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Induction of Cytogenetic Damage in Rodents After Short-Term Inhalation of Benzene

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Experiments were designed to investigate both the induction of sister chromatid exchanges (SCEs) in peripheral blood lymphocytes (PBLs) and micronuclei (MN) in bone marrow polychromatic erythrocytes (PCEs) of mice and rats after inhalation of benzene (BZ). Male DBA/2 mice (17–19 weeks old) were exposed to target concentrations of either 0, 10, 100, or 1,000 ppm BZ for 6 hr. Male Sprague-Dawley rats (11–14 weeks old) were exposed to target concentrations of either 0, 0.1, 0.3, 1, 3, 10, or 30 ppm BZ for 6 hr. Blood was obtained by cardiac puncture 18 hr after exposure, and PBLs were cultured in the presence of lipopolysaccharide (mouse B cells, 60 μg/ml) or concanavalin A (rat T cells, 30 μg/ml) to stimulate blastogenesis for SCE analysis. Femoral bone marrow smears from both species were analyzed for MN in PCEs 18 hr after BZ exposure. Mouse PBLs revealed a significant concentration-related increase in the SCE frequency over controls at 10, 100, or 1,000 ppm BZ. Mouse bone marrow showed a significant concentration-dependent increase in MN over controls after exposure to 10, 100, or 1,000 ppm BZ. Rat PBLs showed a significant increase in the SCE frequency after exposure to 3, 10, or 30 ppm BZ. The statistical significance of the 1 ppm BZ result was borderline and dependent on the statistical test chosen. Rat cells revealed a significant concentration-related increase in MN after inhalation of either 1, 3, 10, or 30 ppm BZ. PBLs from treated mice showed significant concentration-dependent decreases in mitotic indices; however, cell cycle kinetics and leucocyte counts remained unaffected. Rat PBLs showed significant decreases in mitotic activity only after exposure to 3 and 30 ppm BZ, whereas cell cycle kinetics and leucocyte counts were unaffected. These results show that BZ can induce statistically significant cytogenetic effects in PBLs and PCEs of both mice and rats after a 6-hr inhalation of BZ at low concentrations.

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INTRODUCTION

Benzene (BZ) has been implicated as a human leukemogen [Goldstein, 19771. Long-term bioassays using rats or mice exposed to BZ either by inhalation or gavage have resulted in the induction of tumors. Snyder et al [1980] saw a significant increase in the frequency of hematopoietic neoplasms including thymic lymphoma in C57Bl/6 mice inhaling 300 ppm BZ. Goldstein et al [1982] found an increased incidence of leukemias in both Sprague-Dawley rats and CD-1 mice inhaling 100 or 300 ppm BZ. The finding of myelogenous leukemia in the strains of rodents tested suggests that BZ might have played a role in the induction of leukemia, although the increase was not statistically significant [Goldstein et al, 1982].

Maltoni et al [1983] reported significant increases of Zymbal's gland carcinoma, carcinoma of the oral cavity, hepatocarcinoma, and lymphoreticular neuplasias in Sprague-Dawley rats after inhalation of BZ. Cronkite et al [1984] observed a significant increase in the frequency of lymphoma in female C57Bl/6 mice inhaling 300 ppm BZ for 16 weeks. The National Toxicology Program [1984] has reported that B6C3f1 mice exposed to BZ by gavage showed significant increases of Zymbal's gland carcinoma, lymphoma, alveolar/bronchiolar carcinoma, and alveolar/bronchiolar adenomas. In addition, the same study reported that Fischer-344N rats exhibited increased frequencies of Zymbal's gland carcinoma, squamous cell papillomas and squamous cell carcinomas of the skin, and squamous cell papillomas and squamous cell carcinomas of the oral cavity.

