CYTOGENETIC EFFECTS OF INHALED BENZENE IN MURINE BONE MARROW

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INTRODUCTION

Exposure to benzene is both an occupational and an environmental hazard. Health concerns are based on the extensive use of benzene in industry and local commerce (1) and a long-standing association with aplastic anemia and leukemia in occupationally-exposed workers (2-4, see Goldstein, these proceedings). Although negative for genotoxic activity in a variety of short-term in vitro bioassays **(5-7)**, benzene is capable of inducing tumors in animals (8,9). The discrepency between these and other in vitro and in vivo results strongly suggests that a metabolite(s), rather than the parent compound, is primarily responsible for benzene's genotoxic activity.

Both cellular toxicity and carcinogenic potency appear to be causally-related to an agent's clastogenic potential (10,11). Exposure to benzene causes chromosomal aberrations in animals (1-4,12) and is associated with an increased frequency of such clastogenic events in occupationally-exposed workers (1-4,13-15). Several investigators have extended these observations in animals to other cytogenetic manifestations of DNA damage, e.g. micronuclei (16,17) and sister chromatid exchanges (SCEs) **(18)**. Furthermore, two presumptive metabolites of benzene, catechol and hydroquinone, induce SCEs and inhibit cellular proliferation in human cells in vitro (19).

Extrapolating these effects of benzene to an assessment of its impact on human health requires considerably more scientific research. Some of the needed research includes: (i) an understanding of the mechanism(s) by which the various manifestations of benzene toxicity occur, (ii) the acquisition of dose-response information by exposure routes pertinent to human experience, and (iii) an analysis of whether

such factors as age, sex and/or genetic constitution can modulate the magnitude of the response.

Using cytogenetic methodologies based on the incorporation of 5-bromodeoxyuridine (BrdUrd) into DNA **(18,20,21),** we have examined benzene's ability to induce chromosomal aberrations or SCEs and to inhibit cellular proliferation in murine bone marrow. Analysis of SCE frequency appears especially useful since SCEs are, in many instances, an extremely sensitive indicator of direct DNA damage and mutagenic potential **(22-25,** see Galloway and Tice, these proceedings). Inhibition of cellular proliferation may be a sensitive measure of cellular toxicity. Consequently, the former endpoint may indicate leukemogenic potential, while the latter endpoint, bone marrow depression leading to anemia.

We have exposed mice to benzene by inhalation, the normal route for human exposure, and have attempted to assess: (i) benzene's ability to induce different kinds of genotoxic damage, (ii) the importance of concentration, age, sex and genetic constitution in modulating benzene's genotoxic potency, and (iii) the relationship between liver-specific metabolism and extrahepatic metabolism in determining the observed cytogenetic effects.

MATERIALS AND METHODS

In all of these studies, mice were obtained as weanlings from Jackson Laboratory, ME, and fed and housed under the conditions described in detail elsewhere **(18).** Inhalation exposures were conducted in an isolation chamber previously described **(26).** Benzene vapor was generated by bubbling filtered compressed air through liquid benzene and diluting the vapor effluent appropriately. Benzene concentrations were measured at half-hour intervals by a gas chromatograph (Packard, Model **417,** equipped with a column of **10%** silicone SE-30 on Chromasorb W-HP) using an automatic sampling valve. Some animals were injected intraperitoneally (IP) with benzene dissolved in corn oil (Mazola).

With one exception, mice were infused with BrdUrd (Sigma; 50 mg/Kg per hour) as described in detail elsewhere **(21)** beginning one hour after completion of the benzene exposure and extending for up to 30 hours. Two hours prior to animal sacrifice, Colcemid (Gibco; 1 mg/Kg) was injected intravenously into each animal. Femoral bone marrow was obtained by flushing the femurs with phosphate buffered saline (pH **7.0),** incubated in **0.075** M KCl and then fixed with 3:1 methanol:glacial acetic acid. Flame-dried slides were prepared and differentially stained with Giemsa (Harleco) using a modification **(18,27)** of the technique of Goto et al. **(28).** Metaphase spreads were analyzed microscopically for chromosomal aberrations (in **50** first generation metaphase cells), SCEs (in 25 second generation metaphase

