

Effect of Low Benzene Exposure on Neurobehavioral Function, AChE In Blood and Brain and Bone Marrow Picture in Mice

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The purpose of this study was to examine the effect of low level benzene exposure on neurobehavioral functions, AChE in blood and brain, bone marrow picture in Kunming mice. Forty adult Kunming male mice were divided into 4 groups. They were exposed to 12.52, 3.13, 0.78 and 0 ppm benzene for 2 h·d⁻¹ for 30 d. Central nervous system (CNS) function was inhibited by 12.52 ppm and excited by 0.78 ppm benzene exposure, but irregularly affected by 3.13 ppm. AChE in blood and brain was decreased in 12.52, 3.13 ppm group. The weight of liver to body weight ratios in 12.52 ppm group was higher than those of control group significantly. Bone marrow picture revealed inhibited proliferation of white and red cell systems, especially in 12.52 ppm group, consisting of decrease of percentage of myeloblast, promyelocytes, myelocytes, erythroblasts and megakaryocytes, especially in 12.52 ppm group.

INTRODUCTION

Benzene is widely present in industrial environment. Occupational exposure to benzene had widely occurred in China. Benzene exposure leads to dysfunction of hematological and nervous system in human. This fact has been reported by some authors (Ding *et al.*, 1984; Jiang, 1983; He and Zhou, 1986).

Currently, the acceptable level of benzene exposure in industrial environment in China had been decreased to 12.52 ppm, but in USA the corresponding level was 1.25 ppm. Therefore, the study of low-level benzene exposure has special significance.

In recent years, neurobehavioral toxicological method has been adopted in studying effects of benzene exposure. (Dempster *et al.*, 1984). But most experiments involved high exposure levels, not low level benzene exposures.

The purpose of this study was to examine mainly the effect of low level benzene exposure on neurobehavioral function. In addition, changes in AChE in blood and brain, bone marrow picture in mice were also studied.

MATERIALS AND METHODS

Animals and Exposures

Eighty Kunming adult male mice were housed in 16 cages, 5 mice in each. The laboratory was adapted to a 12 h light/dark cycle with light onset at 7 a.m.. The temperature and relative humidity were maintained at 22-25°C and 60±5% respectively, with food and tap water available *ad libitum*. Mice were quarantined for 10 d after arrival at the laboratory.

All inhaled benzene by the ~~exposure~~ exposure method using a ~~800 m³~~ 800 m³ plexiglass chamber. Control mice were exposed to air in another chamber simultaneously.

Benzene exposures were conducted for 2 h·d⁻¹ at 2-4 p.m. 6 d per week for one month, and the benzene concentrations of the four groups were ~~12.52, 3.13, 0.78 and 0~~ 12.52, 3.13, 0.78 and 0 ppm.

Measurements

Benzene level was monitored by ~~gas chromatography~~ gas chromatography every ~~60 min~~ 60 min for ~~the~~ at the beginning of experiment.

Four limb grip strength of mice was determined by persistent time (in seconds) while grasping a vertical iron stick. The Ellman method (Ellman, 1967) was used to determine AChE activity in blood and brain.

Apparatus

The apparatus GJ, 8502 (Tianjin Instrument Factory) was used for measuring locomotor activity. Interruptions of the photobeams by the mouse were counted with a computer.

The apparatus for neurobehavioral test in Y maze was made by Beijing Medical University. There were starting point and safety area. Motivation of mice was electrical shock, 40 volts (80 amperes).

Pretest Training

The time of running of mice from starting point to safety area was called response time. When the response time was within 3 s, it was scored as rapid response. Running smoothly from starting point to safety area within 10 s was scored as correct, running over 10 s or to another area was scored as wrong. Continuous training for 10 times of correct running constituted one cycle. This was done for 3 d.

After pretest training, only ~~40~~ 40 of 80 mice were screened chiefly by response time for neurobehavioral tests. These ~~40~~ 40 mice were randomly divided into 4 groups which were exposed to benzene at 12.52, 3.13, 0.78 and 0 ppm.

Neurobehavioral Test

After exposure to benzene mice were placed individually in maze, in which neurobehavioral function test was conducted. After this, locomotor activity was measured for 15 min and four limb grip strength was determined.

AChE activity in blood and brain (except cerebellum), and bone marrow picture (histologic method) were determined at the end of experiment.

Statistics

Statistical significance was evaluated by *t* value for four limb grip strength locomotor activity measurement, and by *U* value for rapid response frequency.

RESULTS

The most consistent effect of low level benzene exposure were shown by the four limb grip strength test. However, low level benzene exposure also affected frequency of rapid response, 15 min locomotor activity and AChE in blood and brain.

Decreased grip strength occurred at the highest exposure dose. Thus, the grip strength in 12.52 ppm group was lower than in control group, but grip strength in 3.13 and 0.78 ppm was higher than that in control group (Table 1).

The frequency of rapid response in mice exposed to 12.52 and 3.13 ppm was lower than control, but was significantly higher in mice exposed to 0.78 ppm (Table 2).

Decrease and increase of 15 min locomotor activity, in comparison with the control group, were observed in the 12.52 and 0.78 ppm respectively. But the differences was not significant (Table 3).

Decreases of AChE activity in blood and brain were noted with increase of benzene exposure dose, especially in the 12.52 and 3.13 ppm groups. AChE activity in brain of 0.78 ppm group was slightly higher than that of control group (Table 4). Significant differences of the ratios of organ to body weight occurred only with liver and spleen and only in the 12.52 ppm group compared to the control group (Table 5).

