Bull. Environm. Contam. Toxicol. 29, 385–391 (1982)

Toxicity of Chronic Benzene Inhalation: CD-1 Mice Exposed to 300 ppm

C. A. Snyder, B. D. Goldstein, A. Sellakumar, I. Bromberg, S. Laskin, and R. E. Albert

New York University Medical Center, Institute of Environmental Medicine, 550 First Avenue, New York, NY 10016

Human exposure to benzene has been linked to a variety of hematological disorders including pancytopenia and its variants and acute myeloblastic leukemia and its variants (AKSOY 1981; LASKIN & GOLDSTEIN 1977; SNYDER & KOCSIS 1975). Laboratory experiments have generally reproduced the cytopenic effects of benzene in animals most often by parenteral injection (GERARDE & AHLSTROM 1977; GILL et al. 1979; IRONS & MOORE 1980). On the other hand, except for work performed in this laboratory (GOLDSTEIN et al. 1982; SNYDER et al. 1980), attempts to reproduce the leukemogenic effects of benzene have been unsuccessful.

Because of differences in routes of administration, types of animals and durations of exposure, previous toxicological evaluations of benzene have often produced conflicting results (LEONG 1977). In order to better assess the toxic effects of benzene, this laboratory has conducted a series of lifetime exposures to inhaled benzene using three strains of mice and Sprague-Dawley rats. Two previous reports concerned AKR and C57Bl mice and Sprague-Dawley rats exposed to 300 ppm benzene (SNYDER et al. 1978, 1980). This exposure level was more toxic to the mice than to the rats. The mice exhibited decreased survival and weight gain, as well as peripheral blood lymphocytopenia, anemia and neutrophilia. Rats, on the other hand, exhibited only decreased survival and lymphocytopenia, and these effects were not as severe as those observed in the mice.

The AKR and C57Bl strains were chosen in order to study the effects of benzene exposure on different endogenous, lymphoma-producing viruses. The AKR strain carries a virus which spontaneously produces a high incidence of thymic lymphoma (KAHN & NOVAK 1973). The C57Bl strain is believed to carry a virus that produces thymic lymphoma only after treatment with carcinogenic agents (KAPLAN 1967). Benzene exposures did not increase the incidence or shorten the induction time of the spontaneous thymic lymphoma in AKR mice. However, 15% of the benzene-exposed C57Bl mice developed thymic lymphoma while none of the controls developed this tumor (SNYDER et al. 1980).

0007-4861/82/0029-0385 $01.40

In order to put these findings into perspective, it was decided to assess the effects of benzene exposures on a mouse strain which is not known to harbor an endogenous lymphoma virus. To this end, the CD-1, outbred strain was chosen for study. We wish now to report the results of that study.

METHODS

Male, six-week old Charles River CD-1 mice (Charles River, Wilmington, MA) were quarantined for two weeks and observed for aberrant behavior and disease. After culling, mice were randomly distributed into exposed and control groups of 40 animals each. Animals were housed 5 to a box in polycarbonate boxes fitted with wire mesh tops. Food and water were provided ad libitum except during exposure periods.

Benzene exposures were conducted in a 1.6 m^3 stainless steel and plexiglass, dynamic inhalation chamber (DREW & LASKIN 1973). Animals were exposed for 6 hr/day, 5 days/wk. During exposures, mice were housed 10 to a cage in stainless steel wire mesh exposure cages. Control mice were exposed in a duplicate chamber to filtered, conditioned air and underwent the same exposure regime as benzene-exposed mice. Methods of generating benzene vapor and techniques used to analyze chamber atmospheres were as previously described (SNYDER et al. 1978). Animals were observed daily for evidence of morbidity. Weight determinations were performed weekly for the first four weeks of exposure and biweekly thereafter.