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RESULTS AND DISCUSSION

Induction of Genotoxic Damage in Murine Bone Marrow: 3000 ppm Benzene

In our first experiment, male and female DBA/2 mice (approximately 10 months of age) were exposed to 3000 ppm benzene for 4 hours (9 am- 1 pm) (18). A 4 hour exposure to 3000 ppm benzene induced a significant increase in SCEs in bone marrow cells of both sexes, did not induce a significant increase in chromosomal aberrations in bone marrow cells of either sex, and inhibited cellular proliferation in bone marrow of male but not female mice (Table 1). Furthermore, the proliferative inhibition observed in male mice was even greater one day after the exposure period (Table 2). This observation suggests the delayed formation of toxic metabolites and/or a delayed cellular reaction (18).

To examine the effects of enhanced liver metabolism on these cytogenetic manifestations of benzene's genotoxicity, mice were injected with phenobarbital prior to their exposure to benzene. Phenobarbital is a well-known inducer of hepatic mixed function oxidases (29) and has been shown to alter benzene's toxicity in vivo (1-4). While having no effect on control values, pretreatment with phenobarbital: (i) enhanced benzene's ability to induce SCEs in bone marrow cells of female mice but not of male mice, (ii) enabled benzene to induce chromosomal aberrations in bone marrow cells of both sexes, but more dramatically in male mice than in female mice, and (iii) enhanced benzene's ability to inhibit cellular proliferation in bone marrow of male mice but not of female mice (Table 1). The chromosomal aberrations were all of the chromatid- and not of the chromosome-type. There was also no increase in chromosomal rearrangements of any kind (18).

The difference in both the magnitude and the type of genotoxic response observed in the bone marrow of benzene-exposed male and female mice (Table 3) are intriguing for several reasons. First, these results demonstrate a sex difference in the sensitivity of DBA/2 mice to benzene. Second, the ability of phenobarbital pretreatment to alter the magnitude of different genotoxic responses observed in the bone marrow supports the involvement of metabolism in benzene's genotoxic activity. Finally, the spectrum of response in male versus female mice, with and without phenobarbital pretreatment, suggests the involvement of different metabolites of benzene in different genotoxic responses.

Table 1 Cytogenetic effects of inhaled benzene in murine bone marrow (3000 ppm-6 hr)

Exposure Group	Mice (10 mo old)	SCE Frequency[a]	% Abnormal Cells[b]	AGT (hr)[c]
Control	Male	4.6±0.2(6)	8.0±1.2(5)	14.2±1.3(6)
	Female	4.5±0.3(6)	10.0±2.2(5)	13.4±0.9(6)
Benzene (3000ppm-4hr)	Male	8.7±0.4(10)*	12.8±2.0(5)	16.6±0.4(-)*
	Female	8.2±0.7(10)*	12.0±1.3(5)	12.2±0.9(11)
Phenobarbital**	Male	4.1±0.2(-)	10.8±2.0(5)	11.8±0.4(-)
	Female	4.2±0.2(8)	8.6±1.2(5)	12.5±0.2(8)
Phenobarbital** + Benzene (3000ppm-4hr)	Male	8.3±0.4(6)*	42.4±3.6(5)*	20.9±2.2(11)*
	Female	12.7±1.0(7)*	25.6±1.7(5)*	12.1±0.4(7)

[a]Mean SCE frequency ± S.E. between (n) animals; values reflect the presence of both achromatic lesions and chromatid-type aberrations

[b]Mean % abnormal cells ± S.E. between (n) animals; values reflect the presence of both achromatic lesions and chromatid-type aberrations

[c]Average Generation Time = mean cell cycle duration ± S.E. between (n) animals, calculated by dividing the BrdUrd exposure period by the frequency of first generation metaphase cells plus twice the frequency of second generation metaphase cells plus three times the frequency of third generation metaphase cells

*Statistically different from the appropriate control at $p < 0.01$ Student t test

**Animals injected with sodium phenobarbital (50 mg/Kg) ip, twice daily for 3 days prior to exposure to benzene

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Table 2. Average generation time in bone marrow cells during the first and second day after benzene exposure (3000 ppm-4 hr)

