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SHORT COMMUNICATION

Sister Chromatid Exchange in Regenerating Liver and Bone Marrow Cells of Mice Exposed to Styrene

Sister Chromatid Exchange in Regenerating Liver and Bone Marrow Cells of Mice Exposed to Styrene. CONNER, M. K., ALARIE, Y., DOMBROSKE, R. L. (1979). *Toxicol. Appl. Pharmacol.* **50**, 365–367. Following exposure of BDF_1 males to 565 ± 15.8 ppm styrene for 4 days (6 hr/day), sister chromatid exchange frequencies were analyzed simultaneously in regenerating liver and bone marrow cells from exposed mice. Comparisons were made with nonexposed controls. Regenerating liver as well as bone marrow cells showed a three- to four fold increase relative to the control.

The use of 5-bromodeoxyuridine (BrdU)-labeled sister chromatid exchange (SCE) as a sensitive indicator of DNA and chromosomal damage, induced by a wide variety of mutagenic agents, has recently been reviewed (Kato, 1977; Schneider *et al.*, 1978; Wolff, 1977). The ability of SCE to detect metabolic activation is evident from significant increases in SCE frequencies induced in murine bone marrow cells *in vivo* by cyclophosphamide, an agent whose activity requires biotransformation to a potent alkylating agent (Allen and Latt, 1976), as contrasted with its inefficiency in producing SCE *in vitro* (Perry and Evans, 1975). A recently developed technique describes sister chromatid differentiation via BrdU labeling of regenerating liver cells (Conner *et al.*, 1978). The present communication describes, for the first time, the use of a liver preparation for study of chemically induced SCE *in vivo*.

Since liver is implicated in the metabolism of styrene (Ohtsuji and Ikeda, 1971) to styrene oxide, a potent *in vitro* (Chinese hamster ovary cells) SCE inducer whose effectiveness reportedly decreases following liver microsomal treatment (de Raat, 1978), the industrially important monomer, styrene, was selected for study of its possible effect on SCE in regenerating murine liver cells. Its effect was also studied simultaneously in bone marrow cells. Administration of styrene was via inhalation, the primary route for industrial exposure.

METHODS

Styrene (99%), obtained from Aldrich Chemical Company (Milwaukee, Wis.), was used without further purification. Hepatectomized and nonhepatectomized 12- to 14-week-old BDF_1 male mice (26–32 g) offspring from a C57Bl/6J female × DBA/2J male cross were used for exposure and control groups. Two-third partial hepatectomies were performed between 11 : 00 AM and noon as previously described (Conner *et al.*, 1978).

Exposures. Four mice (two hepatectomized and two nonhepatectomized) were exposed at a time. The mice were restrained so that only their heads protruded into a 2.5-liter glass exposure chamber exactly as previously described (Barrow *et al.*, 1977). A pump regulated the airflow at 20 liters/min. The generation system consisted of a large impinger containing approximately 30 ml of styrene at room temperature. Styrene was vaporized by metered flow of dried, filtered air. Styrene concentrations, determined twice daily by gc analysis (Perkin–Elmer Model 3902B, flame ionization detector) of charcoal-adsorbed chamber samples (60–70 min) (NIOSH, 1977), were 565 ± 18.5 (SD) ppm. Exposures of 6 hr/day (9 : 15 AM–3 : 15 PM) for 4 days, corresponded to Days 2–5 post hepatectomy.

BrdU incorporation and slide preparation. Control and exposed mice were each injected ip with 0.2 ml of BrdU (10 mg/ml saline) every hour for 9 hr from

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3 : 30 through 11 : 30 PM on the last day of styrene exposure. At 7 : 30 AM the following day, each mouse was injected ip with 100 μg of colchicine and sacrificed at 11 : 30 AM. Harvest of bone marrow cells from nonhepatectomized mice, liver and bone marrow cell harvest from hepatectomized mice, slide preparation, and staining of slides were carried out as previously described (Conner *et al.*, 1978). Slides from the control and exposed groups were screened (double blind) and 30 cells of each animal and type were scored for SCE per cell.

RESULTS AND DISCUSSION

Table 1 shows the average SCE per cell $\pm$ SD for regenerating liver and bone marrow cells isolated from hepatectomized mice and for bone marrow cells isolated from nonhepatectomized mice following a 4-day (6 hr/day) inhalation exposure to 565 $\pm$ 15.8 ppm styrene. A three-to four-fold increase in SCE frequency relative to the controls was observed in regenerating liver cells of mice exposed to styrene. Thus, using the technique presented by Conner *et al.* (1978), it was possible to detect a definite effect of styrene at this exposure concentration. It is of interest to note that a three- to four-fold increase in SCE frequency was also observed in bone marrow cells of exposed mice, hepatectomized as well as nonhepatectomized. The results demonstrate the feasibility of using an *in vivo* SCE technique for elucidating cytotoxicity on regenerating liver cells.