Epidemiological studies have demonstrated that BZ is clastogenic in peripheral blood lymphocytes of occupationally exposed workers [eg, Tough et al, 1970; Forni et al, 1971; Rinsky et al, 19811. In addition, there is limited information regarding the immunotoxic [Baarson et al, 1984; Rozen et al, 1984] and chromosome-damaging [Cortina et al, 1982; Tice et al, 1982; Styles and Richardson, 1984] effects of inhaled BZ on the rodent hematopoietic system at concentrations relevant to human exposure. DBA/2 male mice were used in the present study because this strain and sex are reported to be sensitive to BZ-induced genotoxicity and cytotoxicity [Tice et al, 1982; Snyder et al, 1983]. B lymphocytes were chosen because they are sensitive indicators of BZ-induced immunotoxicity [Irons and Moore, 1980; Rozen et al, 19841, and polychromatic erythrocytes (PCEs) were selected because they are sensitive indicators of BZ-induced genotoxicity [Hite et al, 1980; Meyne and Legator, 1980; Siou et al, 1982; Toft et al, 1982].

To investigate species and cell-type susceptibility to the effects of BZ, similar studies were done with peripheral blood T lymphocytes and bone marrow PCEs of male Sprague-Dawley rats. Preliminary experimental data showed increased cytogenetic damage after exposure to nominal concentrations of 1 and 3 ppm BZ. Therefore, a second and more definitive experiment was conducted in rats in which concentrations of 0.1, 0.3, 1, and 3 ppm BZ were monitored analytically to confirm the previous findings and accurately define the shape of the dose-response curve at low doses of BZ.

MATERIALS AND METHODS

Animals

Eight- to ten-week-old male DBA/2 mice and Sprague-Dawley rats were obtained from Charles River Breeding Laboratories (Kingston, NY) and placed in quarantine for 2 weeks. Sprague-Dawley rats are commonly used for bioassays [Goldstein et al, 1982; Maltoni et al, 1983] and are desirable for the rat micronucleus (MN) test because of the relatively low numbers of mast cells present in their bone marrow [Gollapudi et al, 19831. The housing conditions and diet were as previously described [Erexson et al, 1983; Kligerman et al, 19831. In the first experiment, male DBA/2 mice (17–19 weeks old) were exposed to atmospheres of 10, 100, or 1,000 ppm BZ for 6 hr in groups of five mice per chamber. In a second experiment, male Sprague-Dawley rats (11–14 weeks old) were exposed to atmospheres of 1, 3, 10, or 30 ppm BZ for 6 hr in groups of five rats per chamber. &cause of initial problems in measuring the 1 and 3 ppm exposures analytically, a third experiment was done to confirm the positive cytogenetic effects seen at these concentrations. In addition, 0.1 and 0.3 ppm BZ concentrations were tested. Mice (three or four per cage) and rats (five per cage) were exposed concurrently with each benzene exposure concentration to chamber room air to serve as controls. Temperature (23.5 ± 0.3°C) and humidity (52.5 ± 5.6%) were monitored each hour during exposures.

Benzene Exposures

Food and water were not given to any of the animals during the 6-hr exposures. Animals were exposed in a 102-liter glass and Teflon chamber [Barrow and Steinhagen, 19821 with a 20-liter/min air flow. Generation of all concentrations except 1,000 ppm was done by metering a BZ-nitrogen mixture (Matheson Compressed Gas, Morrow, GA) from a cylinder to the chamber air supply inlet. Generation of the 1,000 ppm concentration was done by metering liquid BZ with an FMI pump (Fluid Metering Inc., Oyster Bay, NY) at 0.072 ml/min into a heated flask. Chambers were analyzed hourly by continuous monitoring with a Miran 1A infrared gas analyzer (Foxboro Analytical, S. Norwalk, CT) for the 10, 30, 100, and 1,000 ppm BZ concentrations. The 0.1, 0.3, 1, and 3 ppm BZ exposures were analyzed two or three times per hour with a model GC-8APF gas chromatograph (Shimadzu Scientific Instruments, Columbia, MD) equipped with a flame ionization detector. All BZ mixtures were analyzed and certified by Matheson.