Time Period (hr)[a]	Exposure Group	Mice (10 mo old)	AGT[b] (hr)
(i) 1-31	Control	Male	11.9±0.6 (5)
		Female	10.9±0.4 (5)
	Benzene	Male	17.2±1.3 (6)*
		Female	11.1±0.6 (6)
(ii) 21-51	Control	Male	12.2±0.8 (5)
		Female	11.0±0.5 (5)
	Benzene	Male	29.8±1.2 (6)*
		Female	11.4±0.5 (5)

[a] BrdUrd infusion period (30hr) beginning 1 hr (i) or 21 hr (ii) after termination of benzene exposure

[b] Average Generation Time (see Table 1)

*Statistically different from the appropriate control by Students t test at $p < 0.01$

Effect of Partial Hepatectomy on Benzene-Induced SCEs

We decided to further examine the importance of metabolism in determining benzene's genotoxic activity by examining the impact of a partial hepatectomy on benzene's ability to induce SCEs and to inhibit bone marrow proliferation in male DBA/2 mice. The theoretical basis for this experiment was derived from studies by Snyder and his co-workers (30, see Snyder, these proceedings) showing that the removal of most of the liver in rats resulted in the decreased ability of benzene to interfer with red blood cell maturation.

We removed ∿60% of the liver in male DBA/2 mice (10-14 wks of age), allowed recovery for seventeen hours and then exposed the animals (along with the appropriate controls) to 3000 ppm benzene for four hours (9 am-1 pm) (31).

The results (Table 4) were surprising for three reasons. First, instead of the expected doubling in SCE values for benzene-exposed animals without partial hepatectomy, we observed a six-fold increase

dividing the BrdUrd exposure period by the frequency of first generation metaphase cells plus twice the frequency of second generation metaphase cells plus three times the frequency of third generation metaphase cells

*Statistically different from the appropriate control by Students t test at $p < 0.01$

**Animals injected with sodium phenobarbital (50 mg/Kg) ip, twice daily for 3 days prior to exposure to benzene

Table 3. Effect of benzene inhalation on various cytogenetic end-points in bone marrow of DBA/2 mice, (10 mo old) with and without phenobarbitol pretreatment

Sex	Pheno-Na	Cytogenetic End Points[a] SCE	Chromosomal Abberations	Inhibition of Cellular Proliferation
Male	no	+	-	-
	yes	+	*	++
Female	no	+	-	-
	yes	++	+	-

[a]See Table 1 for additional information

- = no significant increase
+ = significant increase at $p<0.01$
++ = another significant increase at $p<0.01$

Table 4. Effect of partial hepatectomy on benzene (3000 ppm-4 hr)-induced SCE

Exposure Group	Partial Hepatectomy[a]	SCE Frequency[b]
Control	-	4.2±0.3(6)
	+	6.1±0.6(6)
Exposed	-	26.2±2.5(4)
	+	25.6±1.7 (6)

[a]~60% of the liver removed 17 hr prior to benzene exposure; male DBA/2 (10 -14 weeks of age)

[b]Mean frequency of SCE/cell±S.E. between (n) animals

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in SCE frequency. Second, bone marrow cell proliferation remained normal in these benzene exposed animals instead of exhibiting the expected inhibition. Finally, partial hepatectomy did not reduce benzene's ability to induce SCEs in bone marrow cells.

The inability of a partial hepatectomy to reduce benzene's genotoxic ability appears to conflict with the results of Snyder et al. (30, see Snyder, these proceedings). In the absence of additional experimental data, the difference in our results may be explained by experimental differences such as animal species (rat vs mouse), route of exposure (subcutaneous vs inhalation), recovery time after partial hepatectomy (7 hours vs 17 hours), dose of benzene (880 mg/Kg vs 12000 ppm-hr) or by the endpoint examined (iron-59 incorporation vs SCEs).

The dose of benzene to which the animals were exposed for four hours (3000 ppm) induced a maximal yield of SCEs and mixed function oxidase P450 levels did not increase in the remaining portion of the liver during the seventeen hour recovery time (data not presented). Thus, the most reasonable explanation for our respective results lies the difference in the biological endpoint under examination. Snyder's study (30, see Snyder, these proceedings) examined the incorporation of iron-59 into maturing red blood cells, a process occurring in a nonproliferating, differentiating cell population and induced by a mechanism probably unrelated to DNA damage. SCE formation, on the other hand, represents events occurring in proliferating cells in response to DNA damage (22-25). In view of our experimental results (cited earlier) suggesting possible metabolite specificity for DNA damage versus inhibition of cellular proliferation, our respective results may be both correct. The results of Snyder and his colleagues may indicate a liver-specific metabolite(s) responsible for delayed erythrocyte maturation while our results may indicate a genotoxic metabolite(s) formed directly from benzene in the bone marrow. Alternatively, and contrary to in vitro data on benzene (19), benzene, itself, may be responsible for the bone marrow-induced SCEs.

