DEPENDENCE OF THE FREQUENCY OF CHROMOSOMAL ABERRATIONS IN MOUSE BONE MARROW CELLS ON CONCENTRATION (DOSE) AND MODE OF ADMINISTRATION OF BENZENE

V. S. Zhurkov, E. G. Fel'dt, and V. V. Kosyakov

UDC 612.6.052:612.419.014.24:575.224.23/:547.532

KEY WORDS: benzene; bone marrow; cytogenetic effect.

Analysis of the mutagenic activity of atmospheric chemical pollutants has become an essential part of toxicologic investigations [1, 6]. Maximal allowable concentrations (MAC) of certain chemicals are still determined today without consideration of their mutagenic effects, although experiments on various test objects have demonstrated their ability to increase the frequency of mutations. One such compound is benzene, whose mutagenicity has been demonstrated in experiments on mammals [2, 3, 5] and in observations on workers in contact with it occupationally [7]. However, dependence of the effect on the concentration (dose) of benzene has not been analyzed previously although it is of the utmost importance for evaluation of the mutagenic risk of substances.

The aim of this investigation was to study dependence of the frequency of appearance of cells with chromosomal aberrations in mouse bone marrow on the concentration (dose) of benzene when inhaled or administered perorally.

EXPERIMENTAL METHOD

Chemically pure benzene (from Soyuzkhimreaktiv) was used. Experiments were carried out on noninbred male albino mice weighing 18-22 g, with 5 or 6 mice in each group. It was shown previously that to predict the cytogenetic effect of chemicals in mammalian bone marrow cells the adequate duration of the experiment is 5-10 days [4], and accordingly exposure to benzene was chosen within these limits.

For continuous inhalation the mice were placed in 10-liter exsiccators. Constancy of concentrations was ensured by the use of dosers of dynamic type. The benzene concentration was monitored daily by a chromatographic method. The mean benzene concentrations in the exsiccators throughout the period of the experiment were 13.9 ± 4.0, 36.8 ± 4.6, and 73.7 ± 9.7 mg/m^3. The duration of exposure was 7 days. The animals were killed 2 h after the end of exposure to benzene.

In experiments with peroral exposure the mice were given a solution of benzene in vegetable oil in doses of 5, 20, and 80 mg/kg (from 0.001 to 0.017 LD_{50}) by gastric tube 10 times with an interval of 24 h between doses. Control animals were given 0.2 ml of vegetable oil alone by gastric tube. The mice were killed 6 h after the last dose of benzene.

Colchicine solution in a dose of 2.5 mg/kg was injected intraperitoneally into the animals 2 h before scarifice. Preparations of metaphase chromosomes of bone marrow cells were obtained by the standard technique. For each mouse 100 metaphases were analyzed. Single and paired fragments and chromatid and chromosomal exchanges were counted. The distribution of metaphases with chromosomal aberrations of the various groups was determined by the χ^2 test. Regression coefficients were calculated by the method of least squares.

Laboratory of Medical Genetics and Laboratory of Atmospheric Air Hygiene, A. N. Sysin Research Institute of General and Communal Hygiene, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR G. I. Sidorenko.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 12, pp. 72-73, December, 1983. Original article submitted August 16, 1982.

TABLE 1. Frequency and Types of Chromosomal Aberrations in Bone Marrow Cells of Mice after Continuous Inhalation of Different Concentrations of Benzene

Average concentration of benzene, mg/m³	Number of mice	Number of cells	Number of cells with aberrations, %	Per 100 cells			
				single fragments	chromatid exchanges	paired fragments	total No. of aberrations
Control	5	500	0,8	0,6	0,0	0,2	0,8
13,9	5	500	2,2	2,0	0,0	0,2	2,2
36,8	5	500	2,6*	1,8	0,4	0,4	2,6
73,7	4	400	7,8†	7,8	0,3	1,8	9,8

Legend. *P < 0.05, †P < 0.01 compared with control.

