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Chromosome aberrations in lymphocytes of mice after sub-acute low-level inhalation exposure to benzene

William W. **Au, V.M.S.** Ramanujam, J.B. Ward Jr. and **M.S.** Legator

Department of Preventive Medicine and Community Health, Division of Environmental Toxicology, University of Texas Medical Branch, Galveston. TX (U.S.A.)

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Summary

Male and female CD-1 mice were exposed to near ambient air concentrations of benzene by inhalation for 22 h per day, 7 days per week for-6 weeks. The concentrations were 0, 40, 100 and 1000 ppb. Significant increases in chromosome aberrations in spleen lymphocytes were observed in exposed compared with control mice except in the high-dose group ($p < 0.05$ for female mice in 2 experiments and for male mice in 1 experiment; $p < 0.15$ for male mice in the second experiment). A lack of increase in aberrations among mice of the high-dose group may be due to an induction of detoxifying enzymes as observed by us in a previous study (Au et al., 1988b). We also found that the female mice were more sensitive to the clastogenic activity of benzene than male mice under our experimental conditions. Our study serves to emphasize the need to conduct subchronic, low-dose in vivo genotoxicity studies using exposure conditions similar to those of humans, for evaluation of potential hazards. Our data suggest that the current occupational exposure concentrations for benzene (< 1000 ppb) may still be hazardous to humans.

Potential health hazards from exposure to mutagens/carcinogens are usually estimated by using data that are generated from human and animal studies. In experimental animal studies, the exposure conditions and the assayed endpoints can be well defined. However, in order to limit the size and complexity of the experiments, the exposure doses for in vivo genotoxicity studies are usually high and the animal exposure durations are short (i.e., acute exposure). Since these exposure conditions are not frequently encountered by the general population the extrapolation of information from experimental studies to predict risk in exposed populations may not be precise. In a recent report, Bailar et al. (1988) showed that even in the same experimental animal, the prediction of carcinogenic outcome from high to low doses cannot be made accurately. Therefore, suggestions have been made to conduct experimental studies by using doses that are as similar to human exposure doses as possible.

Correspondence: Dr. William W. Au, Department of Preventive Medicine and Community Health, Division of Environmental Toxicology, University of Texas Medical Branch, Galveston, TX 77550 **(U.S.A.)**

During the last few years, we have been conducting studies to determine genotoxic effects from exposure to near ambient concentrations of hazardous chemicals. In a previous report, we showed that exposure to 0.05–10 parts per million (ppm) of a chemical mixture containing benzene, chloroprene, epichlorohydrin and xylene caused a significant increase in chromosome aberrations in spleen lymphocytes of male mice (Au et al., 1988b). Subsequently, we attempted to determine the contribution by the individual components to the genotoxic effects. Since benzene is a priority environmental contaminant we have focused on the genotoxic effects of benzene.

Epidemiological studies have shown that exposure to benzene is correlated with the development of cancer in workers (Askoy et al, 1972; Rinsky et al., 1987). Experimentally, benzene is reported to be a multipotent carcinogen (Maltoni et al., 1989). Furthermore, carcinogenic concentrations of benzene can cause chromosome aberrations in experimental animals (Tice et al., 1980; Erexson et al., 1986; Rithidech et al., 1987; Au et al., 1990). Therefore, exposure to benzene remains a major health issue. However, the potential health problems to the general public and to workers who are exposed to lower doses of benzene than those studied previously remain unknown.

We have conducted a lymphocyte cytogenetic study in mice exposed to benzene for 6 weeks by inhalation. The concentrations were 0, 40, 100 and 1000 parts per billion (ppb). Significant increases in chromosome aberrations were detected.

Materials and method

Inhalation apparatus

The inhalation set up that was used in the current study is similar to the one described previously (Au et al., 1988b; Harper et al., 1989).

Animal use and care

Male and female CD-1 Swiss mice of 19–21 g each were purchased from Charles River Laboratories, Wilmington, MA. All mice were certified by the supplier to be negative serologically for 16 common viruses and negative for 6 bacterial and 2 parasitic infections. Mice were acclimatized in standard laboratory animal housing conditions for at least 1 week before being used for study.

Inhalation exposure

Compressed air was purified by passage through a filter and a heatless air dryer (Vacuum Technology, Austin, TX) to remove particulate, moisture and organic contaminants. The air stream was split into 4 channels and the flow rate was regulated for each channel at 12 l/min by mass flow controllers for delivery to the mixing chambers for dilution of benzene.