Lymphocyte Culture and Slide Preparation

Lymphocyte culture methodology and slide preparation for sister chromatid exchange (SCE) analysis were as previously described [Kligerman et al, 1982; Erexson et al, 1983; Wilmer et al, 19831. Briefly, blood was removed by cardiac puncture from anesthetized animals 18 hr after exposure to BZ, and lymphocytes were cultured in the presence of either 60 μg lipopolysaccharide/ml (mouse B cells) or 30 μg concanavalin A/ml (rat T cells) to stimulate blastogenesis. Peripheral blood leucocyte counts were determined from a 20-μl aliquot of washed blood with a Coulter counter as previously described [Kligerman et al, 19821. 5-Bromo-2′-deoxyuridine (mice, 2 μM; rats, 4 μM) was added at 24 hr after culture initiation, and the cultures were harvested at either 52 hr (rats) or 60 hr (mice) following a 4-hr demecolcine (1.35 μM) treatment.

Preparation and staining of slides of femoral bone marrow were a modification of the technique described by Schmid [1976]. The same animals (18 hr after BZ exposure) that were used for the SCE studies were used for the MN analysis. May-Grunwald and Giemsa stains (Bio/medical Specialties, Santa Monica, CA) were used for mouse bone marrow smears. Acridine orange (Polysciences Inc., Warrington, PA) was used to stain rat bone marrow because there is a potential problem of artifacts caused by scoring mast cell granule inclusions as MN when using the classical Giemsa staining technique [Hayashi et al, 19831.

Slide Analysis

Two or three slides were prepared per animal for the SCE experiments. Slides from five treated and three to five concurrent control animals were coded, combined, and randomized prior to cytogenetic analysis. Each exposure concentration and its concurrent control were analyzed separately. For the MN experiments, four slides were prepared per animal. Slides were coded, combined, and randomized as described for the SCE experiments. One to four bone marrow smears were analyzed per animal for MN. The same scorer quantitated the frequencies of SCE and MN to alleviate possible scorer-to-scorer variability. Only second-division metaphases containing the diploid number of chromosomes were used for the SCE analyses. Twenty-five seconddivision metaphases and 1,000–2,000 PCEs were analyzed from each animal for SCE and MN frequencies, respectively, unless noted otherwise. One thousand nuclei and 100 metaphases were scored consecutively for mitotic index and cell cycle kinetics, respectively.

Statistical Analysis

Concurrent control values for the MN frequencies were compared to those of the BZ-exposed groups using Student's t test (one-tailed) [Snedecor and Cochran, 19671. Customarily, the same procedure would be applied to the animal-to-animal mean SCE frequencies. However, analysis of the SCE data revealed that the animal-to-animal variation was consistently less than expected if cell-to-cell variation within animals were the only source of variability. At the present time there is no clear explanation for this unexpected result. Similar observations, however, have also been seen in other data sets from our laboratory. Accordingly, both cell-to-cell and animal-to-animal variability are reported, but the larger cell-to-cell variation was used in the statistical analyses at the individual metaphase level. In addition to the parametric t test, the nonparametric Mann-Whitney U test [Lehmann, 1975] was used, because the SCE distribution was not normal in some instances. The chosen level of significance was 0.05. The raw data are given in Tables II and IV to present a complete view of the experimental results and to facilitate independent assessments of the data.

RESULTS

Mice

BZ induced significant, concentration-related increases in the SCE frequencies at all exposure concentrations examined (Tables I and 11). The concentration-related SCE increase is particularly apparent in Table 11 as a shift towards cells exhibiting higher SCE frequencies and a concomitant decrease in metaphases showing low SCE frequencies. In addition, BZ caused a significant, concentration-dependent decrease

TABLE I. The Induction of Cytogenetic Damage in Male DBA/2 Mice and Sprape-Dawley Rats

