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Sister-chromatid exchanges in lymphocytes are increased in relation to longitudinally measured occupational exposure to low concentrations of styrene

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Summary

A longitudinal investigation of styrene exposure was conducted among 48 workers employed at a reinforced plastic boat manufacturing facility. 8-h time-weighted average (TWA) exposures to styrene and concentrations of styrene in the breath were determined for each individual on 7 randomly chosen days during 1 year. Peripheral blood lymphocytes from each subject were analyzed for sister chromatid exchanges (SCEs) 2 times and micronuclei (MN) 4 times during this period. Individual mean SCEs ranged from 4.7 to 9.5 SCEs per cell with a population mean of 6.4 ± 0.2 SCEs per cell. SCEs were found to be significantly increased with an overall observed increase of 11.7% related to increasing exposure to styrene (mean air concentration 64.2 mg/m^3 ± 71.5; range 0.88–235 mg/m^3) and with cigarette smoking. Examination of the relative contribution of each variable to regression of SCEs showed that smoking contributed about 62% and styrene exposure contributed about 25% of the total variability. Intra-individual lymphocyte MN frequencies did not vary significantly over time nor was a gradient toward increased MN observed with styrene exposure. However, significant inter-individual differences in MN frequencies were observed. Females had significantly higher MN frequencies than did males; MN were also increased with age. This study is highly unusual in that it illustrates the ability to separately quantify the relative contribution of each of two variables — smoking and styrene exposure — to an increase in SCEs in lymphocytes of an exposed human population.

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Styrene (CAS No. 100-42-5) is a commercially important chemical to which about 30 000 US workers are exposed and more than 300 000 workers are potentially exposed (NIOSH, 1983). Styrene has mutagenic properties that are principally attributed to its main reactive metabolite, styrene-7,8-oxide (Vainio et al., 1984; Barale, 1991, review). This compound is direct-acting in

vitro inducing sister-chromatid exchanges (SCEs) (Norppa et al., 1980) and chromosome aberrations (Jantunen et al., 1986) in human whole blood cultures. Styrene oxide adducts have been detected in DNA isolated from lymphocytes (Liu et al., 1988; Bodell et al., 1989) and in hemoglobin (Brenner et al., 1991) of styrene-exposed workers. The greatest human exposures occur when styrene is used as a solvent and reactant in the manufacture of unsaturated polyester products that have been reinforced with fibrous glass.

While some cross-sectional occupational studies have shown increases in SCEs in lymphocytes of workers exposed to relatively high levels of styrene in this industry (above 170–213 mg/m^3 as daily TWAs) (Andersson et al., 1980; Camurri et al., 1983), other studies of small groups of workers exposed to approximately 210 mg/m^3 (Kelsey et al., 1990) and 300 mg/m^3 (Maki-Paakkinen et al., 1991) have not detected significantly increased SCEs. These latter studies did, however, detect increased SCEs related to smoking. Additionally, no increase in SCEs has been reported among small groups of workers at lower exposures of approximately 55, 98 and 47 mg/m^3 respectively (Hansteen et al., 1983; Maki-Paakkanen, 1987; Brenner et al., 1991). Utilizing stimulated lymphocytes without cytokinesis-block, 3 studies have reported significantly increased percentages of micronucleated (MN) cells in workers exposed to the relatively low mean styrene concentrations of 55, 102 and 47 mg/m^3 respectively (Hogstedt et al., 1983; Nordenson and Beckman, 1984; Brenner et al., 1991). A fourth study that found significant increases in the percentage of MN cells did not report the exposure concentrations (Meretoja et al., 1977). Conversely, 4 other studies of styrene exposure (Maki-Paakkanen, 1987; Hagmar et al., 1989; Maki-Paakkanen et al., 1991; Tomanin et al., 1992) did not find significant increases in the percentage of non-cytokinesis-blocked MN cells at mean exposures of approximately 98 mg/m^3 and 55 mg/m^3 nor at exposure to greater than 300 mg/m^3 using cytokinesis-block modifications of the MN method. Differences in study outcomes are likely related to one or a combination of factors among which may be differing exposure conditions, small-study populations, exposure misclassification, and methodological differences related to biomarkers. In the following article, we report a significant styrene exposure-related increase in SCEs, but not in MN in cytokinesis-blocked lymphocytes, at a mean 8-h TWA of 64.2 mg/m^3.

Methods

Subjects

48 individuals who worked in a boat manufacturing facility agreed to participate in the study with informed consent. Health histories containing information on lifestyle factors were obtained by administration of standard questionnaires in a private interview. Information was obtained concerning age, sex and smoking status, intake of caffeinated, decaffeinated and alcoholic beverages, intake of prescriptive and non-prescriptive drugs, intake of nutritional supplements such as vitamin C, etc., exposure to diagnostic X-rays within the past 2 years, recent immunizations or infections, and other potential exposures such as those that may occur from home-repair projects, hobbies, etc.. Occupational histories were also obtained by interview and by examination of company records. At the midpoint of the study, a short questionnaire was administered to ascertain any changes in status which may have arisen during 6 months, however, no significant changes were noted.