Cylinders of benzene were purchased from Big Three Industries (Houston, TX). Stock gas was diluted with compressed air to achieve appropriate concentrations. The diluted gas mixtures were delivered to 4 exposure chambers for each concentration level with an equal flow rate of 31/min. Mice were exposed to benzene continuously for 22 h per day, 7 days per week for 6 weeks. The remaining 2 h of each day were used to clean the chambers and to inspect the condition of the mice.

Two experiments were conducted. In the first one, mice were exposed to 0, 100 and 1000 ppb of benzene for 6 weeks. There were 6 mice per exposure chamber. Exposed animals totaled 12 mice per sex per dose. In the second experiment, mice were exposed to 0, 40, 100 and 1000 ppb for 6 weeks. There were 7 mice per chamber. Twelve mice per sex per dose were used for genotoxic analysis. The remaining mouse in each chamber in the second experiment was used to conduct other tests, e.g., histological studies and analysis for the presence of hepatitis virus.

Animal sacrifice

Mice from the first experiment were injected with colchicine (0.15 ml containing 150 mg per mouse) at 4 h before sacrifice in order to block dividing cells in bone marrow, lung and testes. Subsequently, we found that vinblastine sulfate was more efficient in blocking mitotic lung macrophage cells (Au et al., 1988a). Therefore mice in the second experiment were exposed to vinblastine sulfate for 4 h before sacrifice. Mice were killed by an overdose injection of 0.2 ml Nembutal (50 mg/ml concentration). Lymphocytes obtained from the spleen of each mouse were used for cytogenetic analysis. The data are presented in

this report. Data from assays using other cell types will be reported separately.

Spleen lymphocyte cyiogenetic assay

The procedures used to isolate lymphocytes from spleens and to culture cells have been described in detail previously (Au et al., 1988b). Phytohemagglutinin was used to stimulate lymphocytes to proliferate for our cytogenetic analysis. At 42.5 h after initiation of cultures, 0.05 ml Colcemid (1 mg/ml stock conc.) was added to each tube of 4-ml cultures. 1.5 h later, cells were harvested and cytological preparations were made. Slides were coded and analyzed microscopically to determine the presence of chromosome aberrations. A maximum of 100 metaphases were analyzed per mouse.

Statistical analysis

Data were evaluated using the Kruskal–Wallis and the Mann–Whitney U test as described previously (Au et al., 1988b).

Results

Exposure conditions

The delivered chemical concentrations were sampled at the inlet of each chamber. During the second experiment, samples were also collected inside each chamber to determine the chemical concentrations. As shown in Table 1, the expected and observed benzene concentrations are in close agreement.

The purity of benzene was determined using capillary gas chromatography. A small peak of contaminant was observed in the compressed air which was used for the first experiment. The identity of the contaminant was not determined. During the second study, no such contaminant was detected.

Animal conditions

The basic condition of the mice during the 6 weeks of exposure was as good as we had found in our previous study (Au et al., 1988b). All mice gained weight during the course of our study. None of the mice showed any signs of discomfort such as lethargy or roughness of hair. There was no indication of hepatitis virus infection.

TABLE 1

EXPECTED AND OBSERVED CONCENTRATIONS OF BENZENE INSIDE THE INHALATION CHAMBERS

Concentration (ppb)		
Expected	Inlet [a]	Inside [b]
40	43.4 ± 0.48 (n = 144)	34.2 f0.92 (n = 160)
100	103.4 ± 1.56 (n = 172)	87.8 + 2.62 (n = 152)
1000	1018 ± 32.6 (n = 152)	858 + 38.4 (n = 144)

Concentrations were determined by collecting benzene vapors in ORBO-100 charcoal tubes and using the NIOSH (1977,1984) procedure.

[a] **Determined from the vapors collected at the cage inlets.**

[b] **Mean of determined values at 3 zones: (1) near the animals' breathing zone, (2) near the wall of the chamber, and (3) near the bottom of the chamber where the feces accumulate.**

n = number of samples collected.

