[CANCER RESEARCH 43, 1330–1334, March 1983]
0008-5472/83/0043-0000$02.00

Induction of Sister Chromatid Exchanges and Cell Division Delays in Human Lymphocytes by Microsomal Activation of Benzene[1]

Kanehisa Morimoto[2]

Laboratory of Radiobiology and Environmental Health and Department of Anatomy, University of California, San Francisco, California 94143

ABSTRACT

Metabolic activation of benzene by rat liver microsomes and a reduced nicotinamide adenine dinucleotide phosphate-generating system (S-9 mix) induced sister chromatid exchanges (SCEs) and cell division delays in cultured human lymphocytes. There were optimal concentrations of S-9 mix for the conversion of benzene into the active metabolites that exerted these cytotoxic effects. Reduced glutathione prevented the induction of SCEs by benzene plus S-9 mix in a dose-dependent manner. Reduced glutathione (3 mM) also prevented the induction of SCEs by catechol or hydroquinone, active metabolites of benzene and potent inducers of SCEs, strongly suggesting that glutathione did not simply inhibit the activity of S-9 mix to activate benzene but actually prevented the production of DNA lesions by the active metabolites. Pulse treatment of cells with benzene plus S-9 mix produced the largest number of SCEs when administered at 40 hr of culture (fixed at 72 hr) but did not induce SCEs when administered immediately after the beginning of culture. This indicates that induced DNA lesions that could lead to formation of SCEs are removed in time.

INTRODUCTION

Because benzene, which has a direct association with human cancer, *e.g.*, leukemia (9, 25), is contained in gasoline (8, 22), it is one of the most widely distributed environmental pollutants. Furthermore, the annual production of benzene for industrial use has been increasing worldwide; in the United States alone, an estimated 2 million workers are potentially exposed to it (8). Thus, there is an increased public health concern for the hematotoxicity and leukemogenicity of benzene, and many reviews have appeared on the subject (2, 4, 8, 9, 21, 25). However, the mechanisms by which benzene exerts its effects are not understood.

In the body, benzene is converted metabolically into phenolic compounds, the major metabolites being phenol, catechol, and hydroquinone (4, 23, 25). A previous study (18) showed that catechol and hydroquinone induce SCEs[3] as well as cell cycle delays in human lymphocytes *in vitro*. Because benzene itself is negative in the Ames *Salmonella* test, even with microsomal activation (4), as well as in *in vitro* SCE test (4, 7, 18), it has been suggested (7, 18) that the metabolites of benzene, which also interact with rat DNA (16, 26), might be responsible for its biological effects.

The present study with human lymphocytes demonstrates the mutagenic potential of benzene by means of an *in vitro* metabolic activating system that can produce the phenolic metabolites of benzene (5, 10, 27, 32). After metabolic activation, benzene leads to the induction of both SCE, which is one of the most sensitive methods for detecting the effects of mutagenic carcinogens (3, 13, 15, 22, 28), and cell cycle delays. The results also imply that induced DNA lesions that lead to SCE formation are removed in time.

MATERIALS AND METHODS

Heparinized peripheral blood samples were obtained from healthy adult men. Whole blood (0.2 ml) was added to 5 ml of Roswell Park Memorial Institute Tissue Culture Medium 1640 containing 15% fetal bovine serum and 3% phytohemagglutinin M (Grand Island Biological Co., Grand Island, N. Y.). Bromodeoxyuridine (20 μM) was also present in medium for the entire culture period. The cultures were incubated at 37° for 72 hr in complete darkness. Three hr before fixation, Colcemid (final concentration, 2×10^{-7} M) was added. The cells were then collected by centrifugation, exposed to 0.075 M KCl hypotonic solution for 5 min to spread the chromosomes and hemolyze the RBC, and fixed 3 times in methanol:acetic acid (3:1). Air-dried chromosome preparations were made, and a modification of the fluorescence-plus-Giemsa method (11) was applied to obtain harlequin chromosomes as reported previously (18, 19). Cells dividing for the first (X1), second (X2), and third or more (X3+) time in culture can be determined in such preparations (18, 19, 30). X1 cells contain chromosomes with both sister chromatids stained uniformly darkly. X2 cells contain only harlequin chromosomes with one chromatid darkly stained and its sister chromatid lightly stained, whereas X3+ cells contain some harlequin chromosomes and other chromosomes with both sister chromatids stained uniformly lightly.

