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Attention Mr. Terry O'Bryan
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Dear Mr. O'Bryan:

Enclosed is a copy of a manuscript on the cytogenetic effects of benzene in rodents which may be of interest to you. This manuscript was submitted last week to Environmental Mutagenesis for review and consideration for publication. A copy has also been forwarded to API, OSHA and to Dr. Peter Voytek of EPA.

Sincerely,

A handwritten signature in cursive script, appearing to read "Robert A. Neal".
Robert A. Neal
President

RAN:ewb

Enclosure

INDUCTION OF CYTOGENETIC DAMAGE IN RODENTS AFTER
SHORT-TERM INHALATION OF BENZENE

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Running title: Cytogenetic damage in rodents inhaling benzene

ABSTRACT

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 1000 ppm BZ for 6 h. 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 h. Blood was obtained by cardiac puncture 18 h 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 h after BZ exposure. Mouse PBLs revealed a significant concentration-related increase in the SCE frequency over controls of 1.7, 3.6, or 7.9/metaphase after exposure to 10, 100, or 1000 ppm BZ, respectively. Mouse bone marrow showed a significant concentration-dependent increase in MN over controls of 6.9, 18.2, or 26.0/1000 PCEs after exposure to 10, 100, or 1000 ppm BZ, respectively. Rat PBLs showed a significant increase in the SCE frequency of 2.3, 1.8, or 2.5/metaphase after exposure to 3, 10, or 30 ppm BZ, respectively. 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 over controls of 2.7, 3.1, 4.2, or 5.6/1000 PCEs after inhalation of either 1, 3, 10, or 30 ppm BZ, respectively. 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 h inhalation of BZ at relatively low concentrations.

Key words: benzene, rodents, sister chromatid exchange, micronucleus, bone marrow, peripheral blood lymphocytes.

INTRODUCTION

Benzene (BZ) has been implicated as a human leukemogen [Goldstein, 1977]. 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 may 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 neoplasias 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 [e.g. Tough et al., 1970; Forni et al., 1971; Rinsky et al., 1981]. In addition, there is limited information regarding the immunotoxic [Baarson et al., 1984; Rosen et al., 1984] and chromosome-damaging [Tice et al., 1982; Cortina et al., 1983; Styles and Richardson, 1984] effects of inhaled BZ on the rodent hemato-poietic 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., 1984], 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].

In order 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 suggested increased cytogenetic damage after exposure to nominal concentrations of 1 and 3 ppm BZ. Thus, a more definitive experiment was conducted in rats in which concentrations as low as 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 benzene.

MATERIALS AND METHODS

Animals

Eight to 10 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, 1983; Maltoni et al., 1983] and are desirable for the rat MN test because of the relatively low numbers of mast cells present in their bone marrow [Gollapudi et al., 1983]. The housing conditions and diet were as previously described [Erexson et al., 1983; Kligerman et al., 1983]. Male DBA/2 mice (17-19 weeks old) were exposed to atmospheres of 10, 100, or 1000 ppm BZ for 6 h in groups of 5 mice/chamber. Male Sprague-Dawley rats (11-14 weeks old) were exposed to atmospheres of 0.1, 0.3, 1, 3, 10, or 30 ppm BZ for 6 h in groups of 5 rats/chamber. Control mice (3-4/cage) and rats (5/cage) were exposed concurrently to chamber room air to serve as controls. Temperature ($23.5 \pm 0.3^{\circ}\text{C}$) and humidity ($52.5 \pm 5.6 \%$) were monitored each hour during exposures.

