benzidine and Wright/Giemsa (LoBue *et al.*, 1963). Slides were coded and read blindly. Five hundred nucleated cells per slide were categorized.

5. Data Evaluation

Peripheral and organ blood cell counts were determined on the four animals (two males and two females) randomly selected from each litter. Cell counts from animals randomly selected from matched air control litters were determined each time cell counts were determined from a benzene-treated litter. Thus. each benzene-exposed animal had its own age-matched air control. In almost all cases. there were five litters per exposure. per age group. Differences in the cell counts were evaluated by the Student *t* test using the litter as the experimental unit. Differences greater than the two-tailed, $p < 0.05$, Student *t* value were considered significant.

Differential counts of peripheral blood cells and DPP cells were evaluated as follows. Peripheral and organ slides made from one male and one female progeny were randomly selected from each litter. Slides from five litters per age group per benzene treatment were so selected. Slides from five age-matched air control litters were se-

TABLE I

PERIPHERAL BLOOD CELL INDICES (Mean ± SE)[a]

	RBC/mm³ × 10⁶	MCH[c]	Nucleated cells per mm³	Ratio of HbA major[d] to HbA minor
16-Day fetuses				
5 ppm	5.5 ± 0.4	2.4 ± 0.1	47.677 ± 5.615	1.1 ± 0.1
Air	4.9 ± 0.2	2.8 ± 0.1	35.952 ± 3.374	—
10 ppm	5.4 ± 1.2	2.3 ± 0.3	63,363 ± 17,394	0.8 ± 0.1
Air	3.4 ± 0.3	3.1 k0.3	44,145 ± 9.342	—
20 ppm	4.3 ± 0.4	2.1 ± 0.3	75,749 ± 3.330	1.0 ± 0.1
Air	5.2 ± 0.2	2. 1 ± 0.03	99,332 ± 10,910	0.9 ± 0.1
2-Day neonates				
5 ppm	7.0 ± 0.1[b]	1.8 ± 0.03[b]	29.151 ± 10.003	1.0 ± 0.1
Air	6.4 a 0. 1	20 ± 0.07	28.024 ± 5,302	—
10 ppm	6.4 ± 0.4	2.0 ± 0. 1	4.487 ± 419	1.0 ± 0.1
Air	5.8 ± 0.2	2.3 ± 0.2	5,793 ± 847	—
20 ppm	6.9 ± 0.2	2.1 ± 0.1	5.059 ± 560	1.1 ± 0.2
Air	6.4 ± 0.3	2.0 ± 0. 1	5,075 ± 658	1.0 ± 0.2
6-Week adults				
5 ppm	7.6 ± 0.2[b]	1.9 ± 0.2	8,983 ± 655	1.1 ± 0.1
Air	6.6 ± 0.2	2.2 ± 0.2	9,174 ± 753	—
10 ppm	7.1 ± 0.2	2.2 ± 0.1	9,494 ± 649	1.5 ± 0.1
Air	6.9 ± 0.2	2.3 ± 0.1	9.691 ± 648	—
20 ppm	7.9 ± 0.2[b]	2.0 ± 0. 1	8,476 ± 573	1.5 ± 0.2
Air	7.2 ± 0.2	2.1 ± 0.1	8,142 ± 722	1.2 ± 0.2

[a] Values determined from five individual litters (two males and two females per litter) per treatment. per age group.
[b] Statistically different from control values, $p < 0.05$.
[c] Mean corpuscular hemoglobin.
[d] For all indices except ratios of HbA major to HbA minor. there were age-matched air control litters for each litter exposed to a given benzene concentration. For ratios of HbA major to HbA minor. one set of age-matched litters served as controls for all benzene-exposed litters within a given age group. Methods of statistical analyses for all indices are given under Methods.

lected in a similar fashion. Unlike the cell count data discussed above, one set of air control slides served as control for all three benzene treatments within a given age group. For example, slides from 16-day-old fetuses exposed *in utero* to 5, 10, or 20 ppm benzene were compared to slides from 16-day-old fetuses exposed *in utero* to air. Other age groups were handled analogously. These data were evaluated first using a two-way analysis of variance with treatment and gender as covariates. F ratios were significant for differences in the responses of the genders in only 3 of the 42 two-way analyses of variance performed. In contrast, F ratios were significant for the responses due to benzene treatment in 15 of the 42 two-way analyses of variance performed. For this reason total litter responses (male and female) vs treatment were assessed by one-way analysis of variance followed by Dunnett's tests. Ratios of hemoglobin A major to hernoglobin A minor were also analyzed by one-way analyses of variance followed by Dunnett's tests. Differences between treatments were considered significant when they were greater than the two-tailed, $p < 0.05$ Dunnett's critical value (Dunnett, 1964).