Benzene (Aldrich Chemical Co., Milwaukee, Wis.) was first dissolved in serum-free culture medium to give a 25 mM concentration. Aliquots of this freshly made solution were added to the cultures to give the appropriate final concentration.

Cultures to be treated were first centrifuged, and the supernatant was removed, leaving 0.5 ml of medium which contained the pelleted cells. The cells were then resuspended in serum-free culture medium containing benzene and the metabolic activation system [Ames, S-9 mix, a rat liver microsomal suspension supplemented with an NADPH-generating system (1)]. The S-9 mix consisted of 10% (v/v) S-9 rat liver extract (Litton Bionetics, Inc., Kensington, Md.), prepared according to the procedure described by Ames *et al.* (1); 8×10^{-3} M $MgCl_2$; 3.3×10^{-2} M KCl; 5×10^{-3} M glucose 6-phosphate; 4×10^{-3} M NADP; and 1×10^{-1} M Na_2HPO_4:NaH_2PO_4 (pH 7.4). The proportion of S-9 mix in the medium is expressed as a percentage of undiluted S-9 mix; *e.g.*, 30% S-9 mix consists of 30% undiluted S-9 mix and 70% Roswell Park Memorial Institute Tissue Culture Medium 1640 with supplements. Except as otherwise described, the cells were incubated at 37° with benzene and S-9 mix for 2 hr (40 hr after the beginning of culture) in tightly capped glass tubes in a shaking water bath. This agitation ensured even distribution of active metabolites among the cells (34). After treatment, the medium containing benzene and S-9 mix was removed by centrifugation, and the cells were washed 3 times with prewarmed complete culture medium. The cells were then resuspended in the same culture medium for further incubation.

[1] Supported by the United States Department of Energy.

[2] Recipient of a fellowship from the Japanese Society for the Promotion of Science. Present address: Department of Public Health, Faculty of Medicine, University of Tokyo, Tokyo 113, Japan.

[3] The abbreviations used are: SCE, sister chromatid exchange; GSH, glutathione.

Received February 10, 1982; accepted December 7, 1982.

SCEs were analyzed in 35 consecutive second-division cells for each point. Two hundred metaphase cells were scored to determine the percentage of cells in X1, X2, and X3+ divisions. The S.E. for the percentages of X1, X2, and X3+ cells was determined by the use of polynomial variances.

RESULTS

When cells from cultures exposed to benzene in the presence of metabolic activation were examined, it was found that 10% S-9 mix caused the largest increase in the number of SCEs (Table 1; Chart 1). Cells treated with benzene and 1% or even 90% S-9 mix, or in the absence of activation, showed no increase of SCEs (Chart 1). Treatment with S-9 mix alone also gave an increase in the frequency of SCEs when added at concentrations of more than 10% (Table 1). The data indicate that 10% S-9 mix is the optimal concentration for converting benzene into the active metabolites that might be responsible for the induction of SCEs. Benzene induced SCEs in a clearly dose-dependent manner only after the appropriate activation. It was further shown that treatment with 5 mM benzene plus 10% S-9 mix leads to highly significant increases in the frequency of SCEs in lymphocytes from 4 different individuals (Table 2).

Only when 10 to 30% S-9 mix was used was benzene converted into active form(s) that were cytotoxic in that they delayed cell turnover times (Chart 2). It has been shown before (18) that a chemically induced delay in cell division is clearly manifested as a change in the relative proportion of X1, X2, and X3+ cells. In this study, the ratio of X3+ cells to X2 cells was calculated as another index of cell division delay (Table 1). When cells from untreated cultures fixed at intervals from 60 to 72 hr after the beginning of culture were examined, the base-line frequency of SCEs found in X2 cells was independent of culture time, but the ratio of X3+ to X2 cells increased with increasing culture times (Chart 3). These data are in good agreement with those found previously for untreated cells (18, 19, 30). By comparing the relative frequencies in X1, X2, and X3+ cells or the ratios of X3+ to X2 cells from treated and control cultures, cell division delays can be estimated (18). For instance, treatment with 5 mM benzene plus 10% S-9 mix gave a distribution of 11% X1, 39% X2, and 50% X3 cells at 72 hr (Chart 2), which is given in untreated cultures at 67 hr (Chart 3). It thus appears that this treatment leads to a division delay of 5 hr.