Effect of Co-Administered Toluene on Benzene-Induced SCEs

To determine if unmetabolized benzene was responsible for the formation of SCEs in the bone marrow of exposed animals, we next examined the ability of toluene, a well-known competitive inhibitor of benzene metabolism in vitro and in vivo (32,33, see Snyder, these proceedings), to affect the yield of benzene-induced SCEs.

DBA/2 male mice (10-14 weeks of age) were injected IP with toluene (32.4 mmoles/Kg) immediately prior to a four hour exposure to 3000 ppm benzene (31). Toluene did not induce SCEs or inhibit cell proliferation. Although the yield of benzene-induced SCEs was depressed by ~40% in toluene pretreated animals, many of these doubly

exposed animals exhibited extreme respiratory depression. We thought it likely that the depressed breathing rate may have contributed to the reduction in SCEs and decided to circumvent this possible confounding effect by co-administering benzene and toluene by IP injection.

Benzene was dissolved in corn oil and injected IP into DBA/2 male mice (10-14 weeks of age) one hour prior to the beginning of the BrdUrd infusion, a time corresponding to the end of the inhalation exposure. Using a range of benzene concentrations inducing low to maximal yields of SCEs, we assessed the ability of equi-molar concentrations of co-administered toluene to affect the induction of SCEs by benzene (Table 5). At the lowest concentration of benzene (2.25 mmoles/Kg), toluene had no effect on benzene-induced SCE yields. However, with increasing concentrations of both agents, toluene inhibited benzene's genotoxic activity to an increasing extent. Maximal inhibition of benzene' ability to induce SCEs (~90%) occurred at concentrations of benzene which, in the absence of toluene, induced maximal yields of SCEs (~26 SCE/cell @ 22.5 mmoles/Kg). Presumably, at low levels of benzene and toluene, the enzymes responsible for benzene metabolism are in excess of the total number of benzene and toluene molecules present. Only at agent concentrations exceeding the total number of enzyme binding sites would toluene inhibition of benzene metabolism be expected to occur. Bone marrow cell proliferation and yields of chromosomal aberrations remained at control values throughout this experiment (data not shown).

These results demonstrate an absolute requirement for the metabolic activation of benzene prior to the production of SCEs in bone marrow cells of exposed animals.

Table 5. Effect of co-administered toluene on benzene (2.25-22.5 mmoles/Kg)-induced SCEs in bone marrow cells of DBA/2 male mice (10-14 weeks of age)

Exposure Group	Benzene mmoles/Kg[a]	SCE Frequency[b]	+ Toluene mmoles/Kg[a]	SCE Frequency[b]	% Decrease

Benzene Concentration-SCE Response: Modulating Effect of Sex and Genetic Constitution

Since 3000 ppm benzene induced approximately a six-fold increase in SCEs in bone marrow cells of male DBA/2 mice when 10 to 14 weeks of age, we next examined the ability of benzene to induce SCEs at much lower ambient concentrations. We also examined whether sex and genetic constitution could modulate benzene's genotoxic activity *(34)*. In this series of experiments, male and female mice (10-14 weeks of age) from two isogenic strains--DBA/2 and C57B1/6--were exposed for four hours to concentrations of benzene ranging from 28 to 5000 ppm. These two mouse strains were chosen because they differ at the aryl-hydrocarbon hydroxylase (AHH) inducible locus, a locus associated with carcinogenic sensitivity in mice (35,36) and, perhaps, in man (37).