Peripheral blood counts were performed on venous tail blood from 10 exposed and 10 control animals. Treated and control mice were bled biweekly on the same days within a period of 1.5 hours in order to minimize differences in blood counts caused by circadian rhythms, handling, etc. As mortality increased, exposed and control mice were cycled into those respective exposed and control groups undergoing blood count determinations so that, throughout most of the study, there were usually 10 mice in each group available for blood count measurements. Red cell, white cell and white cell differential counts were performed as previously described (SNYDER et al. 1978).

Tissues routinely sectioned at autopsy included lung, liver, spleen, bone marrow and kidney as well as any abnormally appearing organs. Sections were fixed, mounted and stained as previously described (SNYDER et al. 1980). All slides were read by the same pathologist (A.S.).

RESULTS

The mean daily benzene concentration ± SD for the benzene-exposed mice was 300 ± 2.6 ppm. The mean daily chamber temperature ± SD and relative humidity ± SD for benzene-exposed mice were 68.9 ± 1.6°F and 54.2 ± 6.4%, respectively. These values for sham-exposed mice were 73.3 ± 1.2°F and 51.4 ± 5.6%, respectively.

Animals received 149 exposures over 222 calendar days. At this time, two benzene-exposed mice had developed myelogenous leukemia and several others were exhibiting leukemoid reactions. Exposures were terminated in order to prolong the lives of the survivors so that further cases of leukemia might develop. Nine benzene-exposed mice and 33 sham-exposed mice remained alive when exposures were terminated but no further incidences of myeloid leukemia developed.

Median survival after the first exposure for benzene-exposed and sham-exposed mice was 179 days and 369 days, respectively. Relative to sham-exposed mice, benzene-exposed mice exhibited a slower rate of weight gain from the start of the exposures and exhibited a weight loss after 24 weeks of exposure (Table 1).

TABLE 1

WEIGHT GAIN IN BENZENE-EXPOSED AND SHAM-EXPOSED MICE

Week After First Exposure	Mean Weight (g) ± 2 S.E.	
	Benzene*	Sham*
0	35.7 ± 0.5	36.6 ± 0.5
4	35.7 ± 0.8	37.5 ± 0.6
8	39.2 ± 0.8	40.2 ± 0.6
12	38.1 ± 0.9	40.0 ± 0.7
16	39.6 ± 1.3	43.1 ± 1.0
20	40.8 ± 1.6	43.1 ± 0.9
24	39.1 ± 2.0	42.2 ± 1.1
28	32.6 ± 3.4	41.9 ± 1.4
32	32.3 ± 2.0	39.7 ± 1.6
36	30.8 ± 1.6	39.8 ± 1.6
40	29.7 ± 2.7	40.3 ± 1.7

* Forty mice initially at risk.

Peripheral red blood cell and lymphocyte levels of benzene-treated mice were significantly (± 2SE) depressed relative to sham-exposed (control) values after the first week of exposure and remained so throughout the study. Polymorphonuclear granulocyte levels in benzene-exposed mice were unaffected until 29 weeks after the first exposure when levels became statistically elevated (± 2SE) vs. control values. These quantitative changes

are illustrated in Figures 1 and 2. In addition to the quantitative changes, the following qualitative changes were regularly observed in the peripheral blood of benzene-exposed mice: Howell-Jolly bodies 7 days after the first exposure, anisocytosis after 22 days, poikilocytosis after 92 days and a shift to immature myeloid cells after 217 days. Similar changes were not observed in control mice.

Neoplasms developed in 5 benzene-exposed mice and in 2 sham-exposed mice. Among the benzene-exposed mice, 2 developed malignant lymphoma with thymic involvement, 1 developed acute myeloblastic leukemia, 1 developed chronic myelogenous leukemia and 1 developed a benign lung adenoma. Details of the 2 cases of myeloid leukemia have been reported elsewhere (GOLDSTEIN et al. 1982). Two control mice developed malignant lymphoma with no thymic involvement.