Blood samples were drawn in heparin by venipuncture from subjects at approximately 3-month intervals up to 4 times on each individual during the course of 1 year. To avoid possible bias, subjects were randomly assigned a different code number at each sampling time which was retained through all laboratory tests. Samples were transported to the laboratory within approximately 6 h for analysis of SCE and MN. Other assays performed on aliquots from these samples included those to determine concentrations of styrene and styrene glycol in blood (Rappaport et al. 1991).

Exposure

Personal exposure to styrene for each participant at this facility was rigorously characterized longitudinally over a period of 1 year. Briefly, passive monitors which employ coconut charcoal

to trap styrene, were clipped to the lapel of each worker for the entire work shift. The charcoal was subsequently desorbed with carbon disulfide and analyzed by gas chromatography. Exposures were measured for the same workers at approximately 6-week intervals (on 7 randomly chosen days) during the year.

Styrene was measured in exhaled air with a device especially designed for this study (Rappaport et al., 1991). Briefly, it consisted of a disposable mouthpiece connected to a tube containing 200 mg of coconut charcoal and a Wright respirometer which measured the volume of air passing through the tube. The subject forcibly exhaled through the apparatus in 0.5 l increments until a volume of 3 l was obtained. After collection of exhaled air, the charcoal tube was capped and stored prior to analysis. The analytical procedure was performed according to the same method used for the passive monitors as described above. During each of the 7 surveys, exhaled air samples were obtained from the subjects on 3 separate randomly assigned occasions throughout an 8-h shift, i.e., at least 45 min after work began, before lunch, and at least 45 min after the mid-shift break in the afternoon. Care was taken to collect samples in a documented styrene-free area (usually out-of-doors) within 3 minutes of leaving the work area. Up to 21 exhaled air samples were collected from each participant.

Sister-chromatid exchange

Replicate whole blood cultures were set up in RPMI 1640 medium (Gibco) containing 10% (v/v) fetal calf serum (Hyclone), 1% L-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, 1.5% PHA (HA 15, Burroughs–Wellcome) and 35 μM 5-bromo-2-deoxyuridine (BrdUrd). The same lot number of fetal calf serum was used throughout the study. Cultures were incubated at 37°C for 72 h in the dark. 3 h before the end of incubation, 0.1 ml of 10^{-5} M colcemid was added (final concentration 2×10^{-7} M). Air-dried chromosome preparations were made and the slides stained using a modification of the fluorescence plus Giemsa technique (Perry and Wolff, 1974) for differential chromatid staining. For each coded sample, SCEs were scored by one observer in 80 second-division metaphases containing 44–46 chromosomes. Equal numbers of cells were scored from each replicate culture. The replicative index (RI) was determined by scoring 200 cells per sample for the number of cells that had undergone 1, 2 or 3 or more rounds of DNA replication since culture initiation. Replicative index (RI) was calculated by the formula: $RI = ([1 \times \text{percentage of first-division cells}] + [2 \times \text{percentage of second-division cells}] + [3 \times \text{percentage of third or higher division cells}]/100)$ (Schneider and Lewis, 1981).

Lymphocyte micronuclei

MN in cytokinesis-blocked lymphocytes were analyzed according to the method of Fenech and Morley (1985). Lymphocytes were isolated from whole blood by centrifugation through a Ficoll-Hypaque (Pharmacia) density gradient; cell counts and viability by Trypan Blue dye exclusion were determined. Cells were consistently 98–99% viable. Cells were placed in culture at an initial density of 0.5×10^6 cells/ml in RPMI culture media with 10% fetal calf serum and supplements as above. Cytochalasin B (Sigma) (3 μg/ml final concentration) was added at 44 h of incubation and cells were harvested onto slides at 72 h using a cytocentrifuge. Slides were fixed in methanol, and dried and stained with May-Grunewald Giemsa. A minimum of 1000 binucleated cells per sample were scored (500 per duplicate culture) for MN by one observer utilizing standard scoring criteria. The number of mononucleated, binucleated and cells with more than two nuclei were scored in 400 cells per sample. The usual percentage of binucleated cells ranged from about 25% to 60%. Proliferative index (PI) was calculated by the formula: $PI = ([1 \times \text{percentage of binucleated cells}] + [2 \times \text{percentage of cells with greater than two nuclei}]/100)$. This summary number indicates the average number of divisions per cell that occurred after cytokinesis-block as an estimate of proliferative activity in culture.

