

Mutagenicity study of workers employed in the styrene and polystyrene processing and manufacturing industry

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FLEIG, I. and THIESS, A. M. Mutagenicity study of workers employed in the styrene and polystyrene processing and manufacturing industry. *Scand. j. work environ. & health* 4 (1978): suppl. 2, 254—258. Mutagenicity studies were undertaken on lymphocytes from the following groups of persons exposed to styrene or polystyrene: one group from a styrene manufacturing plant, one from a polystyrene plant, and three from plants processing unsaturated polyester resins with different manufacturing methods. For all the groups the concentration of styrene in the atmosphere of the workplace was measured at various points and the amount of urinary mandelic acid was determined. In each case 100 metaphases were analyzed for chromosomal aberrations. The results were compared with reference groups.

Key words: chemical industrial workers, chromosomal aberrations, mutagenicity study.

A review of the literature shows studies which demonstrate a definite mutagenic effect of styrene and styrene oxide in bacterial systems and in yeast (4, 5, 7, 11) and a high probability that styrene oxide is also an intermediate metabolite of styrene in man. As far as we know, only one mutagenic study, of occupationally exposed workers in Finland, has revealed a clear increase in the rate of chromosome aberrations. The incidence of aberrant cells ranged from 10 to 26 % in the exposed group and was 3 % or less in the unexposed reference group (6). The mandelic acid determinations from urine samples — which were taken as the biological measure of current styrene exposure — referred to an exposure of ~100 ppm. So, at high concentrations, styrene appears to be a substance which,

after biotransformation in the organism, is capable of altering DNA (deoxyribonucleic acid) genetic material.

MATERIAL AND METHODS

Mutagenicity studies were undertaken on lymphocytes from the following groups of persons exposed to styrene or polystyrene: (a) one group from a styrene manufacturing plant, (b) one group from a polystyrene plant, (c) three groups from plants processing unsaturated polyester resins with different manufacturing methods (external plants).

For all the groups the concentration of styrene in the atmosphere of the workplace was measured at various points, and the amount of urinary mandelic acid was determined with the method of

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Engström and Rantanen (1) as slightly modified by Schaller et al. (9). With this method, the limit of detection of mandelic acid is 10 mg/l of urine. Currently, a value of 350 mg/l is being considered as the upper limit permissible for occupational medical purposes.

Before commencing the mutagenicity study, we collected anamnestic data and took into account age, occupational exposure to any other agent known to induce chromosome alterations, medical radiological treatment either for diagnostic or therapeutic purposes, recent viral diseases and vaccinations, consumption of drugs or alcohol, and smoking habits.

A reference group of 20 healthy men not exposed to styrene or polystyrene or to any other known chromosome damaging agent but working in the same factory in different departments (e.g., as clerical staff) were selected. The same questionnaire used for obtaining data on experimental subjects was also used for the referents.

The chromosome studies were performed on peripheral blood lymphocytes (2,8). Heparinized venous blood (0.2 ml) was incubated at 37°C for 70–72 h in 4 ml of TC Chromosome Microtest Medium and Reconstituting Fluid (Difco). For the last 2 h of culture, Colcemide (Ciba) was added at a concentration of 0.25 µg/ml. After hypotonic treatment with 0.0075 M of potassium chloride, the cells were fixed in methanol-glacial acetic acid (3:1). Air dried slides were stained with a 10% solution of Giemsa. A culture period of

70–72 h was chosen. In each case 100 metaphases were analyzed for chromosomal aberrations.

RESULTS

Styrene manufacturing

At the time we examined the cells of workers manufacturing styrene, we felt it was unnecessary to analyze more than five workers and 100 metaphases per person, because of the low exposure and the correspondingly low mandelic acid concentrations in the urine.

The analysis of the metaphases showed the following results. The frequency of aberrant cells was 1.6% (3.8%) in the exposed group and 2.1% (5.5%) in the reference group. The figures in parentheses correspond to the aberrant metaphases of inclusive gaps or isogaps. There was no significant difference in the rate of chromosome aberrations in these two groups. The results are depicted in table 1.

Polystyrene manufacturing

The people selected for investigation from the polystyrene plant consisted of 12 male employees, 22–62 years of age, who had

Table 1. Chromosomal aberrations in the lymphocytes of workers occupationally exposed to monostyrene (BASF) and unexposed referents.

Subject	Age (years)	Exposure (years)	Mandelic acid con- centration (mg/l urine)	Analyzed cells	Aberrant cells (%)		Polyploid cells
					Inclu- sive gaps	Exclu- sive gaps	
75	53	20	—	100	6	3	0
104	42	25	40	100	3	1	1
111	45	24	—	100	3	2	2
112	33	14	19	100	4	2	0
116	41	25	—	100	3	0	0
Mean of the 5 exposed persons	42.8	21.6	—	500 (total)	3.8	1.6	0.6
Mean of the 20 referents	46.6	—	—	2,000	5.5	2.1	1.3

been exposed to styrene for 19—39 years. As an exception two employees had worked in the plant for only three and four years each, but were found to have increased urinary concentrations of mandelic acid (100 and 75 mg/l, respectively).