The lowest concentration of benzene that induced a significant increase in SCEs in male and female mice of both strains was 28 ppm

Table 5. Effect of co-administered toluene on benzene (2.25-22.5 mmoles/Kg)-induced SCEs in bone marrow cells of DBA/2 male mice (10-14 weeks of age)

Exposure Group	Benzene mmoles/Kg[a]	SCE Frequency[b]	+ Toluene mmoles/Kg[a]	SCE Frequency[b]	% Decrease
Control		6.2±0.2(4)			
Corn oil		5.9±0.4(6)			
Toluene			18.50	6.2±0.3(4)	
Toluene			32.40	5.2±0.1(4)	
Benzene	2.25	10.9±0.2(4)	2.25	11.7±0.6(4)	0.0
	5.63	17.1±0.6(6)	5.63	11.2±0.9(5)	-50.1
	11.25	21.4±0.6(6)	11.25	9.6±1.1(4)	-76.0
	22.50	26.4±2.1(5)	22.50	8.1±1.0(5)	-89.2

[a]Dissolved in corn oil and administered IP (0.1 5 gm body weight) one hour before initiation of BrdUrd infusion

[b]Mean frequency of SCE per cell ± standard error of the mean between (n) animals

(Table 6). Concentrations of benzene lower than 28 ppm were not tested. However, differential sex and strain sensitivities to ambient benzene were readily apparent over the concentration range examined. Benzene induced more SCEs in male mice than in female mice and more SCEs in DBA/2 mice than in C57B1/6 mice. SCE frequency increased linearly to 3000 ppm benzene in the DBA/2 mice and to 2000 ppm in the C57B1/6 mice. Higher concentrations, i.e. >3000 ppm for DBA/2 mice and >2000 ppm for C57B1/6 mice, resulted in a less than maximal frequency of SCEs. Because the SCE frequency saturates at high doses of benzene when the agent is injected IP, we believe that the most plausibible explanation for this phenomenon is that the high doses of benzene affected the central nervous system of the animals which resulted in a depressed breathing rate and a decrease in the uptake of benzene via the lungs.

In early epidemiolical surveys an increased susceptibility to the adverse health activity of benzene was ascribed to women *(1-4).* However, no current survey supports a sex-related differential sensitivity in humans for either benzene-induced anemia or leukemia (1-4). This may not be surprising considering the extent of the difference in SCE levels observed between the two sexes and the confounding effect of genetic constitution on SCE yields that we have observed in mice. The ability of benzene at concentrations as low as 28 ppm to induce a significant increase in SCEs in mice, regardless of the sex or genetic constitution of the animal, is especially interesting. OSHA currently considers 10 ppm benzene to be an occupationally safe exposure level for an eight hour working day (Time Weighted Average = 10 ppm with a 25 ppm ceiling) (see Goldstein, these proceedings). These levels are within the same order of magnitude as the lowest exposure level used in our experiments, an exposure level which elicited a positive genotoxic response in mice. Although these positive effects in mice cannot be directly extrapolated to human exposure conditions and to human physiology and the relevance of SCE induction to cancer remains unknown, the data does suggest that more research is needed to evaluate the cvrrent "safe" levels of benzene.

Age-Response to Inhaled Benzene

In evaluating the data from these various studies, one obvious and very significant observation is the age-related differential sensitivity of mice to the genotoxic effects of benzene (Table 7). "Aged" male mice, approximately 10 months of age, exhibited only a doubling in SCE frequency while "young" male mice, 3 months of age, exhibited almost a six-fold increase in SCEs. Furthermore, while exposure to benzene inhibited bone marrow cellular proliferation in the older male mice, inhibition which was enhanced one day after the exposure (Table 2), no inhibition was observed in the younger male mice examined under the same conditions (data not shown, 38).

Table 6. Effect of inhalation of benzene on SCE frequencies in bone marrow cells of mice

	C57B1/6	DBA/2
	SCE Frequency[a]	SCE Frequency[a]
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Table 6 Effect of inhalation of benzene on SCE frequencies in bone marrow cells of mice

Conc. Benzene (ppm/ppm-hr)	DBA/2 SCE Frequency[a]		C57Bl/6 SCE Frequency[a]	
	Male	Female	Male	Female
Control	4.3±0.3(6)	4.6±0.4(4)	4.1±0.5(5)	4.7±0.4(4)
28/112	7.4±0.5(4)	6.6±0.7(4)	6.2±0.9(5)	2±0.5(3)
489/1960	12.7±0.9(4)	9.5±0.6(4)	8.2±0.4(5)	7.5±0.5(3)
1018/4072	16.2±1.4(5)	9.6±1.0(4)	9.8±1.0(5)	7.5±0.5(4)
2082/9541	22.2±1.4(6)	11.2±0.2(3)	16.6±1.8(3)	9.3±0.2(3)
3044/12182	26.2±2.5(4)	11.9±0.5(5)	11.2±1.9(4)	9.0±0.9(5)
5276/21104	27.1±0.9(4)	10.6±0.8(4)	9.3±1.0(4)	8.7±0.7(4)

[a] Mean frequency of SCE per cell ± standard errors of the mean between (n) animals.