RESULTS

The mean daily benzene exposure concentrations ± standard deviations for the three exposures employed were 5.1 ± 0.5, 9.9 ± 0.6, and 20.4 ± 0.9 ppm.

TABLE 2

PERIPHERAL BLOOD CELL DIFFERENTIALS[a]

	Blasts	Dividing granulocytes	Nondividing granulocytes	Early nucleated red cells	Late nucleated red cells	Primitive nucleated red cells	Lymphocytes
16-Day fetuses							
Air	0.00 ± 0.00	0.50 ± 0.16	1.60 ± 0.50	5.10 ± 1.34	0.40 ± 0.22	92.4 ± 1.95	—
5 ppm	0.00r0.00	2.10 ± 0.67	3.60 ± 1.57	5.802 1.88	0.80 ± 0.25	87.4 ± 4.11	—
10 ppm	0.10 ± 0.00	0.90 ± 0.28	1.30 ± 0.33	4.0020.60	1.20 ± 0.39	92.4 ± 1.20	—
20 ppm	0.10 ± 0.00	1.50k0.50	2.20 ± 0.63	3.90r0.79	1.5020.34	90.72 1.48	—
2-Day neonates							
Air	0.00 ± 0.00	3.80 20.66	67.60 ± 2.44	7.30 ± 1.36	6.20 ± 1.79	—	14.0 ± 3.1
5 ppm	0.2020.14	3.10r0.57	72.30 ± 3.09	1.70 ± 0.62[b]	3.6020.88	—	17.9 ± 2.4
10 ppm	0.10 ± 0.10	5.90 ± 1.04	67.90 ± 2.88	0.50 ± 0.22[b]	7.30 ± 0.83	—	16.9 ± 2.0
20 ppm	0.10 ± 0.10	2.10 ± 0.62	80.40 ± 2.67[b]	0.00 ± 0.00[b]	1.60 ± 0.45[b]	—	14.2 ± 2.5
6-Week adults							
Air	0.00 ± 0.00	2.70k0.47	19.3 ± 2.28	0.00r0.00	0.2020.14	—	75.0 ± 3.0
5 ppm	0.00 ± 0.00	1.20 ± 0.47	22.0 ± 2.47	0.10 ± 0.10	0.2020.13	—	12.3 ± 3.1
10 ppm	0.00 ± 0.00	0.60 ± 0.22	24.2 ± 2.59	0.00 ± 0.00	0.10 ± 0.10	—	75.1 ± 2.9
20 ppm	0.10 ± 0.10	2.2020.63	16.7 ± 2.27	0.10 ± 0.10	0.20 ± 0.13	—	77.6 ± 2.4

[a] One hundred cells counted from one male and one female from each of five individual litters per treatment, per age group. Numbers are means ± SE.

[b] Denotes statistically significant from corresponding control values. $p < 0.05$.

1. Maternal Toxicity

There was no evidence of maternal toxicity among dams exposed to any concentration of benzene tested as determined by maternal morbidity, mortality, or weight loss during the exposures.

2. Fetal and Neonatal Toxicity

There was no evidence of nonhematopoietic toxicity among any of the fetal or neonatal progeny exposed *in utero* to any concentration of benzene studied. Litter sizes, male/female ratios, and body weights, as well as the numbers of dead, resorbed. or malformed fetuses, were all within control limits.

3. Hematopoietic Toxicity among Progeny

F ratios indicated significant differences in the responses of the genders to the exposures in only 3 of the 42 analyses of variance performed. These 3 cases were peripheral blood late nucleated red cells of 6-week adults, bone marrow dividing granulocytes of 6-week adults, and bone marrow lymphocytes of 6-week adults. When the total litter responses were evaluated. none of the values for these three indices among benzene-treated animals were statistically different from control values (Tables 2 and 3).

a. Peripheral blood indices. No pattern of statistically significant differences between benzene-exposed and airexposed progeny was observed among any of the peripheral blood counts or hemoglobin indices (Table 1). A small number of statistical differences were observed but these occurred in no discernible pattern and probably have little toxicological significance (Table 1).