Benzene Exposures

Food and water were not given to any of the animals during the 6 h exposures. Animals were exposed in a 102 liter glass and Teflon chamber [Barrow and Steinhagen, 1982] with a 20 liter/minute air flow. Generation of all concentrations except 1000 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 1000 ppm concentration was done by metering liquid BZ with an FMI pump (Fluid Metering Inc., Oyster Bay, NY) at 0.072 ml/minute 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 1000 ppm BZ concentrations. The 0.1, 0.3, 1, and 3 ppm BZ exposures were analyzed 2 to 3 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 SCE analysis were as previously described [Kligerman et al., 1982; Erexson et al., 1983; Wilmer et al., 1983]. Briefly, blood was removed by cardiac puncture from anesthetized animals 18 h after exposure to BZ, and lymphocytes were cultured either in the presence of 60 μ g lipopolysaccharide/ml (mouse B-cells) or 30 μ g concanavalin A/ml (rat T-cells) to stimulate blastogenesis. Peripheral blood leucocyte counts were determined from a 20 μ l aliquot of washed blood with a Coulter counter as previously described [Kligerman et al., 1982]. 5-Bromo-2'-deoxyuridine (mice: 2 μ M and rats: 4 μ M) was added at 24 h after culture initiation, and the cultures were harvested at either 52 h (rats) or 60 h (mice) following a 4 h demecolcine (1.35 μ M) treatment.

Bone marrow slide preparation and staining were a modification of the technique described by Schmid (1976). May-Grunwald's and Giemsa stains (Bio/medical Specialties, Santa Monica, CA) were used for mouse bone marrow smears whereas acridine orange (Polysciences Inc., Warrington, PA) was used to stain rat bone marrow since 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., 1983].

Slide Analysis

For the SCE experiments two to three slides were prepared per animal. 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 was 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 second division metaphases and 1000 to 2000 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 (1-tailed) [Snedecor and Cochran, 1967]. 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 this 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 equivalent nonparametric Mann-Whitney U test [Lehmann, 1975] was used because the SCE distribution was non-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 (Table I, II). The concentration-related SCE increase is particularly apparent in Table II 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 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 1000 ppm (Table I, 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 h exposure to 3, 10, or 30 ppm (Table I, 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 upon 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. Atmospheres of 0.1 or 0.3 ppm BZ did not significantly affect the SCE frequency. While exposure of rats to 0.1, 0.3, 1, or 10 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 kinetics (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 MN at concentrations from 1 to 30 ppm (Table I, IV). As with the SCE frequency there was 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 response 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 benzene.

DISCUSSION

These results confirm and extend the work of several investigators who have also shown 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 μ l BZ/kg/day by gavage and sacrificed 18 h 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 μ l BZ/kg/day by gavage or intraperitoneal injections at 24 h intervals for 3 consecutive days. Also, Siou et al. (1982) observed a significant increase in PCEs containing MN from male Swiss mice after 2 gavages of 25.6 μ l BZ/kg/day at 24 h intervals. Toft et al. (1982) found that NMRI mice exposed to 14 ppm BZ by inhalation continuously for 1 to 8 weeks had a significant increase in PCEs containing MN.

Tice et al. (1982) have demonstrated significant increases of SCE after a 4 h inhalation exposure to 28 ppm BZ in both male and female DBA/2 and C57BL/6 mice (28 ppm X 4 hours = 112 ppm X hours) 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, 1960, 4072, and 9541 ppm x hours BZ, respectively. For comparison, BZ induced an increase of 1.7, 3.6, and 7.9 SCEs/metaphase at 62, 601, 5948 ppm x hours 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, and catechol, 1,2,4-benzenetriol, and p-benzoquinone [Teisinger et al., 1952; Dean, 1969]. The decreases in mitotic indices seen after the inhalation of BZ might be attributed to the formation of hydroquinone and p-benzoquinone [Irons and Neptun, 1980; Irons et al., 1981; Pfeifer and Irons, 1981]. These metabolites react with sulfhydryl groups of cytoskeletal proteins vital for blastogenesis [Irons and Neptun, 1980; Irons et al., 1981; Pfeifer and Irons, 1981]. 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) may 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., 1983]. The formation of MN in bone marrow may 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., 1981], leading to disruption of the spindle and aneuploidy. The idea that chromosome loss may be involved in MN induction by BZ is supported by an in vitro study [Irons et al., 1981], which 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 1000 ppm BZ for 6 h [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 3100 ppm BZ for 4 h after pretreatment with phenobarbital, and other investigators have reported chromosome aberrations in rat bone marrow after exposure to BZ alone [Philip and Jensen, 1970; Lyon, 1976; Dean et al., 1978; Anderson and Richardson, 1981].