Results

The study population was composed of approximately equal numbers of males (54%) and females (46%). There were approximately equal

numbers of subjects in each of 3 age categories: 20–30 ($n = 13$), > 30–40 ($n = 19$), and > 40–60 ($n = 16$). The mean ages of females and males, respectively were 41.1 ± 2.1 and 33.1 ± 1.7 years. Of the 26 males, 22 were less than 40 years old; in contrast, of the 22 females, only 10 were less than 40 years old.

Approximately 46% of the population were nonsmokers. Of the smokers, most (80%) smoked one pack a day or less. 40% of the subjects drank less than 5 caffeinated beverages (coffee and caffeinated soft drinks) per day; 10% of the subjects drank no caffeinated beverages. About 35% of the subjects drank no alcoholic beverages (beer, wine, and/or spirits) while 30% drank less than 5 drinks per week and 35% drank 5 or more per week. Information obtained on other factors including X-rays during the past 2 years, prescriptive and non-prescriptive medications, nutritional supplements, infections and immunizations, and other exposures was unremarkable. The length of employment at this facility ranged from 0.5 to 27 years with an average of 6.4 ± 5.6 (s.d.) years.

Results of the SCE and MN analyses along with measures of cell proliferation are shown in Table 1. Mean SCE values ranged from 4.7 to 9.5 SCEs per cell with an overall mean of 6.4 ± 0.2 (s.e.) per cell. Mean MN values ranged from 1.3 to 33.0 MN per 1000 binucleated cells with an overall mean of 8.9 ± 0.9 (s.e.).

Individual mean values for personal breathing zone styrene concentrations (up to 7 samples per subject) ranged from 0.88 to 235.35 mg/m^3 with a mean of 64.2 ± 71.5 (s.d.) mg/m^3. Breath styrene concentrations (up to 21 samples per subject) ranged from 0 to 7.16 mg/m^3 with an overall mean of 1.65 ± 1.82 (s.d.) mg/m^3.

Data were also categorized by styrene exposure with subjects divided into groups designated as low (TWA < 6 mg/m^3), medium ($6 \leq$ TWA $<$ 118 mg/m^3) and high (TWA > 118 mg/m^3) and by smoking and nonsmoking categories (Table 2). SCEs were increased with regard to both smoking and exposure.

Fig. 1 shows the distribution of mean styrene exposure averaged over the year for the various job categories studied at this facility. The group containing laminators, chopper gun operators and mold repairers, comprising about 40% of the

TABLE 1

INDIVIDUAL MEAN SISTER-CHROMATID EXCHANGES, MICRONUCLEI, AND REPLICATIVE INDICES IN SAMPLES OBTAINED DURING ONE YEAR FROM SUBJECTS EXPOSED TO STYRENE

Subject	Mean SCEs/cell [a]	Mean *RI* [a,b]	*N* [c]	Mean MN /1000 BN cells [a]	Mean *PI* [a,d]
0010	6.30 – [e]	2.26 –	2	10.5±0.5	0.87±0.15
0171	6.13 –	2.37 –	3	18.3±7.1	0.55±0.12
0192	5.02±0.36	2.48±0.05	2	5.0±3.0	0.74±0.06
0346	6.04±0.10	2.61±0.07	4	2.0±1.4	0.61±0.16
0475	6.05±0.71	2.32±0.02	4	13.0±0.4	0.70±0.18
0853	7.19±0.15	2.60±0.05	4	8.3±3.3	0.57±0.14
1272	7.42±0.47	2.66±0.02	3	8.3±3.2	1.02±0.13
1467	7.01 –	2.52 –	3	33.0±2.3	0.71±0.16
1525	8.09±0.09	2.52±0.01	3	6.0±2.3	0.50±0.12
1712	5.30±0.06	2.56±0.01	4	5.5±1.3	0.92±0.16
2046	5.51±0.18	2.35±0.05	3	13.7±1.2	0.59±0.08
2848	6.55±0.85	2.48±0.10	4	11.5±2.9	0.69±0.11
3008	6.20 –	2.24 –	3	5.0±1.0	0.52±0.14
3589	5.96±0.65	2.55±0.01	3	6.0±2.3	0.96±0.16
3606	6.57±0.08	2.45±0.07	3	19.3±3.2	0.90±0.16
3935	6.77±0.38	2.24±0.14	3	12.0±2.5	0.80±0.20
4067	–	–	1	6.0 –	0.81 –
5238	5.32±0.69	2.51±0.04	4	12.7±1.8	0.65±0.07
5786	6.12±0.42	2.58±0.05	3	1.3±0.7	0.83±0.04
5838	6.33±0.84	2.54±0.11	4	7.5±2.0	1.03±0.22
5912	6.24±0.66	2.57±0.03	2	4.5±0.5	1.16±0.12
6074	6.46±0.68	2.53±0.03	4	11.3±2.3	0.85±0.15
6480	5.38 –	2.70 –	3	2.7±0.7	0.48±0.16
6493	6.62±0.19	2.28±0.16	3	7.3±0.9	0.83±0.15
6553	6.19 –	2.72 –	3	6.3±3.2	0.49±0.08
6620	6.56±0.43	2.46±0.15	4	6.8±2.0	0.69±0.16
6657	6.21 –	2.25 –	3	4,7±1.9	0.51±0.12
6844	6.14±0.01	2.53±0.03	3	10.7±1.2	0.55±0.20
6882	6.74±0.36	2.47±0.06	4	6.5±1.0	0.70±0.05
6955	6.57±2.12	2.31±0.02	4	13.0±3.3	0.48±0.15
6965	5.49±0.06	2.44±0.03	4	4.0±1.1	0.80±0.16
7061	6.89±0.68	2.47±0.03	3	6.0±2.9	0.57±0.08
7164	5.85±0.59	2.54±0.08	3	6.3±0.7	0.82±0.08
7659	6.25 –	2.47 –	3	6.7±3.2	0.65±0.17
7715	6.13±0.79	2.49±0.08	4	5.3±2.0	0.59±0.14
7738	8.16±1.00	2.40±0.02	3	7.3±2.8	0.32±0.08
8045	4.73±1.07	2.52±0.08	4	5.8±2.0	0.73±0.16
8122	5.35 –	2.49 –	2	7.5±3.5	0.73±0.18
8723	–	–	1	2.0 –	0.80 –
9317	5.91±0.97	2.43±0.04	4	8.3±1.5	0.65±0.17
9336	7.25±0.50	2.59±0.10	4	10.3±1.8	0.69±0.17
9390	5.75±0.09	2.42±0.20	4	13.3±4.1	0.59±0.11
9418	8.38±0.12	2.20±0.02	4	13.8±3.3	0.67±0.12
9461	8.56±0.28	2.24±0.08	4	26.8±2.8	0.66±0.14
9503	5.29 –	2.32 –	2	4.0±4.0	0.89±0.10