Benzene (ppm/hr)	Number of animals	SCEs/metaphase[a]	Number of MN/1,000 PCEs[a]
DBA/2 mice			
0	10 (SCE), 11 (MN)	5.9 ± 0.2 (0.14)[b]	2.1 ± 0.3
10.3 ± 0.9	5	7.6 ± 0.2 (0.17)*,**	9.0 ± 0.6'
100.1 ± 1.6	5	9.5 ± 0.2 (0.23)*,**	20.3 ± 0.7'
991.2 ± 5.9	5	13.8 ± 0.9 (0.37)*,**	28.1 ± 0.8'
Sprague-Dawley rats			
Experiment 1			
0	10	8.6 ± 0.1 (0.17)	2.0 ± 0.1
10.0 ± 0.2	5	10.4 ± 0.2 (0.27)*,**	6.2 ± 0.4'
30.0 ± 0.3	5	11.1 ± 0.1 (0.23)*,**	7.6 ± 0.4*
Experiment 2			
0	20	8.2 ± 0.1 (0.13)	1.7 ± 0.2
0.1 ± 0.01	5	8.2 ± 0.1 (0.24)	1.6 ± 0.3
0.3 ± 0.02	5	8.2 ± 0.1 (0.19)	2.0 ± 0.3
1.0 ± 0.02	5	9.1 ± 0.2 (0.28)'	4.4 ± 0.3"
3.0 ± 0.05	5	10.5 ± 0.2 (0.25)*,**	4.8 ± 0.4'

[a]Mean ± standard error among animals within a group.
[b]The numbers in parentheses denote the standard error among cells within a group.
'Significantly different from the concurrent controls ($P < 0.05$) using Student's t test (one-sided).
**Significantly different from the concurrent controls ($P < 0.05$) using Mann-Whitney U test (one-sided).

in the mitotic activity but did not significantly affect the leucocyte counts (Table III). No significant effect was seen on the cell cycle kinetics at any exposure concentration examined (data not shown). Femoral bone marrow smears made from the same mice showed that BZ caused significant, concentration-related increases in the number of PCEs containing MN of up to 13.4 times the control value at 1,000 ppm (Tables I and II). The concentration-response curves for SCE and MN are similar in shape.

Rats

BZ induced significant, concentration-dependent increases in the SCE frequency after a 6-hr exposure to 3, 10, or 30 ppm (Tables I and IV). The concentration-related SCE increase is manifested primarily as an increase in the number of cells exhibiting higher SCE frequencies, which appear as "outliers". The significance of the effect seen on SCE induction at 1 ppm is borderline and dependent on the statistical test chosen. The SCE-inducing effect of 1 ppm BZ is statistically significant using the t test (one-sided; $P = 0.036$), but not statistically significant using the Mann-Whitney U test (one-sided; $P = 0.055$). The SCE frequency for the 1-ppm BZ exposure does fall in line between the SCE frequencies observed for the 0.3 and 3 ppm exposures, and all rats exposed to 1 ppm BZ had a higher SCE frequency than all their concurrent controls. Atmospheres of 0.1 or 0.3 ppm BZ did not significantly affect the SCE frequency. Whereas exposure of rats to 0.1, 0.3, 1 or 10 ppm BZ revealed no significant change in mitotic activity, both 3 and 30 ppm BZ caused a significant decrease in mitotic activity (Table III). In addition, no significant effect was observed on the leucocyte counts (Table III) or cell cycle lunetics (data not shown) at any exposure concentration studied.

Femoral bone marrow smears prepared from the same rats revealed that BZ induced significant, concentration-related increases in the number of PCEs containing

TABLE II. Frequency Distribution of the Raw Data Obtained From the Sister Chromatid Exchange (SCE) Assay and Micronucleus (MN) Test Using Male DBA/2 Mice Exposed to Benzene