The present study accurately defines the shape of the dose response curves for these cytogenetic endpoints and indicates that a 6 h exposure to concentrations of approximately 1 ppm BZ and above can induce measurable cytogenetic effects in rodents. It should be noted that the magnitude of the response of BZ was modest relative to other known inducers of SCE. The monitoring of human exposure to BZ with sensitive cytogenetic tests of peripheral blood lymphocytes may be warranted to determine if 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 may be needed to assess the persistence and accumulation of cytogenetic lesions in rodent lymphocytes.

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REFERENCES

- Anderson D, Richardson CR (1981): Issues relevant to the assessment of chemically induced chromosome damage in vivo and their relationship to chemical mutagenesis. *Mutat Res* 90:261-272.
- Baaron KA, Snyder CA, Albert RE (1984): Repeated exposure of C57Bl mice to inhaled benzene at 10 ppm markedly depressed erythropoietic colony formation. *Toxicol Lett* 20:337-342.
- Barrow CS, Steinhagen WH (1982): Design, construction and operation of a simple inhalation exposure system. *Fund Appl Toxicol* 2:33-37.
- Cortina TA, Sica EW, McCarroll NE, Coate W, Thakur A (1982): Inhalation cytogenetics in mice and rats exposed to benzene. In MacFarland HN, Holdsworth CE, MacGregor JA, Call RW, Kane ML (eds): "The Toxicology of Petroleum Hydrocarbons." Washington DC: American Petroleum Institute, pp 122-127.
- Cronkite EP, Bullis JE, Inoue T, Drew RT (1984): Benzene inhalation produces leukemia in mice. *Toxicol Appl Pharmacol* 75:358-361.
- Dean BJ (1969): Chemical-induced chromosome damage. *Lab Anim* 3:157-174.
- Dean BJ (1978): Genetic toxicology of benzene, toluene, xylenes and phenols. *Mutat Res* 47:75-97.

- Erexson GL, Wilmer JL, Kligerman AD (1983): Analyses of sister chromatid exchange and cell cycle kinetics in mouse T- and B-lymphocytes from peripheral blood cultures. *Mutat Res* 109:271-281.
- Erexson GL, Wilmer JL, Kligerman AD (1985): Sister chromatid exchange induction in human lymphocytes exposed to benzene and its metabolites in vitro. *Cancer Res* 45 (in press).
- Forni A, Pacifico E, Limonta A (1971): Chromosome studies in workers exposed to benzene or toluene or both. *Arch Environ Health* 22:373-378.
- Goldstein BD (1977): Hematotoxicity in humans. *J Toxicol Environ Health* Suppl 2:69-105.
- Goldstein BD, Snyder CA, Laskin S (1982): Myelogenous leukemia in rodents inhaling benzene. *Toxicol Lett* 13:169-173.
- Gollapudi BB, Linscombe VA, Sinha AK (1983): A note of the utility of Fischer-344 rat in the micronucleus test. *Toxicol Lett* 17:201-204.
- Greenlee WF, Gross EA, Irons RD (1981): Relationship between benzene toxicity and the disposition of ¹⁴C-labelled benzene metabolites in the rat. *Chem- Biol Interact* 33:285-299.
- Hayashi M, Sofuni T, Ishidate Jr M (1983): An application of acridine orange fluorescent staining to the micronucleus test. *Mutat Res* 120:241-247.

- Heddle JA, Hite M, Kirkhart B, Mavournin K, MacGregor JT, Newell GW, Salamone MF (1983): The induction of micronuclei as a measure of genotoxicity: A report of the U.S. Environmental Protection Agency GENE-TOX Program. *Mutat Res* 123:61-118.
- Hite M, Pecharo M, Smith I, Thornton T (1980): The effect of benzene in the micronucleus test. *Mutat Res* 77:149-155.
- Irons RD, Dent JG, Baker TS, Rickert DE (1980): Benzene is metabolized and covalently bound in bone marrow in situ. *Chem-Biol Interact* 30:241-245.
- Irons RD, Moore BJ (1980): Effect of short term benzene administration on circulating lymphocyte subpopulations in the rabbit: Evidence of a selective B-lymphocyte sensitivity. *Res Comm Chem Pathol Pharmacol* 27:147-155.
- Irons RD, Neptun DA (1980): Effects of the principal hydroxy-metabolites of benzene on microtubule polymerization. *Arch Toxicol* 45:297-305.
- Irons RD, Neptun DA, Pfeifer RW (1981): Inhibition of lymphocyte transformation and microtubule assembly by quinone metabolites of benzene: Evidence for a common mechanism. *J Reticuloendothel Soc* 30:359-372.
- Kligerman AD, Wilmer JL, Erexson GL (1982): Characterization of a rat lymphocyte culture system for assessing sister chromatid exchange. II. Effects of 5-bromodeoxyuridine concentration, number of white blood cells in the inoculum, and inoculum volume. *Environ Mutagen* 4:585-592.