The delay in cell division produced by benzene after activation is evident in both the frequency curves of X1, X2, and X3+ cells (Chart 2) and the ratios of X3+ to X2 cells in treated cultures (Table 1). Benzene exerted a greater cytotoxic effect in the presence of 30% S-9 mix than in the presence of 10% S-9 mix. However, 5 mM benzene plus 30% S-9 mix induced only half as many SCEs as did 5 mM benzene plus 10% S-9 mix (Chart 1). There was no increase in the SCE frequency in

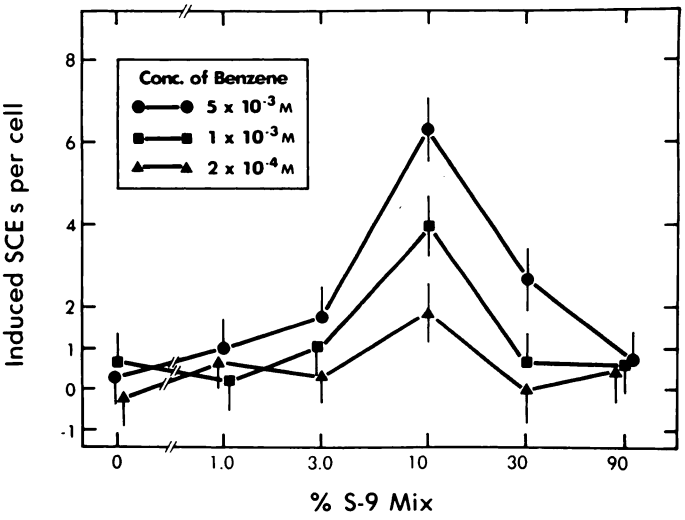

Chart 1. Induction of SCEs in cells exposed to benzene and S-9 mix. Cells were treated for 2 hr (40 hr after the beginning of culture). Induced SCEs/cell were calculated by subtracting the SCE frequency in cells treated with S-9 alone from that with benzene plus S-9 at each S-9 concentration. *Bars*, S.E.

Table 2

Induction of SCEs by benzene plus S-9 mix in lymphocytes from 4 different individuals

Cells were treated from 40 to 42 hr after initiation of cultures. Two cultures from Donors A and B were also exposed to benzene plus S-9 and fixed at 78 hr. They showed 11.57 SCEs and 11.03 SCEs per cell, respectively.

	Treatment			
Donor	Control	10% S-9 mix	5 mM benzene	5 mM benzene + 10% S-9 mix
A	7.17 ± 0.45[a]	7.86 ± 0.47	7.40 ± 0.45	13.23 ± 0.63[b]
B	7.71 ± 0.45	7.11 ± 0.46	6.89 ± 0.47	12.91 ± 0.60[b]
C	6.71 ± 0.49	7.31 ± 0.40	6.26 ± 0.50	13.96 ± 0.64[b]
D	6.83 ± 0.51	7.43 ± 0.46	7.49 ± 0.47	12.66 ± 0.60[b]
Mean[c]	7.11 ± 0.23	7.36 ± 0.19	7.00 ± 0.29	12.72 ± 0.24[b]

[a] Mean ± S.E. of 35 second-division cells.
[b] Significant at $p < 0.001$ (Student's t test).
[c] Mean ± S.E. of 4 individuals.

Table 1

SCEs/cell and ratios of X3+ to X2 cells in human lymphocytes exposed for 2 hr to benzene plus S-9 mix

Cells were treated from 40 to 42 hr after initiation of cultures. The SCE frequency is based on 35 second-division cells for each point.

	Concentration of benzene (M)							
% of S-9 mix	Control		2.0×10^{-4}		1.0×10^{-3}		5.0×10^{-3}	
	SCEs/cell	X3+:X2	SCEs/cell	X3+:X2	SCEs/cell	X3+:X2	SCEs/cell	X3+:X2
0	7.69 ± 0.48[a]	2.44	7.46 ± 0.46	2.13	8.34 ± 0.51	1.91	8.03 ± 0.43	2.03
1	7.46 ± 0.46	2.33	8.14 ± 0.45	2.10	7.86 ± 0.47	1.86	8.49 ± 0.48	2.01
3	8.10 ± 0.45	2.58	8.49 ± 0.51	2.30	9.20 ± 0.55	2.05	9.86 ± 0.55[b]	2.20
10	7.54 ± 0.43	1.71	9.37 ± 0.51[c]	1.83	11.51 ± 0.57[d]	1.65	13.80 ± 0.62[d]	1.28
30	9.69 ± 0.53[c]	1.51	9.60 ± 0.53[c]	1.49	10.31 ± 0.54[b]	1.02	12.34 ± 0.63[d]	0.67
90	10.46 ± 0.57[b]	0.94	10.97 ± 0.59[d]	1.08	11.14 ± 0.59[d]	1.09	11.22 ± 0.59[d]	0.91

[a] Mean ± S.E.
[b] Significant by Student's t test (one tailed) ($p < 0.01$).
[c] $p < 0.05$.
[d] $p < 0.001$.