Benzene (ppm)	N[a]	1[b]	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	≥21[c]	SCE/cell (mean)	SCE/cell (SD)	MN/ 2,000 PCEs[d]
0[e]	25		1				6	4	2	1	1												5.72	1.82	2
0[e]	25		1				3	3	4	1	1												5.88	1.90	4
0[e]	25		3	2	3	3	2	6	3		3												5.88	2.47	3
0[f]	25		1		6	6	3	4	4		1												5.76	1.83	7
0[f]	25			1	4	5	7				2	1											6.08	1.96	4
0[f]	25			3	4	7	6			2	1												5.56	1.87	8
0[f]	ND																						—	—	2
0[g]	25		1		6	4	8		2		1	1	2										6.20	2.61	2
0[g]	16			4	4	1	4	2			1												5.06	1.95	5
0[g]	25	1		1	5	7	1	5	1	2	1		1										5.92	2.41	7
0[g]	14		1	1	1	3		2	1	3	1			1									6.86	3.03	3
10	25				2	2	3	3	7	5	3												7.52	1.78	19
10	25					1	4	4	7	1	5	2	1										8.20	1.85	22
10	22				1	2	4	5	5	3	2												7.27	1.61	15
10	25				2	4	2	3	8	1	4		1										7.44	2.08	18
10	25					2	6	7	5	1	2	1			1								7.56	2.02	16
100	25						2	2	5	7	7	1		1									8.92	1.55	43
100	25			1	1			2	4	7	6	1	2						1				9.16	2.75	44
100	25						3	2	3	3	2	2	5	2	3								10.12	2.65	39
100	25				1	1		1	6	3	6	4	2		1								9.32	2.17	41
100	25			1	1		3	1	4	2	6		2		4	1							9.48	3.23	36
1,000	25								1				5		3	3	4	2	3		2	21,22	15.60	3.30	54
1,000	25										2		3	2	3	1	5	1	2	1	3	22,24	15.88	3.63	52
1,000	25						2		2		3		4	2	4	2	4			1	1		12.84	3.60	55
1,000	15				1	1					2		3		2	1	2	2	1				12.80	4.20	61
1,000	25				1		2	1	1	2	3	1	4	3	2	1	2	2					11.44	3.55	59

[a]Total number of cells for a given concentration.
[b]Denotes the number of SCEs/metaphase.
[c]This column contains the actual SCE frequency of each cell having 21 or more SCEs.
[d]Denotes the number of micronuclei per 2,000 bone marrow polychromatic erythrocytes.
[e]Concurrent controls for the 10 ppm exposure experiment.
[f]Concurrent controls for the 100 ppm exposure experiment.
[g]Concurrent controls for the 1,000 ppm exposure experiment.
ND, not done.

TABLE III. The Effects of Inhaled Benzene on Mitotic Indices and Leucocyte Counts of Male DBA/2 Mice and Sprague-Dawley Rats

Benzene (ppm/hr)	Number of animals	Mitotic index (%)[a]	Leucocytes/ml of whole blood (10^6)[a]
DBA/2 mice			
0	10	4.4 ± 0.3	4.13 ± 0.5
10.3 ± 0.9	5	2.9 ± 0.2'	3.20 ± 0.2
100.1 ± 1.6	5	2.3 ± 0.6'	5.57 ± 1.0
991.2 ± 5.9	5	1.0 ± 0.1'	4.94 ± 0.4
Sprague-Dawley rats			
Experiment 1			
0	20	4.6 ± 0.1	14.80 ± 0.7
10.0 ± 0.20	5	3.7 ± 0.3	14.70 ± 0.9
30.0 ± 0.30	5	3.5 ± 0.2'	15.00 ± 2.2
Experiment 2			
0	20	5.1 ± 0.2	14.10 ± 0.7
0.1 ± 0.01	5	6.1 ± 0.2	14.60 ± 1.9
0.3 ± 0.02	5	5.6 ± 0.2	12.40 ± 1.0
1.0 ± 0.02	5	5.5 ± 0.1	14.00 ± 2.7
3.0 ± 0.05	5	3.9 ± 0.1*	15.60 ± 0.8

[a]Mean ± standard error among animals within a group.
*Significantly different from the concurrent controls ($P < 0.05$) using Student's t test (one-tailed).