Chromosome analyses

In the first experiment, 50 metaphase cells were analyzed per mouse for the presence of chromosome aberrations. In the second experiment, attempts were made to score more cells. Up to a maximum of 100 cells per mouse were analyzed. A summary of the data is shown in Table 2. Dose-dependent increases in chromosome aberration frequencies were observed in both sexes (2.0 ± 0.4, 6.4 ± 1.6 and 9.0 ± 0.3 per 100 cells for males and 2.5 ± 0.6, 8.2 ± 1.6 and 8.7 ± 2.7 for females for 0, 100 and 1000 ppb benzene). One-way analysis of variance showed that the significance of difference between the exposed and control groups was $p < 0.05$. In female mice, the increase in aberrations after exposure to 1000 ppb was much moderated compared to the increase in the 100-ppb dose group. The moderation of dose–response curves at high doses was reproduced in the second experiment for both sexes. In fact, for both male and female mice, the frequencies of aberrations in mice exposed to 1000 ppb were lower than in those exposed to lower concentrations of benzene (40 and 100 ppb). The aberration frequencies per 100 cells for male mice were 2.6 ± 0.6, 4.7 ± 1.2, 4.0 ± 1.1 and 2.0 ± 0.7 for 0, 40, 100 and 1000 ppb respectively. One-way analysis of variance showed that the significance of the difference between the exposed and control groups was $p < 0.15$. The

TABLE 2

CHROMOSOME **ABERRATIONS IN SPLEEN LYMPHOCYTES OF MICE EXPOSED** TO **BENZENE** [a]

Concentrations (ppb)	Number of mice analyzed	Number of cells scored	Chromatid breaks/ 100 cells ± SEM
Experiment 1			
Male			
0	12	600	2.0 ± 0.4 *
100	11	550	6.4 ± 1.6 *
1000	10	500	9.0 ± 0.3 *
Female			
0	11	550	2.5 f0.6 *
100	12	600	8.2f1.6*
1000	11	550	8.7 ± 2.7 *
Experiment 2			
Male			
0	12	926	2.6 ± 0.6 **
40	11	1050	4.7f1.2**
100	8	449	4.0 ± 1.1 **
1000	11	1100	2.0f0.7 **
Female			
0	11	1100	0.4 ± 0.2*
40	6	417	1.7 ± 0.5 *
100	12	1028	2.7f0.8 *
1000	11	1003	1.4 ± 0.4 *

[a] **Mice were exposed by inhalation for 22 h per day, 7 days per week for 6 weeks. One-way analysis of variance shows that the significance of difference between exposed and control groups is * $p < 0.05$, ** $p < 0.15$.**

aberration-frequencies for female mice were 0.4 ± 0.2, 1.7 ± 0.5, 2.7 ± 0.8 and 1.4 ± 0.8 for the 4 concentrations of benzene. Statistically significant differences between exposed and control groups were detected ($p < 0.05$).

Discussion

Our data show that exposure to near ambient concentrations of benzene induces significant increases in chromosome aberrations in lymphocytes of CD-1 mice. In our study, mice were exposed to 0, 40,100 or 1000 ppb benzene for 22 h per day, 7 days a week for 6 weeks. By comparison the recent occupational permitted exposure limit (PEL) is 1000 ppb, and ambient air concentrations of benzene are generally in the range **of** 1–15 ppb with some higher values reported (Nisbet et al., **1984;** Rogers et al., **1988).** Our results suggest, therefore, that exposure to the new occupational standard of benzene may still be hazardous to workers.

In general, the results from our 2 experiments are consistent. The difference in the shape of the dose–response curves may be due to a shift of the curve towards the left for the first experiment. In comparing the conditions for the **2** experiments, we believe that the data from the second experiment are more reliable. In addition, in the second experiment, the unidentified minor contaminant in the air used for dilution of benzene was absent, more doses were used, more cells were scored, the benzene concentrations were better characterized and the health of the mice was better documented than in the first experiment. **A** graph which summarizes the data from both experiments is **shown** in Fig. 1. Our observations serve to emphasize the complexity of responses from repeated exposures to hazardous agents. Complex responses were also reported by us in a previous study using very low doses of a mixture of chemicals **(Au** et **al.,** 1988b)