TABLE 1

SISTER CHROMATID EXCHANGE IN REGENERATING LIVER AND BONE MARROW CELLS OF HEPATECTOMIZED MICE AND IN BONE MARROW CELLS FROM NONHEPATECTOMIZED MICE FOLLOWING INHALATION EXPOSURE TO STYRENE[a]

	Average SCE/cell $\pm$ SD		
	Hepatectomized mice		Nonhepatectomized mice
	Bone marrow	Liver	Bone marrow
Control			
1	2.8 $\pm$ 1.3	3.3 $\pm$ 1.5	2.8 $\pm$ 1.3
2	2.9 $\pm$ 1.8	4.9 $\pm$ 2.5	4.0 $\pm$ 2.6
3	3.3 $\pm$ 2.5	3.2 $\pm$ 1.3	3.1 $\pm$ 1.9
4	[b]	[b]	3.2 $\pm$ 1.5
Mean	3.0 $\pm$ 1.9	3.8 $\pm$ 2.0	3.3 $\pm$ 1.9
Exposed			
1	11.5 $\pm$ 2.6	12.2 $\pm$ 3.5	11.7 $\pm$ 5.2
2	12.0 $\pm$ 3.7	13.5 $\pm$ 3.6[c]	9.8 $\pm$ 3.4
3	12.1 $\pm$ 3.7	11.3 $\pm$ 3.2	12.2 $\pm$ 4.2
4	12.0 $\pm$ 3.6	11.9 $\pm$ 4.3	10.1 $\pm$ 2.2
Mean[d]	11.9 $\pm$ 3.4	12.2 $\pm$ 3.7	11.0 $\pm$ 4.0

[a] Styrene (565 $\pm$ 15.8 ppm) was given for 4 days (6 hr/day). Each value was determined by scoring 30 cells.

[b] Accidental death during BrdU injection.

[c] 21 cells were scored.

[d] All exposed group means are significantly different from their respective control mean (*t* test, $p < 0.05$).

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REFERENCES

ALLEN, J. W., AND LATT, S. A. (1976). *In vivo* BrdU-33258 Hoechst analysis of DNA replication kinetics and sister chromatid exchange formation in mouse somatic and meiotic cells. *Chromosoma* **58**, 325–340.

BARROW, C. S., ALARIE, Y., WARRICK, J. C., AND STOCK, M. F. (1977). Comparison of sensory irritation response in mice to chlorine and hydrogen chloride. *Arch. Environ. Health* **32**, 68–76.

CONNER, M. K., BOGGS, S. S., AND TURNER, J. H. (1978). Comparisons of *in vivo* BrdU labeling methods and spontaneous sister chromatid exchange frequencies in regenerating murine liver and bone marrow cells. *Chromosoma* **68**, 303–311.

DE RAAT, W. K. (1978). Induction of sister chromatid exchanges by styrene and its presumed metabolite styrene oxide in the presence of rat liver homogenate. *Chem.-Biol. Interact.* **20**, 163–170.

KATO, H. (1977). Spontaneous and induced sister chromatid exchanges as revealed by the BrdU labeling method. *Int. Rev. Cytol.* **33**, 55–97.

NIOSH. (1977). *NIOSH Manual of Analytical Methods*, Vol. 1, p. 127. U.S. Department of HEW, Center for Disease Control, Cincinnati, Ohio.

OHTSUJI, H., AND IKEDA, M. (1971). The metabolism of styrene in the rat and the stimulatory effect of phenobarbital. *Toxicol. Appl. Pharmacol.* **18**, 321–328.

PERRY, P., AND EVANS, J. (1975). Cytological detection of mutagen-carcinogen exposure by sister chromatid exchange. *Nature (London)* **258**, 121–125.

SCHNEIDER, E. L., TICE, R. R., AND KRAM, D. (1978). Bromodeoxyuridine differential chromatid staining technique: A new approach to examining sister chromatid exchange and cell replication kinetics. In *Methods in Cell Biology* (D. M. Prescott, ed.), Vol. XX, pp. 379–409, Academic Press, New York.

WOLFF, S. (1977). Sister chromatid exchange. *Annu. Rev. Genet.* **11**, 183–201.

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