- Kligerman AD, Erexson GL, Phelps MC, Wilmer JL (1983): Sister chromatid exchange induction in peripheral blood lymphocytes of rats exposed to ethylene oxide by inhalation. *Mutat Res* 120:37-44.
- Lehmann EL (1975): In "Nonparametrics." San Francisco, CA: Holden-Day, Inc., pp 76-81.
- Lyon JP (1976): Mutagenicity studies with benzene. *Dissert Abstr* 36B:5537.
- Philip P, Jensen MK (1970): Benzene induced chromosome abnormalities in rat bone marrow cells. *Acta Path Microbiol Scand* 78A:489-490.
- Maltoni C, Conti B, Cotti G (1983): Benzene: A multipotential carcinogen. Results of long-term bioassays performed at the Bologna Institute of Oncology. *Am J Ind Med* 4:589-630.
- Meyne J, Legator MS (1980): Sex-related differences in cytogenetic effects of benzene in the bone marrow of Swiss mice. *Environ Mutag* 2:43-50.
- Morimoto K, Wolff S (1980): Increase of sister chromatid exchanges and perturbations of cell division kinetics in human lymphocytes by benzene metabolites. *Cancer Res* 40:1189-1193.
- National Toxicology Program (1984): Technical report on the toxicology and carcinogenesis studies of benzene (CAS No. 71-43-2) in F-344/N rats and B6C3F1 mice (gavage studies). NTP-TR-289, Bethesda, MD: NIH, 328 pp.

- Pfeifer RW, Irons RD (1981): Inhibition of lectin-stimulated lymphocyte agglutination and mitogenesis by hydroquinone: Reactivity with intracellular sulfhydryl groups. *Exp Mol Pathol* 35:189-198.
- Rickert DE, Baker TS, Bus JS, Barrow CS, Irons RD (1979): Benzene disposition in the rat after exposure by inhalation. *Toxicol Appl Pharmacol* 49:417-423.
- Rinsky RA, Young RJ, Smith AB (1981): Leukemia in benzene workers. *Amer J Ind Med* 2:217-245.
- Rozen MG, Snyder CA, Albert RE (1984): Depressions in B- and T-lymphocyte mitogen-induced blastogenesis in mice exposed to low concentrations of benzene. *Toxicol Lett* 20:343-349.
- Schmid W (1976): In Hollaender A (ed): "Chemical Mutagens: Principles and Methods for their Detection." Vol 4, New York: Plenum Press, pp 31-53.
- Siou G, Conan L, Haitem M el (1982): Comparative study of the effect of the carcinogens benzene and methylmethane sulfonate on the mouse and Chinese hamster. *Comm Eur Commun* 7549:353-359.
- Snedecor GW, Cochran WG (1967): In "Statistical Methods." Ed 6, Ames, Iowa: Iowa State University Press, 593 pp.