MN at concentrations from 1 to 30 ppm (Tables I and IV). As with the SCE frequency, there were no observed effects at 0.1 or 0.3 ppm. The concentration-response curves for the rat reveal that the SCE and MN responses are similar in shape. Observations at 10 ppm show that the responses for induced SCE frequencies are similar for both the rat and mouse, whereas mouse PCEs appear to be somewhat more sensitive to the MN-inducing effects of BZ.

DISCUSSION

These results confirm and extend the work of several investigators, who have also observed cytogenetic effects from low-dose BZ exposure on mouse bone marrow. Hite et al [1980] reported significant increases in the number of PCEs with MN in both male and female CD-1 mice given 125 μl BZ/kg/day by gavage and sacrificed 18 hr after the second dose. Similarly, Meyne and Legator [1980] found that male CD-1 mice exhibited higher frequencies of chromosome aberrations and MN than females after exposure to 500 μl BZ/kg/day by gavage or intraperitoneal injections at 24-hr intervals for 3 consecutive days. Also, Siou et al [19821 observed a significant increase in PCEs containing MN from male Swiss mice after two gavages of 25.6 μl BZ/kg/day at 24-hr intervals. Toft et al [1982] found that NMRI mice exposed to 14 ppm BZ by inhalation continuously for 1–8 weeks had a significant increase in PCEs containing MN.

Tice et al [1982] have demonstrated significant increases of SCE after a 4-hr inhalation exposure to 28 ppm BZ in both male and female DBA/2 and C57Bl/6 mice (28 ppm × 4 hr = 112 ppm × hr), which represents the previously reported lowest cumulative concentration of BZ to cause a significant induction of SCE in the bone marrow. Tice et al [1982] observed an induced SCE frequency in male DBA/2 mice of 3.1, 8.4, 11.9, and 17.9/metaphase at 112, 1,960, 4,072, and 9,541 ppm × hr BZ,

TABLE IV. Frequency Distribution of the Raw Data Obtained From the Sister Chromatid Exchange (SCE) Assay and Micronucleus (MN) Test Using Male Sprague-Dawley Rats

Benzene (ppm)	N[a]	1[b]	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	≥21[c]	SCE/ cell (mean)	SCE/ cell (SD)	MN/ 1,000 PCEs[d]
0[e]	25		1			1	1	6	6	2	2	2	3	1									8.48	2.50	1
0[e]	25			1	2	2	5	4	1	5	1		1	1	1	1							7.84	3.12	2
0[e]	25			1	1	2	3	4	2	4	6	1	1										7.92	2.31	3
0[e]	25		1		4	3	1	1	5	2	1	2	1	1	3								8.12	3.56	2
0[e]	25			1		3	4	4	5	2	2	1		2			1						8.04	2.92	0
0[f]	25			2	1	2	1	1	7	4	4	2	1										7.96	2.44	2
0[f]	25				3	3	1	2	3	5	4		2			1						9	9.00	5.17	2
0[f]	25				2	4	3	2	4	4	1	2	3										7.84	2.54	0
0[f]	25				1	2	5	3	4	3	4	2			1								8.00	2.33	2
0[f]	25					3	3	3	10	3	3												7.64	1.50	2
0[g]	25				1	3	4	4	2	2	4		1	2	1		1						8.52	3.16	3
0[g]	25				2	1	3	2	5	5	2	3		1		1							8.44	2.62	1
0[g]	25				1	1	7	1	3	5	3	1	2			1							8.28	2.57	2
0[g]	25			1	2	1		4	6	2	2	4	3										8.40	2.58	2
0[g]	25				3	1	4	3	5		2	1	4		1		1						8.44	3.24	1
0[h]	25				1	2	2	4	3	5	2	1	1	3	1								8.76	2.74	3
0[h]	25			1	3	2	4	1	3	2	2	2	2		2		1						8.32	3.54	2
0[h]	25			1	2		1	3	6	2	4	1	4		1								8.68	2.73	2
0[h]	25		1	3		2	2	2	3	1	5	2	2	2									8.08	3.29	0
0[h]	25		1		1	3	3	5	1	3	1		4	1		1			1				8.52	3.73	2
0[i]	25				1	1	2	4	8	3	2	1	1					1				2	8.88	3.71	3
0[i]	25				1	1	3	1	7	4	7				1								8.40	2.06	2
0[i]	25		1			3	2	4	5	3	2		3	1					1				8.44	3.25	1
0[j]	25						3	3	8	3	4	1	3										8.68	1.82	2
0[j]	25					1	3	6	4	3	4	2	1				1						8.56	2.38	2
0[j]	25		1		2	1	2	1	9	3	1		1			2	1	1					8.72	3.76	2
0[j]	25					3	2	5	6	2	2	1	3		1								8.36	2.41	2
0[j]	25				1		4		7	3	5	1	2	1		1							8.96	2.47	2
0[j]	25				3	1	4		5	4	4		3	1									8.24	2.62	2
0[j]	25				1		5	6		3	3	2	5										8.60	2.47	3
0.1	25				2	3	2	2	2	5	5	2	1	1									8.28	2.51	2
0.1	25					2	4	6	4	1	5	2					1						8.20	2.43	2
0.1	25					2	6	2		9	1	2	1	1	1								7.88	2.07	1
0.1	25				1	3	1	5	7		1	2	2		2							1	8.80	3.57	1