b. *Peripheral blood differentials.* There were marked statistically significant depressions in the numbers of early nucleated red

TABLE 3

CELL DIFFERENTIALS OF HEMATOPOIETIC ORGANS[a]

	Blasts	Dividing granulocytes	Nondividing granulocytes	Early nucleated red cells	Late nucleated red cells	Lymphocytes
16-Day fetuses (hepatic)						
Air	3.40 ± 0.64	13.2 ± 3.1	L49 ± 2.7	19.8 ± 2.5	396.5 ± 8.5	51.6 ± 7.3
5 ppm	4.70 ± 0.83	12.5 ± 2.1	12.1 ± 1.9	21.7 ± 2.3	395.5 ± 6.5	52.9 ± 4.0
10 ppm	3.20 ■1.00	12.7 ± 2.8	14.6 ± 2.5	25.3 ± 5.9	407.4 ± 5.5	35.2 ± 6.1
20 ppm	4.70 ■0.65	5.7 ± 1.4	11.5 ± 2.8	20.2 ± 2.0	418.1 ± 5.3	38.5 ± 4.4
2-Day neonates (hepatic)						
Air	0.80 ± 0.33	6.90 ± 1.1	27.0 ± 4.5	15.0 ± 1.33	286.0-t 13.9	155.5-t 13.6
5 ppm	2.80 ± 0.70'	6.60 ± 0.9	35.0 ± 2.1	14.9 ± 1.93	282.0 ± 9.0	156.1 ± 10.4
10 ppm	0.80 ± 0.29	7.50 ± 0.9	36.6 ± 2.7	10.4 ± 0.97	289.9 ± 4.8	150.2 ± 5.6
20 ppm	2.60 ± 0.56'	17.30 ± 1.4'	52.0 ± 3.2[b]	12.5 ± 1.47	199.4 ± 10.6'	213.8 ± 11.9'
6-Week adults (bone marrow)						
Air	2.22 ± 0.89	6.30 ± 1.0	83.1 ± 8.6	18.8 ± 2.6	123.0f5.2	257.6 ± 11.7
5 ppm	3.30 ± 0.97	9.50 ± 1.2	113.5 ± 10.5	14.3 ± 1.9	134.6 ± 7.5	215.6 ± 15.7'
10 ppm	1.80 ± 0.63	7.60 ± 1.3	93.5 ± 5.8	12.5 ± 1.7	104.6 ± 6.8	272.7 ± 9.9
20 ppm	3.60 ± 0.97	8.90 ± 1.3	78.2 ± 12.3	10.4 ± 1.5[b]	115.6 ± 9.1	271.5 ± 17.7
6-Week adults (spleen)						
Air	0.20 ± 0.13	1.30 ± 0.4	4.80 ± 1.3	2.30 ± 0.4	38.4 ± 9.2	450.9 ± 9.1
5 ppm	0.30 ± 0.15	2.10 ± 0.5	9.40 ± 1.4[b]	4.20 ± 0.8	50.1 ± 9.0	430.9 ± 9.9
10 ppm	0.40 ± 0.22	1.30 ± 0.4	3.40 ± 0.8	3.60 ± 0.8	44.2 ± 3.1	445.3 ± 4.0
20 ppm	1.30 ± 0.45[b]	3.80 ± 0.7'	15.2 ± 2.0[b]	5.50 ± 1.1	41.7 ± 5.3	430.7 ± 8.2

[a] Five hundred cells counted from one male and one female offspring from each of five individual litters per treatment. per age group. Numbers are means ± SE.

[b] Denotes statistically significant from corresponding control values. $p < 0.05$.

cells (basophilic normoblasts) in the peripheral blood of 2-day neonates exposed ***in utero*** to all concentrations of benzene studied (Table 2). These depressions of erythroid precursors occurred in a dose/response pattern. Those neonates exposed ***in utero*** to ***20*** ppm benzene also exhibited depressed numbers of late nucleated red cells (polychromatic normoblasts and their nucleated progeny) and elevated numbers of nondividing granulocytes (granulocytes differentiated to at least the metamyelocyte stage).

c. ***Cell differentials of hematopoietic organs.*** There were several statistically significant changes in the numbers of recognizable hepatic hematopoietic precursor cell types among 2-day neonates exposed *in utero* to 20 ppm benzene (Table 3). These benzene-exposed neonatal mice exhibited elevated numbers of blasts, dividing granulocytes (promyelocytes and myelocytes), nondividing granulocytes, and lymphocytes. In contrast, the numbers of late nucleated red cells (polychromatic erythroblasts and their nucleated progeny) were depressed in these animals.