Statistically significant increase over the appropriate control by Student's t test at $p < 0.01$

Table 7. Effect of age on benzene-induced bone marrow damage in DBA/2 male mice

Age (mo)	Control SCE Frequency[a]	Control AGT (hr)[b]	Benzene* SCE Frequency[a]	Benzene* AGT (hr)[b]
3	4.3±0.3(4)	12.2±0.3(4)	26.222.5(4)[c]	13.520.5 (4)
10	4.6±0.2(6)	14.2±0.3(6)	8.5+0.6(7)[c]	16.6±0.4(7)[c]

*3000 ppm for 4 hours

[a]Mean frequency of SCE/cell ± standard error of the mean between (n) animals

[b]Average Generation Time = mean cell cycle duration ± S.E. between (n) animals; see Table 1 for additional information

[c]Statistically different by Student's t test at $p<0.01$

The biological basis €or these age differences is not known. The differences may result from alterations in metabolism, distribution of metabolites or parent compound, target cell sensitivity or to levels of DNA repair. There is no appropriate epidemiological information for age-related occupational exposure of humans to benzene suggesting an age-related differential sensitivity. However, the extensive genetic heterogeneity among humans would make the identification of age-related effects difficult to discern.

Route of Exposure

In one additional analysis, SCE data obtained from the two routes of exposure to benzene--inhaled and IP injection--were compared (Table 8). SCE frequency increased in a linear fashion as a function of benzene concentration for both routes of exposure. For one manifestation of genotoxic damage--SCEs--and under our experimental conditions, an ambient exposure of 120 ppm benzene for four hours equaled the effect of an IP injection of 1.0 mmoles/Kg. Experiments to determine the relative potency of benzene by these two routes of exposure are in progress. However, based on the known pharmacokinetic data for benzene metabolism in vivo (33,39), we have tentatively concluded that a greater bone marrow genotoxic effect results from inhaled benzene than when injected IP (40).

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Table 8. Effect of benzene exposure on bone marrow damage in male DBA/2 mice (10-14 weeks of age)

Inhalation*		IP Injection**	
Conc. (ppm)	SCE Frequency[a]	Conc. mmoles/Kg	SCE Frequency[a]
Control	4.3±0.3(6)	Control	6.1±0.3(6)
28	7.4±0.5(4)	.22	9.4±0.8(4)
217	10.320.9(4)	2.25	10.920.2 (4)
489	12.7±0.9(4)	5.63	17.0±0.6(6)
1018	16.2±1.4(5)	11.2s	21.420.6 (6)
2082	22.221.4 (6)	22.50	26.4±2.1(5)
3044	26.2±2.5(4)	33.75	36.0±3.3(4)
5276	17.1±0.9(4)	45.00	36.4±2.1(4)

*3000 ppm for 4 hours.

**Single IP injection of benzene dissolved in corn oil, total volume 0.1 mL/5 gm

"Mean frequency of SCE/per cell 2 S.E. between (n) animals

CONCLUSION

The results of our cytogenetic investigation into the genotoxic potential of benzene in murine bone marrow can be summarized as follows:

(i) The different responses to benzene observed in male and in female DBA/2 mice (with and without phenobarbital pretreatment), suggest that different metabolites of benzene are responsible for the induced chromosomal aberrations and SCEs and for the inhibition of cellular proliferation in bone marrow tissue in older mice.

(ii) The results from the partial hepatectomy and toluene studies indicate that while metabolism of benzene is necessary before genotoxic damage can occur in bone marrow cells, the liver may not be the main site of production for the SCE-inducing metabolite.

(iii) A four-hour exposure to ambient concentrations of benzene as low as 28 ppm induces a significant increase in bone marrow genotoxic damage as measured by SCE formation.