Bone marrow picture showed decrease of percentages of precursors of WBC and RBC with increase of the inhaled benzene level (Table 6).

Body weight and consumption of food and water in all exposure groups were not affected.

TABLE 1
Four Limb Grip Strength

Dose (ppm)	Strength (s)	t
0.00	70.96	—
0.78	125.71	10.077 ^a
3.13	96.71	6.161 ^a
12.52	63.09	1.987 ^a

^aP < 0.05, ^aP < 0.001.

TABLE 2
Rapid Response

Dose (ppm)	Frequency (%)	U
0.00	33.70	—
0.78	43.83	3.961 ^a
3.13	29.43	2.699 ^a
12.52	25.49	2.488 ^a

^aP < 0.05.

TABLE 3
Comparison of 15 Min Locomotor Activity

Dose (ppm)	Activity	t
0.00	659 ± 112.52	—
0.78	735 ± 108.81	1.460
3.13	666 ± 108.07	0.135
12.52	587 ± 103.74	1.411

$\bar{x} \pm SD$.

TABLE 4
AChE Activity in Blood and Brain

Dose (ppm)	Blood (IU · ml ⁻¹)	Brain (IU · g ⁻¹)
0.00	1.124 ± 0.364	11.790 ± 1.044
0.78	1.050 ± 0.365	12.181 ± 2.370
3.13	0.888 ± 0.212	10.584 ± 1.747
12.52	0.851 ± 0.302	10.248 ± 2.083 ^a

Note. IU = International units. $\bar{x} \pm SD$. ^a $P < 0.05$.

TABLE 5
Ratio of Organ and Body Weight

Dose (ppm)	Organ (wt / BW)			
	Brain	Liver	Spleen	Kidney
0.00	0.637 ± 0.120	4.713 ± 0.528	0.539 ± 0.173	1.546 ± 0.272
0.78	0.597 ± 0.044	4.961 ± 0.443	0.423 ± 0.123	1.636 ± 0.163
3.13	0.584 ± 0.059	4.830 ± 0.285	0.433 ± 0.106	1.510 ± 0.130
12.52	0.567 ± 0.074	5.273 ± 0.292 ^a	0.398 ± 0.094 ^a	1.693 ± 0.153

^a $P < 0.05$, compared with the control group.

TABLE 6
Comparison of BM Picture in Different Dose of Benzene Exposure

Dose (ppm)	Myel. blast	Pre-myel.	Myel. myel.	Meta.	Stab.	Seg.	Lym. blast	Lym.	Mono.	Megak.	Reti.	Ery. blast	Norm. W / R
0.00	3.2	2.5	3.3	7.2	39.0	0.0	0.6	31.5	4.2	0.1	3.0	2.0	15.07
0.78	2.9	2.9	1.3	4.8	50.6	0.2	1.6	27.8	3.1	0.2	0.8	0.8	20.83
3.13	2.5	2.4	3.3	9.0	38.2	0.0	0.9	28.1	4.9	0.0	2.1	0.3	11.04
12.52	0.3	0.9	0.9	1.7	56.3	0.0	0.5	32.6	4.1	0.0	0.0	0.0	41.84

Note. W / R = white / red blood cell system; Myel., myeloblast; Premyel., premyelocyte; Myel., myelocyte; Meta., metamyelocyte; Stab., stab. neutrophile; Seg., segment neutrophile; Lym. blast., lymphoblast; Lym., lymphocyte; Mono., monocyte; Megak., megakaryocyte; Reti., reticulum; Ery. blast., erythroblast; Norm., normoblast of erythrocyte.

DISCUSSION

Neurobehavioral function, four limb grip strength, 15 min locomotor activity, AChE in blood and brain, ratio of organ to body weight and bone marrow picture were all affected by benzene. However, the behavioral function changes were observed on the first 1 or 2 d of exposure. It shows that neurobehavioral function was affected the earliest. Locomotor activity was affected by benzene exposure, revealed elevation being increased in 0.78 ppm group, but depressed in 12.52 ppm group. These findings were different from the work of Dempster *et al.* (1984) in which the mice were exposed to much higher concentrations of benzene (100, 300, 1000, 3000 ppm). During the 15 min locomotor activity test, the mice exposed to 0.78 ppm benzene revealed irritability, but those exposed to 12.52 and 3.13 ppm benzene were depressed.

The decreased frequency of rapid response and increased frequency of slow response observed in this study suggest decreased response rate, hence depressed learning and memory functions.

In this study, stimulative effect was observed at 0.78 ppm benzene exposure on CNS function, but depressive effect at 12.52 and 3.13 ppm.

The mechanism of toxic effect on CNS function was not clear. Yamawaki *et al.* (1982) considered that neurotransmitters in CNS may be affected by mixed benzene with its metabolic products. Consequently AChE in blood and brain had been studied in our experiment.

AChE activity in blood and brain was affected by benzene exposure, the neurobehavioral effects may be related to the variation of AChE activity.

Based upon a large number of reports, adverse effect of benzene on hematological system has been demonstrated (Mason, 1989). But data of low level benzene exposure on bone marrow picture was less frequently studied. The present study demonstrates depression on hematopoietic function associated with relatively low level of benzene exposure.

The increase of organ weight to body weight ratios at 12.52 ppm benzene exposure, might be related to the hematological effect of benzene.

In summary neurobehavioral effect: muscle strength, rapid or slow response time, locomotor activity, and AChE activity in blood and brain, organ weight to body weight ratios and bone marrow picture changes occurred in low level benzene (12.52, 3.13, 0.78 ppm) exposure. The benzene induced neurobehavioral changes may be related to effects on neurotransmitter AChE.

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