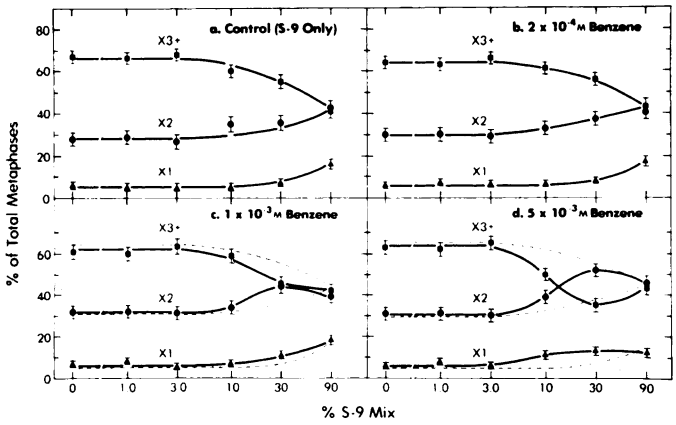

Chart 2. Percentage of X1, X2, and X3+ cells in cultures exposed to benzene and S-9 mix. The frequency curves in control cultures (*a*) are shown by *broken lines* in *c* and *d* for comparison. *Bars*, S.E.

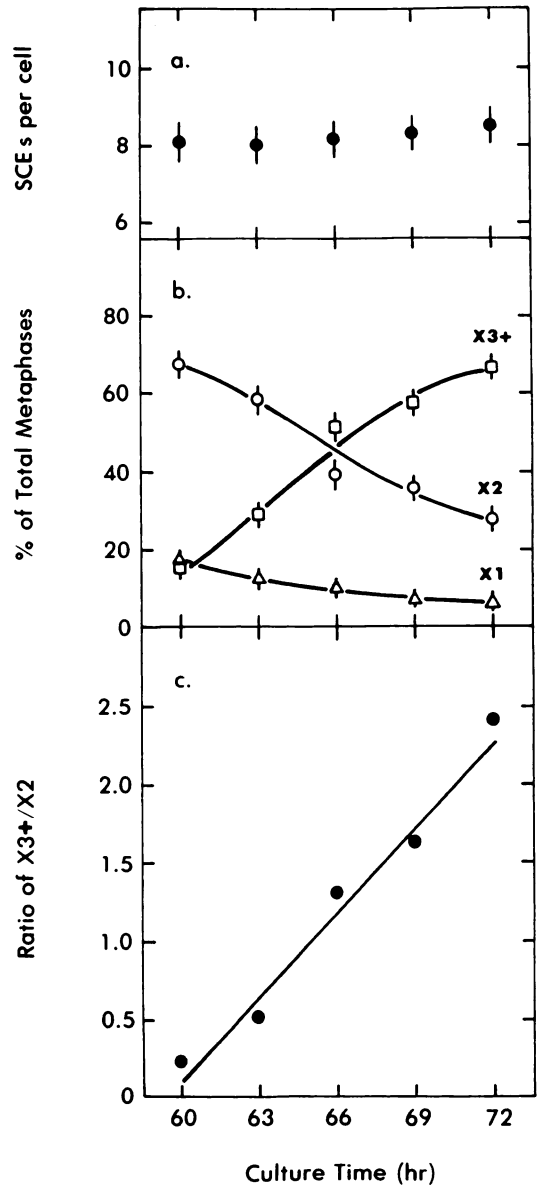

Chart 3. Frequency of SCEs/cell (*a*); the percentage of X1, X2, and X3+ cells (*b*); and the ratio of X3+ to X2 cells (*c*) in untreated cultures fixed at various times. *Bars*, S.E.

cells exposed to 1 mM benzene plus 30% S-9 mix, which induced about the same amount of delay as did 5 mM benzene plus 10% S-9 mix (Chart 2), whereas 1 mM benzene plus 10% S-9 mix still caused the production of SCEs (Chart 1). Thus, we have excluded the possibility that a longer division delay induced by exposure to benzene plus 30% S-9 mix would have caused an apparently smaller induction of SCEs. These data therefore strongly suggest that the metabolites predominantly responsible for cell division delay might be different from those that induce SCEs.