TABLE 1 (continued)

Subject	Mean SCEs/cell [a]	Mean RI [a,b]	N [c] cells [a]	Mean MN /1000 BN	Mean PI [a,d]
9545	9.47±0.12	2.58±0.05	2	2.5±2.5	0.87±0.31
9647	6.63 –	2.49 –	4	14.5±1.8	0.98±0.15
9658	5.89 –	2.47 –	3	6.3±2.6	0.97±0.24
Grand means	6.41±0.14	2.46±0.02		8.9±0.9	0.72±0.03

[a] All data are expressed as the mean ± standard error within each subject. SCE analysis was performed once or twice for each subject.
[b] RI is replicative index: $RI = (1 \times \text{percentage of first division cells}) + (2 \times \text{percentage of second division cells}) + (3 \times \text{percentage of third or higher division cells})/100$.
[c] N is number of determinations of MN.
[d] PI is proliferative index: $PI = (1 \times \text{percentage of binucleated cells}) + (2 \times \text{percentage of cell with greater than two nuclei})/100$.
[e] Indicates missing value.

TABLE 2

SCEs AND SMOKING STATUS IN SUBJECTS GROUPED BY LOW (TWA ≤ 6 mg/m^3), MEDIUM ($6 \leq$ TWA < 118 mg/m), AND HIGH (TWA ≥ 118 mg/m^3) EXPOSURE TO STYRENE

Group	N	Mean exposure (mg/m^3)	Mean SCEs/cell [a]
Low			
Nonsmokers	8	3.5 (±1.7) [b]	5.99 (±0.28) [c]
Smokers [d]	7	3.1 (±1.3)	6.39 (±0.19)
Total	15	3.3 (±1.5)	6.13 (±0.18)
Medium			
Nonsmokers	10	41.2 (±37.6)	6.02 (±0.18)
Smokers [d]	10	46.7 (±39.6)	6.80 (±0.41)
Total	20	44.0 (±37.7)	6.48 (±0.21)
High			
Nonsmokers	4	169.1 (±44.7)	6.38 (±0.33)
Smokers [d]	7	172.9 (±30.9)	6.97 (±0.48)
Total	11	171.5 (±43.3)	6.85 (±0.26)

[a] SCE analysis was performed on samples from 46 subjects.
[b] Mean TWA (mg/m^3) ± standard deviation.
[c] Mean SCEs per cell ± standard error.
[d] Average number of cigarettes per day for smokers in low exposure group: 20.0±7.1 (s.d); in medium group: 22.4±9.2 (s.d.); in high group: 20.0±7.1 (s.d.).