(iv) The modification of the magnitude of the benzene-induced SCE frequency in murine bone marrow cells by age, sex and genetic constitution emphasizes the importance of considering these parameters when attempting to extrapolate animal data to human health risks.

(v) The ability to directly correlate two different routes of benznene exposure for genotoxic damage should permit a better understanding of the pharmacokinetic distribution of benzene and its metabolites in animals and a better evaluation of benzene data for human health risks.

(vi) Our results suggest that the pharmacokinetic, metabolic and molecular mechanisms involved in benzene's ability to cause anemia and leukemia are extremely complex. If different metabolites of benzene are responsible for the induction of chromosomal aberrations, SCEs and cellular proliferation inhibition, then a good animal model for anemia may not be a good animal model for leukemia and vice-versa. Considering benzene's occupational and environmental importance, research in this area is not only of considerable interest but also of economic and societal importance. Furthermore, an understanding of the biological processes involved in benzene's genotoxic activity may lead to a better understanding of the human health hazards associated with the many other genotoxic agents to which man is exposed.

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DISCUSSION

Q. M (Western Illinois University): I wasn't sure whether the 3,000 ppm benzene exposure was in one application into an enclosed chamber or as a continuous flow?

A. Tice (BNL): It was a continuous flow measured throughout the four-hour period and represents an integrated dose.

Q. Sivak (Arthur D. Little): Would you put a couple of thousand ppm of benzene in context with work-place and environmental concentrations.

A. Tice (BNL): Historically, typical exposure levels were in excess of a thousand ppm in certain factories in Italy and in the U.S. In a Science article published a few years ago, benzene levels were measured in a garage during a normal paint stripping operation and the levels were on the order of a couple of hundred parts per million. The current OSHA standards set occupational exposure levels at 10 ppm for an eight-hour day. We observed a significant increase in SCEs after a four-hour exposure to 28 parts per million in the 10 to 14 week-old mice, an exposure level not greatly different from the current OSHA "safe" limits.

Q. Drew (BNL): Along these same lines the Hematology Group at Brookhaven has been looking at stem cells viability in bone marrow and we see a reduction in the number of stem cells after nine daily exposures to 25 ppm.

Q. Snyder (Thomas Jefferson): Ray, one question about the relationship between the benzene by IP and by inhalation. In recent years, one concept in the area of metabolic activation and induction of reactive intermediates is that toxicity seems to occur when you exceed detoxication mechanisms. This would suggest that the kinetics of the various pathways change as the dose increases. I wonder if at the lower end of your dose response curves the kinetics aren't quite different than what they are at the higher end?

A. Tice (BNL): I also feel sure that the spectrum and ratios of various metabolites of benzene alters as a function of dose. From the shape of the dose response curve and for one end point--SCEs--I get a much greater induction of SCEs at the low end of the concentration scale than I would expect from the linear regression analysis. I might interpret that to mean that the responsible reactive intermediate occurs more frequently at low doses of benzene than at higher doses. At higher concentrations of benzene the metabolite(s) capable of inducing chromosomal aberrations or of inhibiting cell proliferation kinetics may be more prevalent.

Q. Wieland (BNL): Ray, do you have any hypothesis on what may account for the difference between sexes or between young and old animals? For example, do you think it might be a manifestation of a change in DNA repair capacity?

A. Tice (BNL): DNA repair capacity is said to change as a function of age, although to what extent is still questionable. I expect that the differences are due more to changes in metabolism. There's a fair amount of data which shows that as animal age, the capacity to metabolize alters. I don't have a good answer for the difference between sexes. Hopefully, we will be able to examine the sex-dependent response by neonatal imprinting. I also don't know of any good benzene data in humans which suggests that there is a sex-related difference in metabolism or, for that matter, an age-related

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difference. Perhaps, like with a lot of other things, it just has not been looked at closely enough. Bob, do you have any comment.

Q. Snyder (Thomas Jefferson): The only interesting note in that regard is that in the early benzene literature there is a good deal of emphasis on the greater susceptiblity of women than men. However, there is no good evidence now to indicate any difference of susceptibility between males and females.

Q. Maltoni (Italy): There are experimental data suggesting that female rats are more susceptible than males to benzene's tumorigenic effects. Zymbal Gland carcinoma, which is the most common tumor in benzene-treated animals occurs only in females and not in males.