When benzene is incubated with S-9 mix, metabolites are formed that bind irreversibly to biomacromolecules, and the addition of reduced GSH to the incubation mixture prevents this binding in a dose-dependent manner (31). In the present study, the addition of GSH to the culture caused a dose-dependent decrease in SCEs in cells exposed to benzene and S-9 mix (Chart 4). The induction of SCEs was almost completely prevented by 3 mM GSH. In the repeated experiment (Table 3), it was further shown that 3 mM GSH treatment also completely prevented the induction of SCEs by catechol or hydroquinone, major phenolic metabolites of benzene and potent inducers of SCEs (18), as well as that by benzene plus S-9 mix. This strongly suggests that GSH treatment did not merely prevent activation of benzene by inhibiting the S-9 mix activity but really prevented the induction of SCE-leading lesions by the metabolites of benzene. These data are in excellent agreement with the finding of Tunek *et al.* (31) that 2 mM GSH prevented 90 to 95% of the irreversible binding of benzene metabolites to biomacromolecules.

When cells from cultures exposed to 5×10^{-3} M benzene plus 10% S-9 mix at various times were examined for SCEs, it was found that different numbers of SCEs were induced at different times of treatment (Chart 5). After treatment at 0 hr, no SCEs were induced. After treatment at subsequent times, however, the frequency of SCEs increased, peaking at 40 hr, and then decreased at later treatment times. The data thus indicate that 40 hr after the beginning of culture is the most effective treatment time for the induction of SCEs by benzene plus S-9 mix.

In earlier experiments, cells in culture were exposed to

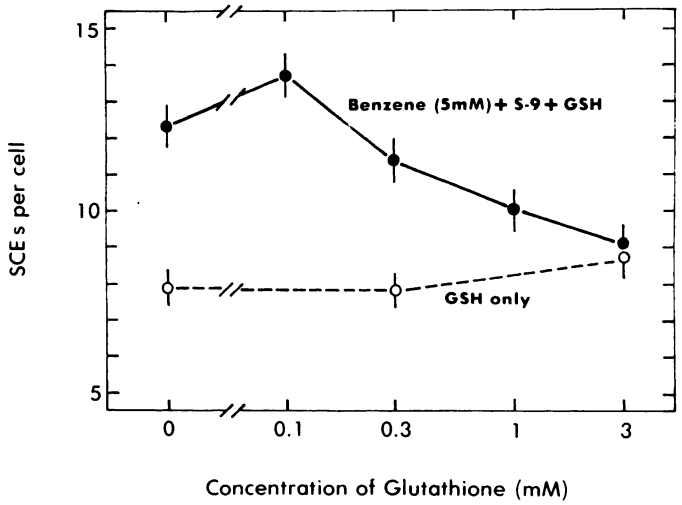

Chart 4. Effects of GSH on the induction of SCEs by benzene plus S-9 mix. Cells were treated with various concentrations of GSH along with benzene (5×10^{-3} M) and 10% S-9 mix from 40 to 42 hr after initiation. *Bars*, S.E.

Table 3

Preventive effects of GSH on the induction of SCEs by catechol, hydroquinone, or benzene plus S-9 mix

Cells were treated in the same protocol as in Chart 4. Each value was based on 35 second-division cells.

Donor	GSH (3 mM) treatment	Treatment: None	Benzene (5 mM) + S-9 mix (10%)	Catechol (0.3 mM)	Hydroquinone (1 mM)
A	–	7.17 ± 0.45[a]	13.23 ± 0.61	13.26 ± 0.79	11.37 ± 0.57
	+	7.86 ± 0.43	8.26 ± 0.55	7.54 ± 0.53	8.63 ± 0.57
	t test	NS[b]	$p < 0.001$	$p < 0.001$	$p < 0.001$
B	–	7.71 ± 0.45	12.91 ± 0.61	12.83 ± 0.61	12.69 ± 0.57
	+	7.11 ± 0.49	6.91 ± 0.53	7.60 ± 0.50	7.54 ± 0.50
	t test	NS	$p < 0.001$	$p < 0.001$	$p < 0.001$

[a] Mean ± S.E.
[b] NS, not significant ($p = 0.05$).