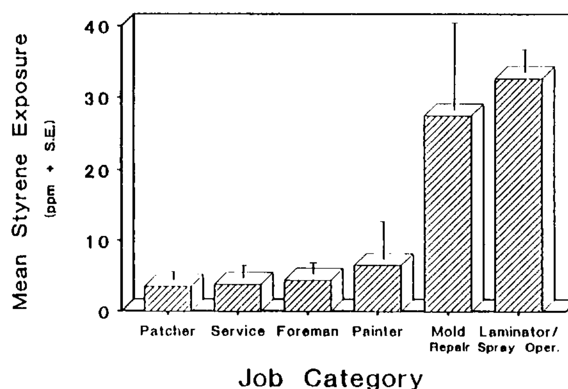

Fig. 1. Styrene exposure was determined by air sampling; mean exposure (ppm ± S.E.) was then determined for the job categories shown. (Note: 1 ppm styrene = 4.25 mg/m^3). Relative percentages of workers in each category were: patcher, 15%; service, 27%; foreman, 8%; painter, 13%; mold repair, 6%; and laminator/spray operator, 31%. Mold repairers and laminators/spray operators together comprised about 38% of the study population and were exposed at a mean of 132 mg/m^3 (31 ppm) while the remainder of job categories were exposed at 13–26 mg/m^3 (3–6 ppm).

study population, received the highest exposure at an average of about 132 mg/m^3 while the remaining categories were exposed at about 13–26 mg/m^3.

Linear regression analysis (SPSS/PC +, SPSS Inc., Chicago, IL) was performed on SCE data with regard to exposure to styrene as well as to other variables described above that were ascertained from questionnaires. Results showed that SCEs were significantly increased with smoking (Fig. 2) as well as with exposure to styrene expressed both as an air concentration (Fig. 3A) and as a breath concentration (Fig. 3B). This was expected since air concentrations and breath concentrations of styrene were highly correlated ($r = 0.94$). Results of multiple regression analysis using competing models showed that only smoking and exposure to styrene contributed significantly to regression of SCEs ($Y = 5.78 \pm 0.041\ X_1 + 0.00416\ X_2$ where Y is mean SCEs, X_1 is number of cigarettes per day and X_2 is mean styrene concentration (mg/m^3) in breathing zone air; $R^2 = 0.38$, $p \ll 0.001$; and $Y = 5.78 + 0.037\ X_1 + 0.184\ X_2$ where Y is mean SCEs, X_1 is cigarettes

per day and X_2 is styrene concentration (mg/m^3) in exhaled air; $R^2 = 0.40$, $p \ll 0.001$. Using beta output from SPSS multiple and simple linear regression in which variable coefficients are expressed in standard deviation units rather than the original measurement units, it is possible to calculate the relative strengths of the contribution of smoking and styrene exposure to regression of SCEs and then directly compare them as shown in Fig. 4. By this means, it was estimated that approximately 25% of the "explained" variability in SCEs in this population was accounted for by exposure to styrene as measured in the breathing zone and 62% by cigarette smoking. The joint positive effect of the two variables accounted for about 13% of the variability, however, the interaction term was not significant. Lack of interaction means that separate effects for smoking and exposure to styrene are not contradicted by these data.

MN were measured 4 times during the year. Since means for the 4 sampling times were not significantly different using the Kruskal–Wallis non-parametric one-way analysis of variance test, mean MN data for each individual were used in subsequent analyses. Fig. 5 shows mean MN values ± S.E. for the 20 individuals who were sampled all 4 times. There was no significant difference in the mean MN values over time (nonparametric one-way ANOVA, $p = 0.09$) nor with log-transformed MN (one-way ANOVA, SPSS, $p = 0.14$). The frequency distribution of mean MN

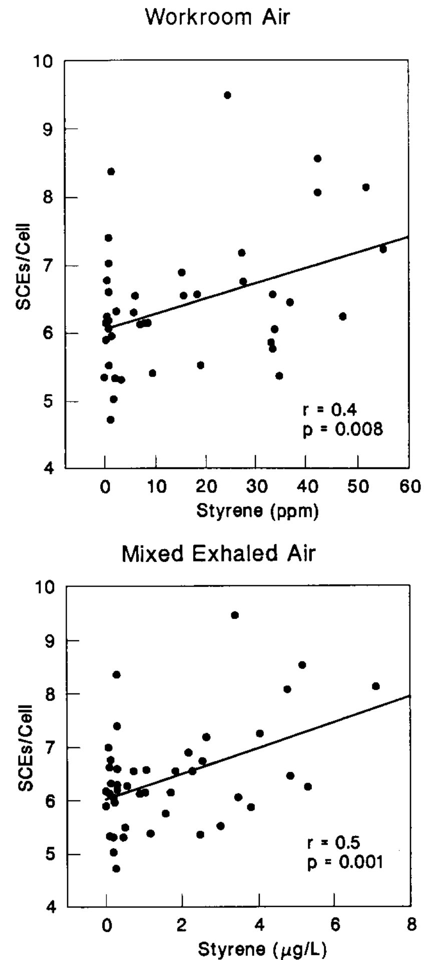

Fig. 3. Regression of mean SCEs per cell on styrene exposure. (A) Significant increase in mean SCEs with styrene concentration (ppm) in air ($Y = 6.094 + 0.022\ X$, $R^2 = 0.150$, $p = 0.007$. (B) Significant increase in mean SCEs with styrene concentration (mg/m^3) in breath ($Y = 6.035 + 0.243\ X$, $R^2 = 0.211$, $p = 0.0013$). The concentration of styrene in air and breath were highly correlated ($r = 0.94$).