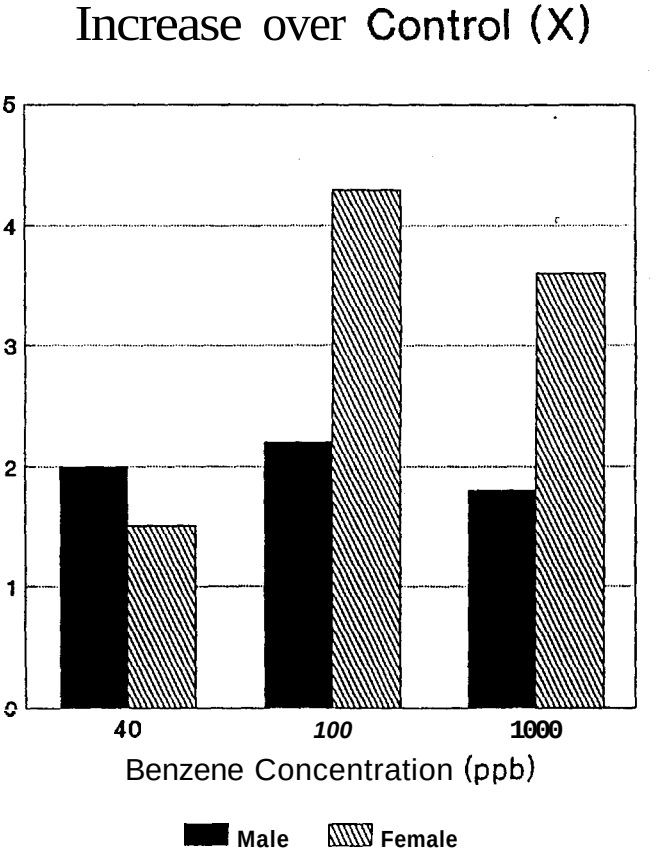

Fig. 1. **Increase of chromosome aberrations in exposed over control animals. Data from 2 experiments are combined.**

and by others using high doses of benzene (Snyder et al., 1988; Henderson et al., 1989). Similarly complex dose–response relationships for carcinogenesis are described by Bailar et al. (1988).

The shape of the dose–response curve in the induction of chromosome aberrations by benzene was unexpected. With the exception of the response in male mice in the first experiment, the response to 1000 ppb of benzene was less than expected. In addition, a reduced response was observed in both sexes in the second experiment. The reduced response may be due to a shift in splenic lymphocyte populations from the exposure, however this explanation is likely if the mice were exposed to high and acute doses of benzene. On the other hand, the observed phenomenon is more likely due to an induction of detoxifying enzymes as documented by us in our previous study of mice exposed to a mixture of chemicals (Au et al., 1988b). Although benzene has not been demonstrated to be capable of inducing detoxification enzymes it has been shown to induce its own metabolism at high doses (Gollmer et al., 1984). Therefore, the suggested detoxification mechanism may exist in mice under our exposure conditions.

We are not aware of other experimental studies using doses as low as ours. Nevertheless some studies also document more severe effects from exposure to low than to high doses of benzene. Snyder et al. (1988) reported that lifetime exposure, even at a lower concentration (300 ppm), is more tumorigenic than a short-term exposure (1200 ppm) to benzene. On the other hand, Henderson et al. (1989) observed that more toxic metabolites are formed in mice at lower exposure concentrations.

In our second experiment, the relative increase of chromosome aberrations over concurrent control was higher for female mice than for male mice. The sex differences observed after our subacute low-dose exposure is the reverse of responses seen after acute high-dose exposure as reported by Gad-El Karim et al. (1986). The apparent discrepancy may be due to the difference in exposure conditions, such as route and levels of exposure. The female sensitivity is confirmed by us in the *hprt* gene mutation assay using lymphocytes from the same exposed mice (manuscript in preparation). Female specific sensitivity has been reported after exposure of rodents to lindane. In these studies, more tumors were found in females than in males (Smith et al., 1985).

In summary, our study shows that the current occupational exposure limit for benzene may still be hazardous. Cellular responses from repeated exposures to hazardous agents in vivo are more complex than predicted from results after acute exposure. The responses after repeated exposures are dependent upon induction of metabolizing enzymes and detoxifying enzymes. The responses are also sex-dependent. Therefore, these results are unlikely to be predicted or duplicated by using cells in culture. More emphasis should be placed upon conducting studies using animals exposed sub-acutely to low doses of potentially hazardous agents. Unless experimental data are generated by exposing animals under conditions similar to those of humans the extrapolation of animal data to humans for prediction of risk may not be precise.

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