- Snyder CA, Goldstein BD, Sellakumar AR, Bromberg I, Laskin S, Albert RE (1980): The inhalation toxicology of benzene: Incidence of hematopoietic neoplasms and hematotoxicity in AKR/J and C57Bl/6J mice. *Toxicol Appl Pharmacol* 54:323-331.
- Snyder R, Longacre SL, Sammett D, Witmer CM, Kocsis JJ (1983): Relationship between the toxicity and metabolism of benzene. In Mehlman MA (ed): "Carcinogenicity and Toxicity of Benzene." New Jersey: Princeton Scientific Publishers Inc., pp 23-27.
- Styles JA, Richardson CR (1984): Cytogenetic effects of benzene: Dosimetric studies on rats exposed to benzene vapour. *Mutat Res* 135:203-209.
- Teisinger J, Bergerova-Fiserova V, Kudma J (1952): The metabolism of benzene in man. *Prac Lek* 4:175-188.
- Tice RR, Costa DL, Drew RT (1980): Cytogenetic effects of inhaled benzene in murine bone marrow: Induction of sister chromatid exchanges, chromosomal aberrations, and cellular proliferation inhibition in DBA/2 mice. *Proc Natl Acad Sci USA* 77:2148-2152.
- Tice RR, Vogt TF, Costa DL (1982): Cytogenetic effects of inhaled benzene in murine bone marrow. In Tice RR, Costa DL, Schaich KM (eds): "Genotoxic Effects of Airborne Agents." New York: Plenum Publishing Company, pp 257-275.

Toft K, Olofsson T, Tunek A, Berlin M (1982): Toxic effects on mouse bone marrow caused by inhalation of benzene. Arch Toxicol 51:295-302.

Tough IM, Smith PG, Court Brown WM, Harnden DG (1970): Chromosome studies on workers exposed to atmospheric benzene. Europ J Cancer 6:49-55.

Wilmer JL, Erexson GL, Kligerman AD (1983): Implication of an elevated sister-chromatid exchange frequency in rat lymphocytes cultured in the absence of erythrocytes. Mutat Res 109:231-248.

Table I. The induction of cytogenetic damage in male DBA/2 mice and Sprague-Dawley rats

Benzene (ppm/hour)	Number of animals (N)	SCEs/Metaphase ^a	Number of ^a MN/1000 PCEs
DBA/2 Mice			
0.0 ± 0.0	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) ^{c,d}	9.0 ± 0.6 ^c
100.1 ± 1.6	5	9.5 ± 0.2 (0.23) ^{c,d}	20.3 ± 0.7 ^c
991.2 ± 5.9	5	13.8 ± 0.9 (0.37) ^{c,d}	28.1 ± 0.8 ^c
Sprague-Dawley Rats			
0.0 ± 0.0	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) ^c	4.4 ± 0.3 ^c
3.0 ± 0.05	5	10.5 ± 0.2 (0.25) ^{c,d}	4.8 ± 0.4 ^c
0.0 ± 0.0	10	8.6 ± 0.1 (0.17)	2.0 ± 0.1
10.0 ± 0.2	5	10.4 ± 0.2 (0.27) ^{c,d}	6.2 ± 0.4 ^c
30.0 ± 0.3	5	11.1 ± 0.1 (0.23) ^{c,d}	7.6 ± 0.4 ^c

^aMean ± standard error among animals within a group.

^bThe numbers in parentheses denote the standard error among cells within a group.

^cSignificantly different from the concurrent controls (p < 0.05) using Student's t-test (one-sided).

^dSignificantly different from the concurrent controls (p < 0.05) using the Mann-Whitney U test (one-sided).

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 ^d	0 ^e	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	>21 ^f	SCE/cell		PCEs	
																								Mean	St. Dev.		
0 ^a	25		1	1	6	3	6	4	2	1	1													5.72	1.90	2	
0 ^a	25		1	5	7	3	3	4	1	1														5.88	1.90	4	
0 ^a	25		3	2	3	3	2	6	3	3														5.88	2.47	3	
0 ^b	25		1	6	6	3	4	4	1	1														5.76	1.83	7	
0 ^b	25		1	4	5	7	5	2	2	1														6.08	1.96	4	
0 ^b	25		3	4	7	6	1	1	2	1														5.56	1.87	8	
0 ^b	Not done																							--	--	2	
0 ^c	25		1	6	4	8	2	1	1	2														6.20	2.61	2	
0 ^c	16		4	4	1	4	2	1	1															5.06	1.95	5	
0 ^c	25		1	5	7	1	5	1	2	1	1													5.92	2.41	7	
0 ^c	14		1	1	1	3	2	1	3	1	1													6.86	3.03	3	
10	25		2	2	3	3	7	5	3	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	2														7.27	1.61	15	
10	25		2	4	2	3	8	1	4	1	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	1	2	4	7	6	1	2													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	1	6	3	6	4	2													9.32	2.17	41	
100	25		1	1	3	1	4	2	6	2	4													9.48	3.23	36	
1000	25																								15.60	3.30	54
1000	25																								15.88	3.63	52
1000	25																								12.84	3.60	55
1000	15		1	1	1	1	2	2	2	2	1	2	2	1	2	2	1	2	2	1				12.80	4.20	61	
1000	25		1	2	1	1	2	3	1	4	3	2	1	2	2	2	1	2	2					11.44	3.55	59	