per 1000 binucleated cells for all individuals was fit to the Poisson distribution using the fitting procedure of the Statgraphics Statistical Package (Statistical Graphics Corporation, Rockville, MD). However, the chi-square "goodness-of-fit" test showed the Poisson distribution to be rejected at $p = 0.0001$. The data were then log-transformed and found to fit a normal distribution.

Linear regression of log-transformed MN values on the variables previously described, includ-

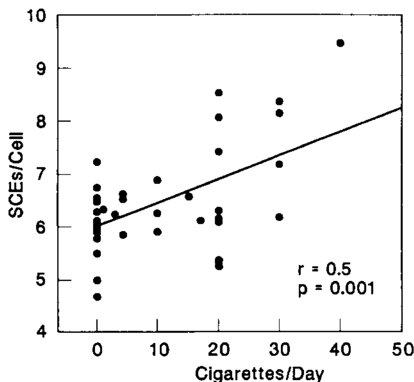

Fig. 2. Regression of mean SCEs per cell on smoking expressed as cigarettes per day ($Y = 6.004 \pm 0.045\ X$, $R^2 = 0.286$, $p = 0.0001$).

ing exposure, showed that gender ($p < 0.0001$) and age ($p = 0.04$) contributed significantly to regression. After adjusting for gender by using analysis of covariance (Statgraphics), no effect of exposure to styrene on MN was detected.

The frequency distribution of MN per cell for the total study population by sex is shown in Fig. 6. This figure illustrates that, in both sexes, the majority of cells contain one or two MN with very few multiple-micronucleated cells. Curve-fitting procedures for these data again showed the Poisson distribution to be rejected at $p < 0.0001$.

Previous studies have shown that older subjects tend to have higher MN rates (Fenech and Morley, 1986; Yager et al., 1988). It has also been shown that hypodiploidy is significantly higher in older females most probably due to loss of an X-chromosome (Galloway and Buckton, 1978). Since females in the study population were, on average, 8 years older than the males, 14 age-matched pairs of males and females were compared for MN as shown in Table 3. These data indicate that at a mean age of 37.5 years for both sexes, females have about a 2.3-fold higher number of MN per 1000 binucleated cells than do males. Since most cells contain only one MN (see Fig. 6), the relationship for micronucleated cells is comparable.

In terms of cell proliferation, *RI* values observed from whole blood SCE cultures were significantly higher overall than *PI* values obtained from the separated lymphocyte MN cultures (Table 1). The *RI* for SCEs was significantly negatively correlated with age ($r = -0.50$, $p = 0.001$), whereas the *PI* for MN was negatively correlated with styrene concentration in breath ($r = 0.37$,

TABLE 3

AGE, MEAN MICRONUCLEI PER 1000 BINUCLEATED CELLS, AND PERCENT MICRONUCLEATED CELLS IN 14 FEMALE AND MALE SUBJECTS

Females			Males			Mean MN difference
Age	Mean MN [a]	% MN cells [b]	Age	Mean MN [a]	% MN cells [b]	
22.4	7.3	0.63	22.7	2.0	0.15	5.3
28.8	6.8	0.60	28.7	6.0	0.30	0.8
32.7	13.7	1.27	32.8	7.3	0.63	6.4
32.6	19.3	1.67	31.8	6.0	0.53	13.3
33.1	6.0	0.50	33.6	5.8	0.55	0.2
34.2	12.8	1.08	34.2	2.0	0.20	10.8
34.8	10.3	0.98	34.6	7.5	0.60	2.8
35.0	10.7	1.03	34.8	2.5	0.25	8.2
35.4	11.5	1.03	35.5	4.7	0.40	6.8
36.1	11.3	1.00	35.8	6.5	0.60	4.8
41.3	10.5	0.95	41.6	6.3	0.57	4.2
43.6	5.5	0.45	43.4	5.3	0.43	0.2
51.3	26.8	2.25	53.3	5.0	0.40	21.8
60.8	12.0	1.10	60.6	4.0	0.35	8.0
Grand means						
37.3	11.8	1.04	37.4	5.1	0.43	6.7
S.E. [c]						
2.6	1.5	0.13	2.6	0.5	0.04	1.6

[a] Mean micronuclei per 1000 binucleated cells averaged over all sampling times.
[b] Percent of cells containing micronuclei in 1000 binucleated cells averaged over all sampling times.
[c] Standard error.