A similar pattern was observed among hematopoietic cell types of 6-week adults ex-

posed *in utero* to 20 pprn benzene (Table 3). These animals exhibited elevated numbers of splenic blasts, dividing granulocytes, and nondividing granulocytes accompanied by depressed levels of marrow early nucleated red cells (basophilic normoblasts).

DISCUSSION

Exposure of pregnant mice to 20 ppm benzene clearly induced hematotoxic responses in the offspring. Toxicity was evident by depressions in the numbers of erythroid precursor cells and elevations in the numbers of granulocytic precursor cells in both neonatal and 6-week-old offspring. It is noteworthy that the alterations in the numbers of the precursor cell types persisted in the adult mice 7 weeks after their *in utero* exposures. Decrements of erythroid precursor numbers accompanied by increases of granulocytic precursor numbers have been observed previously in this laboratory among mice undergoing benzene exposures (Snyder *et al.*, 1980; Baarson *et al.*, 1982; Rosenthal and Snyder, 1984). However, in the previous studies, mice were exposed as adults and the exposure concentrations were an order of magnitude higher than those employed in the present study with *in utero*-exposed mice.

Neonates exposed to all concentrations of benzene studied exhibited depressed levels of peripheral early nucleated red cells (Table 2). During the hepatic phase of fetal hematopoiesis, erythroid precursor cells compose 50–70% of the nucleated hematopoietic cells found in the liver (Paul *et* al., 1969; Tarbutt and Cole, 1970). Our exposures were therefore conducted during a time of preponderance of erythroid cells and it is perhaps likely that the erythroid cells would show the greatest toxic response. Although there were marked depressions in the numbers of recognizable erythroid precursor cells among 2-day neonates and 6-week adult mice exposed *in utero* to benzene, these depressions in erythroid precursor cells did not affect the levels of circulating red cells in these animals. The erythron has a large capacity to deal with an erythropoietic insult. It is known, for example, that during phases of acute anemia, the cell cycle time of recognizable erythroid precursor cells shortens (Hanna *et* al., 1969). This can lead to a lower steady-state number of erythroid precursors while at the same time producing normal or near normal numbers of mature functioning red cells. Such a compensatory mechanism may be in effect among the *in utero* benzene-exposed mice in this study.

It is interesting to note that the 2-day neonates and 6-week adults exposed *in utero* to benzene exhibited changes in recognizable hematopoietic precursor cells but the 16-day fetuses did not. This is especially interesting because the fetuses were assayed 1 day after their last exposure while the neonates and adults were assayed 1 week and 7 weeks, respectively, after their last exposures. The rapidly expanding fetal erythron (Chui *et al.*, 1971) may be able to temporarily mask any damage inflicted by exposure to benzene. However, it appears that some persistent damage is induced by the exposures which becomes evident when expansion of the erythron ceases as the animals age.

Exposures to benzene *in utero* induced no observable changes in the HbA major/HbA minor ratio. Such changes are indicative of functional defects in the normal maturational process of erythroid precursor cells. Increases in HbA minor have been shown to accompany erythropoietic stress in mice (Alter et *al.*, 1982; Pappas and Cauchi, 1982) and are analogous to increases in fetal hemoglobin seen in some erythropoietically stressed humans.

Although benzene is apparently not a teratogen for common laboratory animals (Murray *et al.*, 1979; Mehlman *et al.*, 1980), our data indicate that maternal exposures to low concentrations of benzene induce changes in the developing murine fetal hema-

topoietic system. Although many aspects of human and murine hematopoietic ontogeny are similar, extrapolation of our findings to the possible risk for humans is difficult for two reasons. First, resting mice respire about 10 times more air per minute per gram body weight than resting humans (Guyton, 1947). Thus, for a given air concentration of benzene, a mouse would be expected to inhale a larger dose of benzene than a human. On the other hand, a human fetus has a greater capacity to metabolize xenobiotics than a mouse fetus (Gillette and Stripp, 1975; Netter, 1976). Thus, for a given dose of benzene reaching fetal circulations, a human fetus may form greater amounts of the toxic benzene metabolites believed responsible for benzene's toxicity (Rushmore *et al.*, 1984) than a mouse fetus.

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