^aConcurrent controls for the 10 ppm exposure experiment.

^bConcurrent controls for the 100 ppm exposure experiment.

^cConcurrent controls for the 1000 ppm exposure experiment.

^dTotal number of cells for a given concentration.

^eDenotes the number of SCEs/metaphase.

^fThis column contains the actual SCE frequency of each cell having 21 or more SCEs.

^gDenotes the number of micronuclei per 2000 bone marrow polychromatic erythrocytes.

Table III. The effects of inhaled benzene on mitotic indices and leucocyte counts of male DBA/2 mice and Sprague-Dawley rats

Benzene (ppm/hour)	Number of animals (N)	Mitotic index ^a (%)	Leucocytes/ml of whole blood (10 ⁶) ^a
DBA/2 Mice			
0.0 ± 0.0	10	4.4 ± 0.3	4.13 ± 0.5
10.3 ± 0.9	5	2.9 ± 0.2 ^b	3.20 ± 0.2
100.1 ± 1.6	5	2.3 ± 0.6 ^b	5.57 ± 1.0
991.2 ± 5.9	5	1.0 ± 0.1 ^b	4.94 ± 0.4
Sprague-Dawley Rats			
0.0 ± 0.00	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 ^b	15.60 ± 0.8
0.0 ± 0.0	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 ^b	15.00 ± 2.2

^aMean ± standard error among animals within a group.

^bSignificantly different from the concurrent controls (p < 0.05) using Student's t-test (1-tailed).

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 ^o	0 ^h	>21 ¹																			SCE/cell		MN/2000J PCEs	
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Mean		St. Dev.
0 ^a	25	1	1	1	1	1	6	2	2	2	2	3	1	1	1	1							8.48	2.50	1
0 ^a	25	1	2	2	5	4	1	5	1	1	1	1	1	1	1	1							7.84	3.12	2
0 ^a	25	1	1	1	2	3	4	2	4	6	1	1	1	1	1	1							7.92	2.31	3
0 ^a	25	1	4	3	1	1	1	5	2	1	2	1	1	3									8.12	3.56	2
0 ^b	25	1	1	3	4	4	5	2	2	1	1	2											8.04	2.92	0
0 ^b	25	2	1	2	1	1	7	4	4	2	1	1	2										7.96	2.44	2
0 ^b	25	3	3	1	2	3	5	4	4	1	2	3											9.00	5.17	2
0 ^b	25	2	4	3	2	4	4	1	2	3													7.84	2.54	0
0 ^b	25	1	2	5	3	4	3	4	2	3													7.84	2.54	0
0 ^b	25	1	2	5	3	3	3	10	3	3													8.00	2.33	2
0 ^c	25	1	3	4	4	2	2	4	2	4													7.64	1.50	2
0 ^c	25	1	3	4	4	2	2	4	2	4													8.52	3.16	3
0 ^c	25	2	1	3	2	5	5	2	2	3	1	2	1	1	1	1							8.44	2.62	1
0 ^c	25	1	1	1	7	1	3	5	3	1	2	1	1										8.28	2.57	2
0 ^c	25	1	2	1	4	6	2	2	4	3	1	2	4	3									8.40	2.58	2
0 ^c	25	3	1	4	3	5	2	2	1	4	1	4	1	4	1	1							8.44	3.24	1
0 ^d	25	1	2	2	4	3	5	2	1	1	1	3	1	1	1	1							8.76	2.74	3
0 ^d	25	1	3	2	4	1	3	2	2	2	2	2	2	2	2	1							8.32	3.54	2
0 ^d	25	1	2	1	3	6	2	4	1	4	1	4	1	1	1	1							8.68	2.73	2
0 ^d	25	1	3	2	2	2	3	1	5	2	2	2	2	2	2	2							8.08	3.29	0
0 ^e	25	1	1	3	3	5	1	3	1	1	4	1	1	1	1	1							8.52	3.73	2
0 ^e	25	1	1	2	4	8	3	2	1	1	1	1	1	1	1	1							8.88	3.71	3
0 ^e	25	1	1	1	3	1	7	4	7	7	4	7											8.40	2.06	2
0 ^e	25	1	3	2	4	5	3	2	2	1	3	1	1	1	1	1							8.44	3.25	1
0 ^e	25	1	3	6	4	3	4	1	3	4	1	3	3	3	3	3							8.68	1.82	2
0 ^f	25	1	2	1	2	1	9	3	1	1	1	1	1	1	1	1							8.56	2.38	2
0 ^f	25	1	2	1	2	1	9	3	1	1	1	1	1	1	1	1							8.72	3.76	2
0 ^f	25	1	3	2	5	6	2	2	2	1	3	1	1	1	1	1							8.36	2.41	2
0 ^f	25	1	4	4	7	3	5	1	2	1	2	1	1	1	1	1							8.96	2.47	2
0 ^f	25	3	1	4	5	4	4	4	4	4	3	1	1	1	1	1							8.24	2.62	2
0 ^f	25	1	1	5	6	3	3	3	3	3	2	5	3	2	3	2							8.60	2.47	3