Benzene (ppm)	N[a]	1[b]	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	≥21	SCE/ cell (mean)	SCE/ cell (SD)	MN/ 1,000 PCEs[d]
0.1	25				3	1	3	4	4	3	4	1	1				1						8.04	2.75	2
0.3	25					3	4	3	8	1	2	1	1			2							8.24	2.70	3
0.3	25					2	4	4	3	3	6	2		1									8.32	2.10	2
0.3	25						4	3	7	5	3	1	1				1						8.60	2.20	2
0.3	25				2		1	4	8	6	2		1	1									8.20	1.98	2
0.3	25					3	3	4	8	3	2	1	1										7.80	1.80	1
1	25					2	5	2	5	5	2	1				1				1		21	8.96	3.86	4
1	25				2		4		1	3	6	1	4	1	1		1				1		9.96	3.66	4
1	25				2	2	2	1	5	4	4	1	2	1	1								8.60	2.68	4
1	25					1	3	4	7	1	7			1						1			8.68	2.64	5
1	25					1	4	1	4	1	8	1	3	1	1								9.24	2.40	5
3	25					1	1	2	2	1	6	2	3	2	1	1	1	1			1		11.04	3.53	5
3	25						3	2	3	1	6	1	4	4				1					10.12	2.74	4
3	25							3	5	2	3	4	4	1	1		1				1		10.56	3.04	4
3	25								5	3	6	3	5	1		1				1			10.64	2.36	6
3	25			1			1	1	2	2	6	7	1		4								10.20	2.57	5
10	15							1	3		2	3	5								1		10.93	3.06	6
10	21								4	4	7	3	1	1	1								10.00	1.61	5
10	25						1	1	4	2	7	2	2	1	2		2					21	10.92	3.33	7
10	25							3	7	1	4	3	4	1	1	1							9.96	2.28	6
10	25				1		1	1	4	2	7	3	3		2							24	10.32	3.65	7
30	25						1	1	2	3	5	3	5		1			1	1		2		11.52	3.91	8
30	25						1		3	1	5	4	7	2	2								10.84	1.97	7
30	25							2	2	1	9		3	3	3	1	1						11.08	2.47	7
30	25							1	2	3	6	2	5	2	1	1	1	1					11.20	2.50	7
30	25								2	1	9	3	6	1	2	1							11.04	1.77	9

