

Increased frequency of chromosome aberrations in workers exposed to styrene

by BENKT HÖGSTEDT, M.D.,^{1,2} KARIN HEDNER, M.D.,² EVA MARK-VENDEL, B.Sc.,² FELIX MITELMAN, M.D., Ph.D.,² ANDREJS SCHÜTZ, B.Sc.,¹ and STAFFAN SKERFVING, M.D.¹

HÖGSTEDT, B., HEDNER, K., MARK-VENDEL, E., MITELMAN, F., SCHÜTZ, A. and SKERFVING, S. Increased frequency of chromosome aberrations in workers exposed to styrene. *Scand. j. work environ. & health* 5 (1979) 333—335. Chromosomal aberrations in lymphocytes from peripheral blood were significantly more frequent in six workers from a plant manufacturing polyester resin boats (average 10.8 per 100 cells) than in six age- and sex-matched referents (5.2 per 100 cells). The contamination of the workroom air with styrene, as measured on three occasions within three years in different areas of the plant, was 50—400 mg/m³.

Key words: chromosomal aberrations, human, lymphocytes, polyester plastic production, styrene.

Styrene and styrene oxide, which is an intermediate metabolite of styrene, have proved mutagenic in a series of test systems (2, 5, 6, 7, 9, 12). In workers exposed to styrene an increased frequency of chromosomal aberrations in lymphocytes has been reported in studies of considerably exposed groups (3, 8), while no such effect has been found in a slightly exposed one (11). In these studies the degree of contamination was uncertain, as it was estimated from single determinations of styrene metabolites in the urine.

This paper reports cytogenetic findings concerning workers from a plant in which the contamination of the workroom air

had been measured on several occasions during the last few years.

MATERIAL AND METHODS

Production

The study was performed in a small plant manufacturing fiberglass-reinforced polyester resin boats. The polyester precursors were phthalic acid anhydride, maleic acid anhydride, propylene glycol, and styrene. The hardener used was methyl ethyl ketone peroxide, and the catalyst was a cobalt salt. Acetone was used for cleaning purposes.

The production started in the late 1960s. The gel coat and laminate were sprayed, while the top coat was rolled on. Ventila-

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tors, although rather primitive, were installed before 1973, and spray boxes in 1976–1977.

Measurements of contamination

The styrene levels in the air were measured on several occasions in individual samples from the breathing zones of workers. In 1971 samples were obtained in impinger flasks (ethanol and 0.1 ppm tertiary butyl pyrocatechol). In 1973 and 1977 samples were collected in charcoal tubes and eluted by carbon disulfide. In 1973 urine samples, obtained at the end of a day's work, were analyzed spectrophotometrically (10) for styrene metabolites (mandelic acid and phenylglyoxylic acid) and creatinine.

Subjects

The exposed group consisted of six males, aged 21–56 (mean 33) years. They had been employed at the plant for 0.5–10 (mean 4) years. The six age- and sex-matched referents were selected from workers at a nearby paper factory where there was no contamination with chemicals. Both groups were questioned regarding smoking habits, diseases (especially virus infections), use of drugs, and exposure to ionizing radiation, heavy metals, and organic solvents. Three of the exposed subjects and three of the referents were smokers. No other agents possibly capable of damaging chromosomes were known.

Cytogenetic methods

Venous blood specimens were collected in connection with the atmospheric samples in 1977. The samples from both groups were obtained and analyzed simultaneously. Lymphocyte cultures were incubated about 20 h after the collection of the blood samples. A standard microculture technique was used. The cultivation medium was a mixture of 80 % McCoy's 5a and 20 % fetal calf serum. Phytohemagglutinin was used as the mitogen substance. The cultivation time was 72 h. All preparations were coded, mixed, and analyzed by one observer. Two hundred metaphases from each individual were analyzed. The chromosomal aberrations were recorded according to the classification system recommended by the World Health Organization (1).

RESULTS

The time-weighted average concentration of styrene in the air was 60 mg/m³ in 1971 (total sampling time 42 h), 310 mg/m³ in 1973 (45 h), and 115 mg/m³ in 1977 (41 h). Much higher levels, up to 800 mg/m³, were recorded for shorter periods.

In 1973 the mandelic acid concentration in the urine of nine workers ranged from 225 to 2,100 (mean 490) mg/g of creatinine and that of phenylglyoxylic acid from 82 to 560 (mean 205) mg/g of creatinine.

The average frequency of different types of aberrations was higher for the exposed group than for the referents (fig. 1). This result applied to gaps (3.2 vs. 2.4 per 100 cells), breaks (chromatid and isochromatid 6.9 vs. 2.5 per 100 cells; $p = 0.008$, Mann-Whitney U-test, one-tailed), the sum of gaps and breaks (10.2 vs. 4.9 per 100 cells; $p = 0.001$), hyperdiploidy (0.7 vs. 0.3 per 100 cells), and the sum of all aberrations (10.8 vs. 5.2 per 100 cells; $p = 0.001$).

There was no obvious relation between exposure time and total number of aberrations.