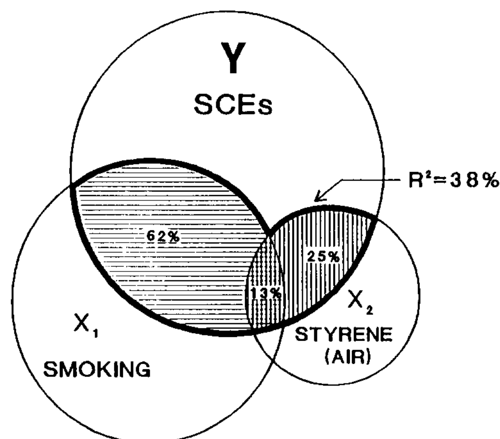

Fig. 4. Relative contribution of smoking (X_1, average number of cigarettes per day) and styrene exposure (X_2, styrene concentration in breathing zone air) to the increase in sister chromatid exchanges (Y, SCEs). For styrene in air (ppm): $Y = 5.78 + 0.041\ X_1 + 0.018\ X_2$, $R^2 = 0.38$, $p \ll 0.001$. Coefficients were transformed via SPSS beta output to units of standard deviation. The heavy line encompasses R^2; the shaded areas indicate the portion of total variability attributable to each variable. The cross-hatched area indicates the proportion of variability due to the joint positive effect of the two variables.

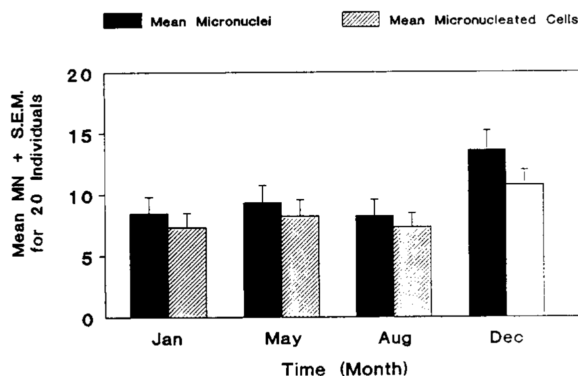

Fig. 5. No significant difference over time was shown for the mean number of micronuclei and micronucleated cells per 1000 binucleated cells in 20 individuals who were sampled at each of 4 sampling times during the year. (Kruskal–Wallis one-way ANOVA, $p = 0.09$; log-transformed MN, one-way ANOVA (SPSS), $p = 0.14$).

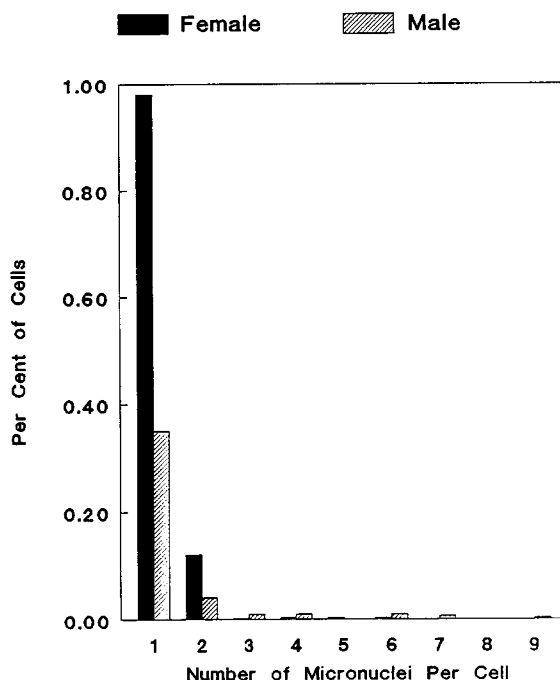

Fig. 6. Frequency distribution of micronuclei per cell in females and males. The X-axis shows the number of micronuclei per cell; the Y-axis indicates the per cent of cells in each category by sex. The Poisson distribution was rejected at $p < 0.0001$ using the Chi-square "goodness of fit" test for both distributions.

$p = 0.01$) and styrene concentration in air ($r = 0.34$, $p = 0.01$).