[a]Total number of cells for a given concentration.
[b]Denotes the number of SCEs/metaphase.
[c]This column contains the actual SCE frequency of each cell having 21 or more SCEs.
[d]Denotes the number of micronuclei per 1,000 bone marrow polychromatic erythrocytes.
[e]Concurrent controls for the 0.1 ppm exposure experiment.
[f]Concurrent controls for the 0.3 ppm exposure experiment.
[g]Concurrent controls for the 1 ppm exposure experiment.
[h]Concurrent controls for the 3 ppm exposure experiment.
[i]Concurrent controls for the 10 ppm exposure experiment.
[j]Concurrent controls for the 30 ppm exposure experiment.

respectively. For comparison, BZ induced an increase of 1.7, 3.6, and 7.9 SCEs/metaphase at 62, 601, 5,948 ppm x hr BZ, respectively in the present study. In addition, the present study demonstrates that BZ is capable of inducing SCE in lymphocytes and increasing MN in bone marrow at a substantially lower time-weighted average concentration than previously examined.

BZ is metabolized in mammals to phenol, hydroquinone, catechol, 1,2,4-benzenetriol, and p-benzoquinone [Teisinger et al, 1952; Dean, 19691. The decreases in mitotic indices seen after the inhalation of BZ might be attributed to the formation of hydroquinone and p-benzoquinone; these metabolites react with sulfhydryl groups of cytoskeletal proteins vital for blastogenesis [Irons and Neptun, 1980; Irons et al, 1981; Pfeifer and Irons, 19811. Possibly, a majority of the SCEs induced in vivo in the mouse B lymphocytes and rat T lymphocytes are caused by catechol, p-benzoquinone, and 1,2,4-benzenetriol; these compounds are relatively efficient SCE inducers in cultured human T lymphocytes exposed in vitro [Morimoto and Wolff, 1980; Erexson et al, 1985]. The data obtained in the present study show parallel induction of SCE and MN in both mice and rats, and this indicates that a common BZ metabolite(s) might be involved.

MN induction is indicative of either spindle disruption or chromosome breakage leading to lagging acentric fragments or entire chromosomes at anaphase [Heddle et al, 19831. The formation of MN in bone marrow might be a result of either the accumulation of phenol, hydroquinone, and catechol at this site or the minor metabolism of BZ and its metabolites by the marrow [Rickert et al, 1979; Irons et al, 1980; Greenlee et al, 19811, leading to disruption of the spindle and aneuploidy. The idea that chromosome loss is involved in MN induction by BZ is supported by an in vitro study [Irons et al, 1981] that has shown that microtubule assembly of rat brain tubulin is inhibited by hydroquinone.

A number of studies also support the hypothesis that the MN are a result of chromosome breakage. Evidence of a concentration-dependent increase of chromosome aberrations in bone marrow of male Wistar rats has been reported after exposure to concentrations of 1, 10, 100, or 1,000 ppm BZ for 6 hr [Styles and Richardson, 1984]. Tice et al [1980] found significant increases of chromatid-type aberrations in bone marrow cells of DBA/2 male and female mice exposed to 3,100 ppm BZ for 4 hr after pretreatment with phenobarbital, and other investigators have reported chromosome aberrations in rat bone marrow after exposure to BZ alone [Philip and Jensen, 1979; Lyon, 1976; Dean, 1978; Anderson and Richardson, 19811.

The present study accurately defines the shape of the concentration-response curves for these cytogenetic endpoints and indicates that a 6-hr exposure to 1 ppm BZ and above can induce measurable cytogenetic effects in rodents. It should be noted that the shape of the concentration-response curves for both rats and mice for both endpoints are nonlinear, with the greatest slope at the lower concentrations. A semilogarithmic plot of the data yields a straight line. The magnitude of the SCE response to BZ, although modest, was greater than that observed for rats exposed to ethylene oxide by inhalation [Kligerman et al, 19831. The continued monitoring of human exposure to BZ with sensitive cytogenetic tests of peripheral blood lymphocytes might be warranted to determine whether or not these rodent models accurately approximate the human situation. These results suggest that further studies on the cytogenetic effects of repeated exposure to low concentrations of BZ are needed to assess the persistence and accumulation of cytogenetic lesions in rodent lymphocytes.

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