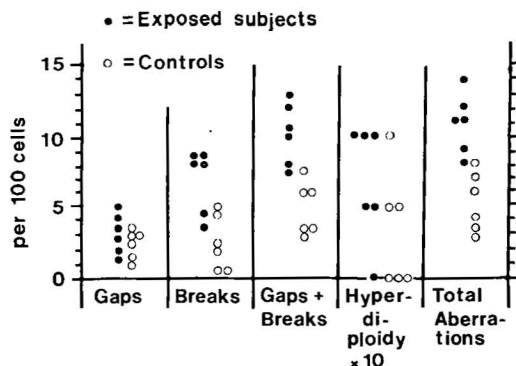


Fig. 1. Chromosomal aberrations in polyester resin workers and referents.

DISCUSSION

Although the present material is small, it clearly shows that the manufacture of polyester resin boats is associated with an increased frequency (approximately a doubling) of chromosomal aberrations. This phenomenon is presumably due to exposure to styrene although the possibility of exposure to other chemicals may have contributed to the increase in the frequency of the aberrations. The concentrations of styrene were approximately at, or even below, the maximum limits for the substance (Sweden 170 mg/m³).

The finding of an increased frequency of chromosomal aberrations is in accord with earlier findings. The present results agree closely with those published by Fleig and Thies (3). Compared with the findings given by Meretoja et al. (8), our frequencies of aberrations were lower (especially when compared with those of the referents), despite the probably higher level (as judged from the atmospheric levels of styrene and urinary levels of mandelic acid) and longer duration of exposure in our study.

The exposure levels measured in the present study are common in many plastic industries. Thus the finding of an increased frequency of chromosomal aberrations at these levels may have serious implications. The possibility of cancer must be borne in mind, although no epidemiologic evidence is available. There are indications of an embryotoxic effect in man (4), an effect which may have a cytogenetic background. In addition there is a risk of a genotoxic effect on germ cells that could possibly not become clinically manifest for several generations.

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REFERENCES

1. BUCKTON, K. E. and EVANS, H. J. *Methods for the analysis of human chromosome aberrations*. World Health Organization, Geneva 1973. 65 p.
2. FABRY, L., LEONARD, A. and ROBERFROID, M. Mutagenicity tests with styrene oxide in mammals. *Mutat. res.* 51 (1978) 377—381.
3. FLEIG, I. and THIESS, A. M. Mutagenicity study of workers employed in the styrene and polystyrene process and manufacturing industry. *Scand. j. work environ. & health* 4 (1978): suppl. 2, 254—258.
4. HOLMBERG, P. Central nervous defects in two children of mothers exposed to chemicals in the reinforced plastic industry: Chance or casual relation? *Scand. j. work environ. & health* 3 (1977) 212—214.
5. LINNAINMAA, K., MERETOJA, T., SORSA, M. and VAINIO, H. Cytogenetic effects of styrene and styrene oxide on human lymphocytes and *Allium cepa*. *Scand. j. work environ. & health* 4 (1978): suppl. 2, 156—162.
6. LOPRIENO, N., ABBONDANDOLO, S., BONATTI, S., BRONZETTI, G., CAMMELLINI, A., CORSI, C., CORTI, G., FREZZA, D., LEPORNI, C., MAZZACARO, A., NIERI, R., ROSSELINI, D. and ROSSI, A. M. Mutagenicity of industrial compounds: Styrene and its possible metabolite styrene oxide. *Mutat. res.* 40 (1976) 317—324.
7. LOPRIENO, N., PRESCIUTTINI, S., SBARNA, I., STRETTI, G., ZACCARO, L., ABBONDANDOLO, A., BONATTI, S., FIORIO, R. and MAZZACARO, A. Mutagenicity of industrial compounds: VII. Styrene and styrene oxide: II. Point mutations, chromosome aberrations and DNA repair induction analyses. *Scand. j. work environ. & health* 4 (1978): suppl. 2, 169—178.
8. MERETOJA, T., VAINIO, H., SORSA, M. and HÄRKÖNEN, H. Occupational styrene exposure and chromosomal aberrations. *Mutat. res.* 56 (1977) 193—197.
9. MILVY, P. and GARRO, A. J. Mutagenic activity of styrene oxide (1,2-epoxyethylbenzene), a presumed styrene metabolite. *Mutat. res.* 56 (1977) 147.
10. OHTSUJI, H. and IKEDA, M. A rapid colorimetric method for the determination of phenylglyoxylic and mandelic acids: Its application to urine analysis of workers exposed to styrene vapours. *Br. j. ind. med.* 27 (1970) 150—154.
11. THIESS, A. M. and FLEIG, I. Chromosome investigations on workers exposed to styrene/polystyrene. *J. occup. med.* 20 (1978) 747—749.
12. VAINIO, H., PÄÄKKÖNEN, R., RÖNNHOLM, K., RAUNIO, V. and PELKONEN, O. A study on the mutagenic activity of styrene and styrene oxide. *Scand. j. work environ. & health* 2 (1976) 147—151.