The results are demonstrated in table 2. The frequency of aberrant metaphases in the exposed and reference groups was 1.9 % (5.1 %) and 2.1 % (5.5 %), respectively. The figures in parentheses correspond to the aberrant metaphases of inclusive gaps or isogaps. There was no increase in the rate of aberrations of the exposed group and no significant difference for either inclusive or exclusive gaps between the two groups.

Plants processing unsaturated polyester resins with different methods

The statistical evaluation for the groups of workers from plants processing unsaturated polyester resins with different methods revealed a significant difference between the rate of chromosome aberrations in the exposed workers and those in the reference group. The frequency of aberrant metaphases in the exposed group was 5.3 % for exclusive gaps and

9.2 % for inclusive gaps, in comparison to 2.1 % and 5.5 %, respectively, in the referent group. The individual data are depicted in table 3.

The technical data and the respective recordings of the workplace concentrations have been reported in another presentation in these proceedings (11).

DISCUSSION

The analysis showed no induction of chromosome aberrations in the group of persons employed in the BASF styrene manufacturing and processing plants. The incidence of aberrant cells ranged from 2 to 10 %. Correspondingly low mandelic acid values in the urine (5—100 mg/l) were found. The rates of aberrant cells in the reference group were between 1 and 9 %.

Contrary to these findings, chromosome aberration rates of 4 to 20 % were found in workers taken at random from processes employing predominantly manual finishing techniques, which means higher rates of exposure to styrene with mandelic acid concentrations from 102 to > 1,500 mg/l of urine (42 to 1,500 mg/g of creatinine).

Table 2. Chromosomal aberrations in the lymphocytes of workers exposed to polystyrene (BASF) and unexposed referents.

Subject	Age (years)	Exposure (years)	Mandelic acid con- centration (mg/l urine)	Analyzed cells	Aberrant cells (%)		
					Inclu- sive gaps	Exclu- sive gaps	Polyploid cells
178	62	19	—	100	7	2	0
180	45	25	< 5	100	6	1	1
181	40	25	29	100	2	0	0
182	28	3	100	100	5	3	1
184	51	18	—	100	5	2	0
185	22	4	75	100	3	2	1
186	61	39	16	100	4	2	2
193	47	15	< 5	100	5	3	0
194	42	22	21	100	10	2	0
195	53	34	—	100	6	3	3
196	46	21	—	100	4	2	1
197	42	19	< 5	100	4	1	2
Mean of the 12 exposed persons	44.9	20.3	—	1,200 (total)	5.1	1.9	1.1
Mean of the 20 referents	46.6	—	—	2,000 (total)	5.5	2.1	1.3

Table 3. Chromosomal aberrations in the lymphocytes of workers occupationally exposed to monostyrene (external plants) and unexposed referents.

Subject	Age (years)	Exposure (years)	Mandelic acid concentration		Analyzed cells	Aberrant cells (%)		Polyploid cells %
			mg/l urine	mg/g creatinine		Inclu- sive gaps	Exclu- sive gaps	
<i>Plant A</i>								
416	44	8	117	260	100	7	4	1
418	38	5	248	292	100	6	4	1
419	38	6	392	276	100	14	8	1
<i>Plant B</i>								
420	40	5	1,038	564	100	5	3	1
421	42	9	1,377	1,027	100	8	4	1
422	47	6	> 1,500	> 1,500	100	4	3	0
423	52	24	908	726	100	11	4	0
424	37	22	869	621	100	20	10	1
425	41	2	581	390	100	5	4	1
<i>Plant C</i>								
426	45	2	578	255	100	12	7	2
428	29	2	102	42	100	6	4	1
429	42	5	> 1,500	> 1,500	100	11	7	1
430	41	7	1,370	710	100	10	5	1
431	38	7	265	145	100	10	8	0
Mean of the 14 exposed persons	41	7.9	—	—	1,400 (total)	9.2	5.3	0.9
Mean of the 20 referents	46.6	—	—	—	2,000 (total)	5.5	2.1	1.3

Therefore our results confirm those of Meretoja et al. (6). They found an incidence of aberrant cells ranging from 11 to 26 % in the lymphocytes of exposed subjects with mandelic acid concentrations from 23 to 3,257 mg/g of creatinine and 3 % or less in those of the reference group. Both their investigation and ours, in plants A, B and C, dealt with workers occupied in processing of unsaturated polyester resins, where peroxides are used for the curing process.