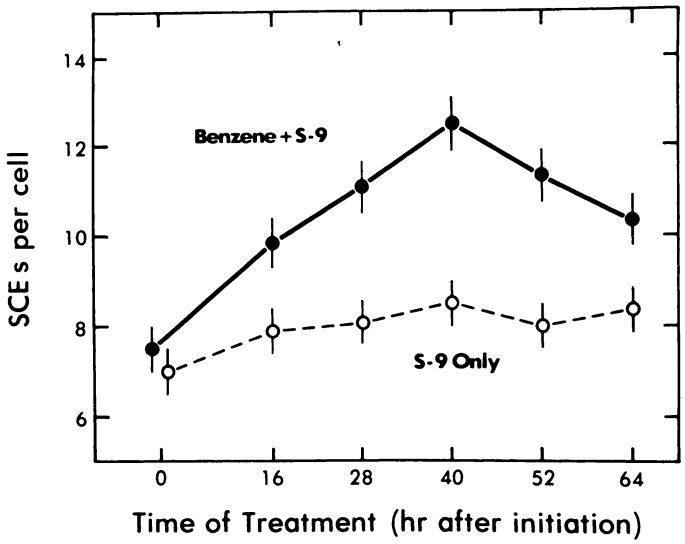

Chart 5. Effects of the time of treatment with benzene plus S-9 mix on the induction of SCEs. Cells were treated with 5 mM benzene plus 10% S-9 mix for 2 hr at various times and fixed at 72 hr after the beginning of culture. *Bars*, S.E.

benzene plus S-9 mix under various conditions of treatment. The results have shown (Table 4) that both tight sealing and agitation of culture bottles during treatment were essential for producing a marked increase in the SCE frequency. These results were repeatable.

Table 4

Effects of treatment conditions on the induction of SCEs by benzene plus S-9 mix

Cells were treated with 5×10^{-3} M benzene plus 10% S-9 mix from 40 to 42 hr after the beginning of culture.

Treatment condition: Type of cap	Agitation	SCEs/cell
Silicone[a]	+	8.66 ± 0.49[b]
Silicone	+	12.46 ± 0.60[c]
Silicone	–	9.43 ± 0.50
Cork	+	9.26 ± 0.55
—[d]	+	9.14 ± 0.52

[a] Incubated without benzene plus S-9 treatment.
[b] Mean ± S.E.
[c] Significant at $p < 0.001$ (Student's *t* test).
[d] Incubated without a stopper.

DISCUSSION

The present study demonstrates that, after appropriate *in vitro* metabolic activation, benzene causes both an increase in SCEs and a delay in cell division in human lymphocyte cultures. Previous experiments (18) showed that the phenolic metabolites, rather than benzene itself, are the cytotoxic substances that induce SCEs and cell cycle delays. Because it has been shown (24, 31, 32) that benzene is converted into the phenolic metabolites *in vitro* when incubated with rat liver microsomes in the presence of an NADPH-generating system (S-9 mix), the present results indicate that the formation of phenolic metabolites might be the cause of benzene toxicity. This is consistent with the findings of Tunek *et al.* (31), that incubation of cultures with GSH hardly affected the formation of phenol but did decrease the irreversible binding of benzene metabolites to biomacromolecules. With the present finding that GSH led to a dose-dependent decrease in SCEs induced by catechol and hydroquinone as well as benzene plus S-9 mix, this strongly suggests that further metabolites of the phenolic metabolites, possibly benzo(semi)quinones (32), might be the ultimate mutagenic toxicants that induce irreversible binding, SCEs, and cell cycle delays and thus might be leukemogenic.

There are optimal concentrations of S-9 mix for the conversion of benzene into reactive forms that induce SCEs and cell cycle delays, because only after appropriate activation does benzene exert these effects in a clearly dose-dependent manner. Similar results with known promutagens such as benzo(*a*)pyrene (1, 14, 29), aflatoxin B_1 (14, 17, 29), furylfuramide (20), and dimethyl nitrosamine (6, 14, 17) indicate the critical importance of S-9 concentrations in mutagenesis assays. The existence of optima for concentrations of S-9 mix to metabolize benzene may result from the balance between activation of benzene into reactive forms and their subsequent reactions (detoxication) (12). At lower than optimal concentrations of S-9 mix, benzene cannot be converted into the phenolic metabolites at sufficient concentrations. At higher concentrations, both the conversion of benzene into phenolic compounds and their subsequent reactions would be so fast that the metabolites could not be accumulated sufficiently. But at optimal concentrations of S-9 mix, benzene can be metabolized to produce sufficient amounts of the compounds that enter cells and cause damage in DNA and other macromolecules, resulting in a marked increase in SCEs and cell cycle delays.