TABLE 2. Frequency and Types of Chromosomal Aberrations in Bone Marrow Cells of Mice Receiving Different Doses of Benzene by Gastric Tube

Dose of benzene, mg/kg	Number of mice	Number of cells	No. of cells with aberrations, %	Per 100 cells			
				single fragments	chromatid exchanges	paired fragments	total number of aberrations
Control	6	600	0,5	0,7	0,0	0,0	0,7
5	5	500	1,6	1,6	0,0	0,0	1,6
20	5	500	3,8*	3,6	0,4	0,6	4,6
80	6	600	10,7*	11,8	0,2	0,5	12,5

Legend. *P < 0.01 compared with control.

EXPERIMENTAL RESULTS

The external appearance and behavior of the animals in all versions of the experiments were indistinguishable from those in the control. The levels of benzene studied, by both methods of administration, had no effect on mitotic activity in the bone marrow.

Data on the frequency and types of chromosomal aberrations in the bone marrow cells of mice inhaling benzene are given in Table 1. The frequency of cells with chromosomal aberrations increased with an increase in the benzene concentration. The minimal active concentration of benzene was 36.8 mg/m³ and the maximal inactive concentration was 13.9 mg/m³. Dependence of the frequency of cells with chromosomal aberrations (y) on the mean benzene concentration (x) was described closely by the straight line: $y = 0.536 + 0.089x$ ($F_{regression} = 18.4$; $F_{0.05;1;17} = 4.5$). The principal types of aberrations were single and paired fragments. Single chromatid exchanges were observed. There were no chromosomal exchanges.

The results of cytogenetic analysis of the mouse bone marrow cells after administration of benzene by gastric tube are given in Table 2. The frequency of cells with chromosomal aberrations (y) increased with an increase in the dose of benzene (x) and the relationship was described closely by the linear equation $y = 0.86 + 0.124x$ ($F_{regression} = 25.95$; $F_{0.05;1;20} = 4.35$). The minimal active dose was 20 mg/kg and the maximal inactive dose — 5 mg/kg. The relative proportions of the types of chromosomal aberrations were similar to those observed after inhalation of benzene.

The results of these experiments on mice confirm data [3, 8] on the cytogenetic activity of benzene for mammalian somatic cells after inhalation and peroral administration. Analysis of the effect—concentration (dose) dependence of benzene not only enabled the minimal active doses to be estimated, but also permitted an analytical description of these relationships, which was in the range of exposures studied to be described closely by a straight line.

On the basis of these results the allowable concentration (dose) of benzene causing the spontaneous mutation rate to increase by 1% compared with the control can be calculated [4]. Using regression levels, they were 0.06 mg/m^3 for inhalation and 0.07 mg/kg (1.4 mg/liter) for peroral administration. In accordance with the formula MAD/200 proposed by Zhurkov [4] (MAD denotes the minimal allowable dose of mutagen causing a significant increase in the mutation level in the experimental group compared with the control), the allowable concentration of benzene by inhalation was 0.18 mg/m^3, and by peroral administration 0.1 mg/kg (2 mg/liter). Comparison of the calculated allowable concentrations (doses) of benzene with the MAC of benzene in atmospheric air (0.1 mg/m^3) and in reservoir water (0.5 mg/liter) shows that their level is the same.

LITERATURE CITED

1. N. P. Bochkov, R. Ya. Shram, N. P. Kuleshov, et al., Genetika, No. 10, 156 (1975).
2. V. B. Dobrokhotov, Gig. Sanit., No. 10, 36 (1972).
3. V. B. Dobrokhotov and M. M. Enikeev, Gig. Sanit., No. 1, 32 (1977).
4. V. S. Zhurkov, in: Medical Problems of Environment Protection [in Russian], Moscow (1981), p. 88.
5. A. A. Lyapkalo, Gig. Tr., No. 3, 24 (1973).
6. G. I. Sidorenko, Gig. Sanit., No. 5, 4 (1981).
7. B. J. Dean, Mutat. Res., 47, 75 (1978).
8. J. Meyne and M. S. Legator, Environ. Mutagen., 2, 43 (1980).