In the most recent investigations in our laboratories it was found that, due to the use of peroxides other than styrene, a considerable amount of styrene oxide is generated. Apart from this, all persons employed in these branches usually come in contact with solvents, especially methylene chloride and acetone, some of them also with epoxide resins and their hardeners.

Since styrene oxide (5) and methylene chloride (3) act as direct mutagens in bacterial systems, it seems questionable whether the observed chromosomal aber-

rations are due to styrene exposure. Styrene, on the contrary, has proved to be mutagenic only after metabolic activation.

Further research is planned to clarify the etiology of the findings demonstrating chromosomal damage in workers processing polyester resins. In addition to the monitoring of styrene oxide in plants A, B and C, experimental work is in progress at the BASF laboratories. It includes mutagenic assays in mammals with styrene, styrene oxide and methylene chloride as a comparison.

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REFERENCES

1. ENGSTRÖM, K. and RANTANEN, J. A new gas chromatographic method for determination of mandelic acid in urine. *Int. Arch. Arbeitsmed.* 33 (1974) 163—167.
2. DE JONG, G. J. and ANDERS, P. A. Lymphocyte cultures from Chinese Hamster (cricetus griseus). *Exp.* 28 (1972): 1, 86—87.
3. KRAYBILL, H. F., HELMES, C. T. and SIGMAN, C. Biomedical aspects of bio-refractories in water. Presented at the 2nd international symposium on aquatic pollutants, Nordwijkerhout (Amsterdam), Netherlands 1977.
4. LOPRIENO, N., ABBONDANDOLO, A., BARALF, R., BARONCELLI, S., BONATTI, S., BRONZETTI, G., CAMMELLINI, A., CORSI, C., CORTI, G., FREZZA, D., LEPORINI, C., MAZZACCARO, A., NIERI, R., ROSSELLINI, D. and ROSSI, A. M. Mutagenicity of industrial compounds: Styrene and its possible metabolite styrene oxide. *Mutat. res.* 40 (1976) 317—324.
5. MEESTER, C. DE, PONCELET, F., ROBERFROID, M., RONDELET, J. and MERCIER, M. Mutagenicity of styrene and styrene oxide. *Mutat. res.* 56 (1977) 147—152.
6. MERETOJA, T., VAINIO, H., SORSA, M. and HÄRKÖNEN, H. Occupational styrene exposure and chromosomal aberrations. *Mutat. res.* 56 (1977) 193—197.
7. MILVY, P. and GARRO, A. J. Mutagenic activity of styrene oxide (1,2-epoxyethylbenzene), a presumed styrene metabolite. *Mutat. res.* 40 (1976) 15—18.
8. MOORHEAD, P. S., NOWELL, P. C., MELLMAN, BATTIPS, D. M. and HUNGERFORD, D. A. Chromosome preparations of leukocytes cultured from human peripheral blood. *Exp. cell res.* 20 (1960): 3, 613—616.
9. SCHALLER, K. H., GOSSLER, K., BOST, H. P. and VALENTIN, H. Gaschromatographische Methoden zur Bestimmung von Styrol — Blut sowie von Mandelsäure und Phenylglyoxylsäure in Urin. *ASP* 1 (1976) 24—26.
10. THIESS, A. M. and FRIEDHEIM, M. Morbidity among persons employed in styrene production, polymerization and processing plants. *Scand. j. work environ. & health* 4 (1978): suppl. 2, 203—214.
11. 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.

QUESTIONS AND ANSWERS

Question to Prof. THIESS

Dr. SORSA:

I do not want to ask a question, but, since Prof. Thies ended by suggesting the specific role of styrene oxide in producing the chromosomal aberrations in workers of reinforced plastic plants, I would like to present the preliminary findings that we have obtained from the in vivo exposure of Chinese hamsters to styrene oxide vapor. The results at present are negative concerning the role of styrene oxide in the induction of chromosomal aberrations. They are negative also for the induction of sister chromatid exchange. So far we have analyzed 16 animals treated with concentrations of 25, 50, 75 and 100 ppm, the treatment time being 2 to 4 d. In the control animals (seven males) the mean aberration frequency in bone marrow cells was 2.2 ‰, while in the treated animals it was 1.9 ‰. It may be possible that the exposure time has not been long enough and a test with longer exposure time is currently in progress — but in comparison to our in vitro results with human lymphocytes this could also mean that it is styrene or its other metabolite rather than styrene oxide that is a more potent clastogen.

Question to Dr. HILDEBRAND

Dr. RINZEMA:

You mentioned methylene chloride as a mutagen. Has this been proved? In how many and what kind of tests?

Dr. HILDEBRAND:

Kraybill and coworkers (Kraybill et al., Presentation at the 2nd International Symposium on Aquatic Pollutants, Amsterdam 1977) reported on EPA data, according to which methylene chloride is positive in the Ames test in strain TA 100 without metabolic activation.