Discussion

An exposure-related increase in SCEs was detected in this population at a lower mean styrene air concentration than has been found previously. We attribute this finding to the fact that multiple full-shift measurements were performed on each subject on 7 randomly selected days during 1 year. We therefore obtained an extremely precise estimate of exposure for each individual worker and significantly reduced the possibility of exposure misclassification that is commonly encountered in this type of study and that then can lead to a loss of power to detect an effect even if one exists. Collection of exposure data in this manner then allowed application of linear regression analysis techniques that are more sensitive than the statistical methods that can be applied to

categorical exposure data. In contrast, the vast majority of occupational studies to date have used customary exposure assessment methods wherein workers are assigned to exposure categories by various means including use of exposure measurements combined with job titles or job categories. However, air samples used for estimating exposure are not determined precisely for individual workers. Often, air samples are obtained cross-sectionally by job category but it is unclear whether exposure measures are obtained on the same subjects from whom biological samples are acquired for cytogenetic or other biomarker analyses. Further, most reported studies have usually included smaller numbers of workers than the present study.

Most importantly, in this present study, individual exposure data were combined with smoking data to allow the separate contributions of each of these factors to increased SCEs to be estimated. To our knowledge, this is the first instance in which the individual contributions of different variables on induction of SCEs has been shown in a human population.

Examination of Table 2 shows group mean SCEs per cell for low and high styrene exposed groups to be 6.13 and 6.85 SCEs per cell, respectively. Therefore, an increase in SCEs of about 12% was detected in this study. This is in agreement with estimates by Hirsch et al. (1984) indicating that with 20–40 subjects per group, and 80 cells scored per individual, increases in mean SCE frequency are detectable in the range of 8–12% with probability (i.e., power) equal to 0.95. It should also be noted here that smokers and numbers of cigarettes per day are evenly distributed throughout the exposure groups; thus there are a reasonable number of non-smokers with higher styrene exposure and smokers with lower styrene exposure. From these observations, it can be seen that smoking and exposure to styrene are not correlated; this is important in enabling us to estimate their separate effect on SCEs. There was no significant effect of duration of employment on SCE levels.

These data clearly illustrate that SCEs are extremely sensitive biomonitors of exposure. Interestingly, examination of the distribution of SCEs per cell for the 3 exposure groups did not reveal a difference in the proportion of high frequency cells as defined in Carrano and Moore (1982) by either exposure or smoking, as might have been expected. In this instance, mean SCE value appears to be more sensitive than the proportion of high frequency cells.

After statistical adjustment for gender, no effect of styrene exposure on MN was detected. These results are in agreement with four recent studies cited earlier in which exposures to styrene were not associated with an increase in MN. As expected, MN were also observed to increase with age in this population. The effect was slight ($p = 0.04$) perhaps because of truncation at the extremes of age and because of the relatively large proportion of young males.

Cell culture proliferation indices (Table 1) for SCEs (*RI*) and MN (*PI*) are roughly comparable measures since each expresses an estimate of the average number of replications per cell. It can be seen that the *PI* was consistently lower than the *RI*. It seems likely that whole blood cultures provide a more favorable environment for lymphocyte growth (contain autologous serum, etc.) than that of separated lymphocyte cultures. Additionally, BrdUrd is added to SCE cultures upon initiation thereby allowing incorporation into early-dividing cells, whereas Cytochalasin-B is added at 44 h of culture. This may allow some early-dividing cells to escape block and therefore be lost to enumeration. In spite of these apparent differences in proliferation, MN cultures consistently yielded from 25 to 60% binucleated cells which is more than adequate for ease of scoring.

SCE *RI* was significantly negatively correlated with age indicating a decreased proliferative capacity with increasing age. A significant increase in SCE *RI* was not observed with smoking, however, as has been previously found (Husgafvel-Pursiainen, 1987). Interestingly, MN *PI* was negatively correlated with styrene exposure implying that proliferative ability for separated lymphocytes may be slightly decreased at relatively high styrene exposure concentrations. Verification in other styrene- or solvent-exposed populations would be desirable in order to confirm this observation.

Lymphocyte MN frequencies in cytokinesis-blocked cells were significantly increased in fe-

males and with increasing age for the total population, but were not found to be higher with styrene exposure. Comparison of a subset of 14 males and females at an average age of 37.5 years indicated that females had an approximately two-fold higher frequency of MN than males (Table 3). Increased aneuploidy (predominantly hypodiploidy) in cultured lymphocytes of older females compared with males has been observed (Jacobs et al., 1963; Galloway and Buckton, 1978; Martin et al., 1980). Premature centromere division of the X-chromosome has been hypothesized as one of the mechanisms of non-disjunction that leads to aneuploidy in older females (Fitzgerald, 1975). The relatively high frequency of MN observed in females compared with males in this study is consistent with these earlier observations. It is hypothesized that a relatively constant proportion of the increased numbers of centric and acentric fragments observed in older females form MN which are then observed in first division interphase cells (Martin et al., 1980). The rather large difference observed between females and males in this study may be all the more striking because the cytokinesis-block modification allows scoring of only first division cells thereby increasing the sensitivity of the assay by excluding non-proliferating cells as well as those in second or third division.

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