Among those animals dying without evidence of neoplasia, benzene-exposed mice exhibited higher incidences of bone marrow hypoplasia, bone marrow hyperplasia and splenic hemosiderin pigments compared to control mice. Benzene-exposed mice dying with bone marrow hypoplasia survived an average of 156 days after the first exposure whereas benzene-exposed mice dying with bone marrow hyperplasia survived an average of 231 days after first exposure. The histopathological findings are summarized in Table 2.

TABLE 2

HISTOPATHOLOGICAL EVALUATION OF BENZENE-EXPOSED AND SHAM-EXPOSED MICE

	Incidence		
	Benzene	Sham	X^2
Neoplasms	5/40	2/40	1.41
Bone Marrow Hyperplasia	9/35	1/38	8.21 $p<0.005$
Bone Marrow Hypoplasia	11/35	0/38	14.06 $p<0.001$
Splenic Hemosiderin Pigments	6/35	0/38	7.10 $p<0.01$
Splenic Hyperplasia	19/35	14/38	2.24

DISCUSSION

Chronic exposures to 300 ppm benzene were clearly toxic to these mice. Mortality and weight gain, as well

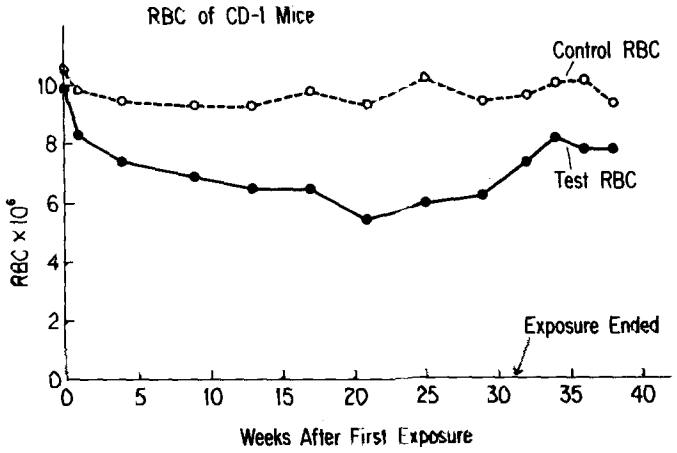

FIGURE 1

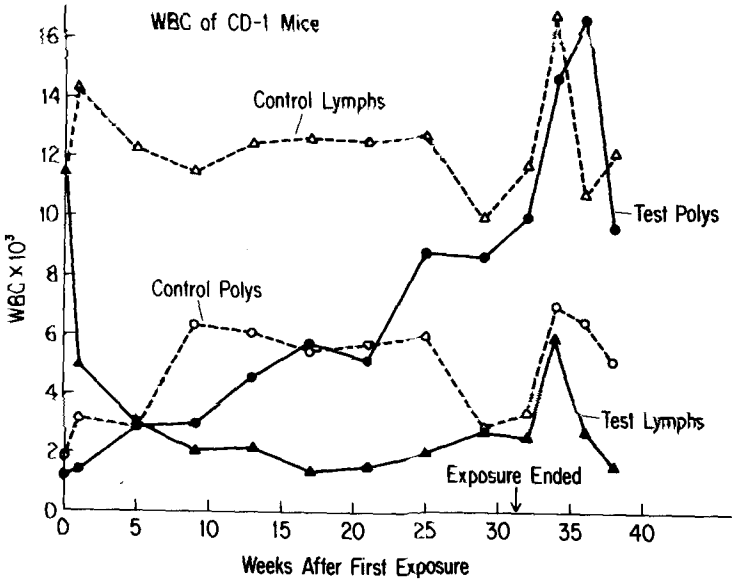

FIGURE 2

as circulating red cell and lymphocyte levels, were significantly reduced in exposed mice. The hematotoxic responses in these mice were similar to those previously observed in AKR and C57Bl mice exposed to benzene (SNYDER et al. 1978; 1980). There was an immediate depression of peripheral lymphocytes and red blood cells and a maintenance of the depressed levels throught the exposures. As exposures progressed, there was also an increase in circulating granulocytes. All of these peripheral blood changes had been previously observed in the AKR and C57Bl strains.