Table IV. (cont'd.)

Benzene [ppm]	N ^g	0 ^b																				SCE/cell		MM/2000 ^j PCEs		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	>21 ⁱ	Mean		St. Dev.	
0.1	25																							8.28	2.51	2
0.1	25	2	2	3	2	2	2	2	5	5	2	1	1											8.20	2.43	2
0.1	25																							7.88	2.07	1
0.1	25	1	3	1	5	7	1	2	1	2	1	1	1											8.80	3.57	1
0.1	25	3	1	3	4	4	3	4	1	1	1	1												8.04	2.75	2
0.3	25																							8.24	2.70	3
0.3	25	2	4	4	3	3	6	2	1	1														8.32	2.10	2
0.3	25																							8.60	2.20	2
0.3	25	2	1	4	3	7	5	3	1	1	1	1											8.20	1.98	2	
0.3	25																							7.80	1.80	1
1	25																							8.96	3.86	4
1	25	2	5	2	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	1										8.60	2.68	4	
1	25	1	3	4	7	1	7	1	7	1	1	1	1										8.68	2.64	5	
1	25	1	4	1	4	1	8	1	3	1	1	1	1										9.24	2.40	5	
3	25																							11.04	3.53	5
3	25	1	1	2	2	1	6	2	3	2	1	1	1	1									10.12	2.74	4	
3	25	3	2	3	1	6	1	6	1	4	4	4	4										10.56	3.04	4	
3	25																							10.64	2.36	6
3	25	1	1	1	2	2	6	7	1	4	1	1	1										10.20	2.57	5	
10	15																							10.93	3.06	6
10	21																							10.00	1.61	5
10	25																							10.92	3.33	7
10	25																							9.96	2.28	6
10	25	1	1	1	4	2	7	3	3	3	2	1	1	1									10.32	3.65	7	
30	25																							11.52	3.91	8
30	25																							10.84	1.97	7
30	25																							11.08	2.47	7
30	25																							11.20	2.50	7
30	25																							11.04	1.77	9

^a Concurrent controls for the 0.1 ppm exposure experiment.
^b Concurrent controls for the 0.3 ppm exposure experiment.
^c Concurrent controls for the 1 ppm exposure experiment.
^d Concurrent controls for the 3 ppm exposure experiment.
^e Concurrent controls for the 10 ppm exposure experiment.
^f Concurrent controls for the 30 ppm exposure experiment.
^g Total number of cells for a given concentration.
^h Denotes the number of SCEs/metaphase.
ⁱ This column contains the actual SCE frequency of each cell having 21 or more SCEs.
^j Denotes the number of micronuclei per 1000 bone marrow polychromatic erythrocytes.