It has been reported recently (33) that there was no increase in SCEs in cultured lymphocytes obtained from benzene-exposed workers, although there was a significant increase in

chromosome aberrations. One should be careful in interpreting such results, because the present study suggests that the DNA lesions leading to SCE formation that are induced by benzene metabolites are repairable. It is necessary for cells to pass through the S phase before DNA damage in the cells can result in the formation of SCEs (35). If induced DNA damage is repaired completely before cells enter S phase, then those cells show no increase in SCE frequency. It has been noted (19) that second-division metaphase cells in 72-hr cultures enter their first S phase at about 48 hr after the beginning of culture. In the present experiments, benzene plus 10% S-9 mix, which induced a 5-hr delay in cell division, gave the largest increase in SCEs when added for 2 hr immediately before cells entered their first S phase, *i.e.*, at 40 hr of culture. The same treatment at 0 hr of culture resulted in no increase in SCEs because there was enough time for repair of the induced DNA damage that would have led to SCE formation. This suggests the possibility that, even when mutagenic carcinogens such as benzene produce DNA damage in the circulating blood (G_0) lymphocytes, the damage can be repaired completely before the cells enter S, resulting in no increase of SCEs in cultured lymphocytes.

ACKNOWLEDGMENTS

I thank Professors S. Wolff and A. Koizumi for continuing guidance and critical reading of the manuscript.