There was no significant increase in tumor incidence in exposed mice. The lifeshortening effects of these exposures may have precluded the development of tumors. The toxic effects of the exposures were quite severe since suspending exposures after 31 weeks did not retard the mortality rate of the nine surviving benzene-exposed mice. Lower exposure concentrations or less frequent exposures would be required to collect adequate data for benzene-induced tumors in these animals.

Exposed mice dying with bone marrow hypoplasia survived, on the average, 75 fewer days than mice dying with bone marrow hyperplasia. This may indicate that bone marrow hypoplasia is an early response to exposure followed by bone marrow hyperplasia. It may also indicate two different responses to the exposures.

The appearance of hemosiderin pigments in the spleens of some treated mice is evidence for benzene-induced hemolysis. The exposures could cause lysis of mature red cells or cause the production of short-lived red cells (ineffective erythropoiesis). There is evidence for decreased red cell membrane elasticity in animals exposed to benzene. Glycerol lysis time is markedly increased in red cells taken from animals exposed to benzene (GOLDSTEIN et al. 1980). This inelasticity may lead to greater splenic sequestering of affected cells and result in the formation of hemosiderin pigments in the spleen.

Acknowledgements

This investigation was supported by Contract U-150-14(PS-7) from the American Petroleum Institute and by Contract NO1 CP 33260 from the National Cancer Institute and is part of a center program supported by grant ES 00260 from the National Institutes of Environmental Health Sciences and grant CA 13343 from the National Cancer Institute.

The authors wish to thank Robert Kelly for conducting the inhalation exposures and Maureen Freitag for typing the manuscript.

REFERENCES

AKSOY, M.: Reg. Toxicol. Pharmacol. 1, 147 (1981).

DREW, R.T. and S. LASKIN: Methods of Animal Experimentation, Vol. 4, New York, Academic Press (1973).

GERARDE, M.W. and D.B. AHLSTROM: Toxicol. Appl. Pharmacol. 9, 185 (1966).

GILL, D.P., R.R. KEMPEN, J.B. NASH and S. ELLIS: Life Sciences 25, No. 19, 1633 (1979).

GOLDSTEIN, B.D., M.G. ROZEN and C.A. SNYDER: Abstracts of papers, Society of Toxicology, Abstract No. 116 (1980).

GOLDSTEIN, B.D., C.A. SNYDER, S. LASKIN, I. BROMBERG, R.E. ALBERT and N. NELSON: Tox. Letters, In Press (1982).

IRONS, R.D. and B.J. MOORE: Rev. Comm. Chem. Pathol. Pharmcol. 27, 147 (1980).

KAHN, R.R. and D. NOVAK: J. Natl. Cancer Inst. 51, 683 (1973).

KAPLAN, H.S.: Cancer Res. 27, 1325 (1967).

LASKIN, S. and B.D. GOLDSTEIN: Benzene toxicity, a critical evaluation. J. Toxicol. Environ. Health Suppl. 2 (1977).

LEONG, B.K.J.: Experimental benzene intoxication. J. Toxicol. Environ. Health Suppl. 2, 45 (1977).

SNYDER, R. and J.J. KOCSIS: CRC Crit. Rev. Toxicol. 265 (1975).

SNYDER, C.A., B.D. GOLDSTEIN, A. SELLAKUMAR, S.R. WOLMAN, I. BROMBERG, M.N. ERLICHMAN and S. LASKIN: J. Toxicol. Environ. Health 4, 605 (1978).

SNYDER, C.A., B.D. GOLDSTEIN, A.R. SELLAKUMAR, I. BROMBERG, S. LASKIN and R.E. ALBERT: Toxicol. Appl. Pharmcol. 54, 323 (1980).

Accepted August 8, 1982