REFERENCES

1. Ames, B. N., McCann, J., and Yamazaki, E. Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. Mutat. Res., *31:* 347–364, 1975.
2. Berlin, M., Gage, J., and Jonnson, E. Increased aromatics in motor fuels: a review of the environmental and health effects. Work-Environ. Health, *11:* 1–20, 1974.
3. Carrano, A. V., Thompson, L. H., Lindl, P. A., and Minkler, J. L. Sister chromatid exchange as an indicator of mutagenesis. Nature (Lond.), *271:* 551–553, 1978.
4. Dean, B. J. Genetic toxicology of benzene, toluene, xylenes, and phenols. Mutat. Res., *47:* 75–97, 1978.
5. Drew, R. T., and Fouts, J. R. The lack of effects of pretreatment with phenobarbital and chlorpromazine on the acute toxicity of benzene in rats. Toxicol. Appl. Pharmacol., *27:* 183–193, 1974.
6. Frantz, C. N., and Malling, H. V. The quantitative microsomal mutagenesis assay method. Mutat. Res., *31:* 365–380, 1975.
7. Gerner-Smidt, P., and Friedrich, U. The mutagenic effect of benzene, toluene, and xylene studied by the SCE technique. Mutat. Res., *58:* 313–316, 1978.
8. Goldstein, B. D. Introduction to "benzene toxicity." J. Toxicol. Environ. Health, *2* (Suppl.): 1–4, 1977.
9. Goldstein, B. D. Hematotoxicity in humans. J. Toxicol. Environ. Health, *2* (Suppl.): 69–105, 1977.
10. Gonasun, L. M., Witmer, C., Kocsis, J. J., and Snyder, R. Benzene metabolism in mouse liver microsomes. Toxicol. Appl. Pharmacol., *26:* 398–406, 1973.
11. Goto, K., Maeda, S., Kano, Y., and Sugiyama, T. Factors involved in differential-Giemsa staining of sister chromatids. Chromosoma (Berl.), *66:* 351–359, 1978.
12. Jerina, D., Daly, J., Witkop, B., Zaltzman-Nirenberg, P., and Udenfriend, S. Role of the arene oxide-oxepin system in the metabolism of aromatic substrates. I. *In vitro* conversion of benzene oxide to a premercapturic acid and a dihydrodiol. Arch. Biochem. Biophys., *128:* 176–183, 1968.
13. Kato, H., and Shimada, H. Sister chromatid exchanges induced by mitomycin C: a new method of detecting DNA damage at chromosomal level. Mutat. Res., *28:* 459–464, 1975.
14. Kuroki, T., Malaveille, C., Drevon, C., Piccoli, C., Macleod, M., and Selkirk, J. K. Critical importance of microsome concentration in mutagenesis assay with V79 Chinese hamster cells. Mutat. Res., *63:* 259–272, 1979.
15. Latt, S. A. Sister chromatid exchanges, indices of human chromosome damage and repair: detection by fluorescence and induction by mitomycin C. Proc. Natl. Acad. Sci. U. S. A., *71:* 3162–3166, 1974.
16. Lutz, W. K., and Schlatter, C. Mechanism of the carcinogenic action of benzene: irreversible binding to rat liver DNA. Chem.-Biol. Interact., *18:* 241–245, 1977.
17. Malaveille, C., Kuroki, T., Brun, G., Hautefeuille, A., Camus, A., and Bartsch, H. Some factors determining the concentration of liver proteins for optimal mutagenicity of chemicals in the *Salmonella*/microsome assay. Mutat. Res., *63:* 245–258, 1979.
18. Morimoto, K., and Wolff, S. Increase of sister chromatid exchanges and perturbations of cell division kinetics in human lymphocytes by benzene metabolites. Cancer Res., *40:* 1189–1193, 1980.
19. Morimoto, K., and Wolff, S. Cell cycle kinetics in human lymphocyte cultures. Nature (Lond.), *288:* 604–606, 1980.
20. Nakamura, N., Suzuki, N., and Okada, S. Mutagenicity of furylfuramide, a food preservative, tested by using alanine-requiring mouse L5178Y cells *in vitro* and *in vivo*. Mutat. Res., *46:* 355–364, 1977.
21. National Research Council, Committee on Toxicology. Health Effects of Benzene: A Review. Washington, D. C.: National Academy of Sciences, 1976.
22. Perry, P., and Evans, H. J. Cytological detection of mutagen-carcinogen exposure by sister chromatid exchange. Nature (Lond.), *258:* 121–125, 1975.
23. Rusch, G. M., Leong, B. K. J., and Laskin, S. Benzene metabolism. J. Toxicol. Environ. Health, *2* (Suppl.): 23–36, 1977.
24. Sloane, N. H. Hydroxymethylation of the benzene ring. 1. Microsomal formation of phenol via prior hydroxymethylation of benzene. Biochim. Biophys. Acta, *107:* 599–602, 1965.
25. Snyder, R., and Kocsis, J. J. Current concepts of chronic benzene toxicity. CRC Crit. Rev. Toxicol., *3:* 265–288, 1975.
26. Snyder, R., Lee, E. W., and Kocsis, J. J. Binding of labeled benzene metabolites to mouse liver and bone marrow. Res. Commun. Chem. Pathol. Pharmacol., *20:* 191–194, 1978.
27. Snyder, R., Uzuki, F., Gonasun, L., Bromfeld, E., and Wells, A. The metabolism of benzene *in vitro*. Toxicol. Appl. Pharmacol., *11:* 346–360, 1967.
28. Stetka, D. G., and Wolff, S. Sister chromatid exchange as an assay for genetic damage induced by mutagen-carcinogens. II. *In vitro* test for compounds requiring metabolic activation. Mutat. Res., *41:* 343–350, 1976.
29. Takehisa, S., and Wolff, S. Induction of sister chromatid exchanges in Chinese hamster cells by carcinogenic mutagens requiring metabolic activation. Mutat. Res., *45:* 263–270, 1977.
30. Tice, R., Schneider, E. L., and Rary, J. M. The utilization of bromodeoxyuridine incorporation into DNA for the analysis of cellular kinetics. Exp. Cell Res., *102:* 232–236, 1976.
31. Tunek, A., Platt, K. L., Bentley, P., and Oesch, F. Microsomal metabolism of benzene to species irreversibly binding to microsomal protein and effects of modifications of this metabolism. Mol. Pharmacol., *14:* 920–929, 1978.
32. Tunek, A., Platt, K. L., Przybylski, M., and Oesch, F. Multi-step metabolic activation of benzene: effect of superoxide dismutase on covalent binding to microsomal macromolecules, and identification of glutathione conjugates using high pressure liquid chromatography and field desorption mass spectrometry. Chem.-Biol. Interact., *33:* 1–17, 1980.
33. Watanabe, T., Endo, A., Kato, Y., Shima, S., Watanabe, T., and Ikeda, M. Cytogenetics and cytokinetics of cultured lymphocytes from benzene-exposed workers. Int. Arch. Occup. Environ. Health, *46:* 31–41, 1980.
34. White, A. D., and Hesketh, L. C. A method utilizing human lymphocytes with *in vitro* metabolic activation for assessing chemical mutagenicity by sister-chromatid exchange analysis. Mutat. Res., *69:* 283–291, 1980.
35. Wolff, S., Bodycote, J., and Painter, R. B. Sister chromatid exchanges induced in Chinese hamster cells by UV irradiation at different stages of the cell cycle: the necessity for cells to pass through S. Mutat. Res., *25:* 73–81, 1974.