

Chromosome aberrations in lymphocytes of workers exposed to styrene

by TYTTI MERETOJA, Ph.L. (†), HILKKA JÄRVENTAUS, MARJA SORSA, Ph.D., and HARRI VAINIO, M.D.¹

MERETOJA, T., JÄRVENTAUS, H., SORSA, M. and VAINIO, H. Chromosome aberrations in lymphocytes of workers exposed to styrene. *Scand. j. work environ. & health* 4 (1978): suppl. 2, 259—264. Workers exposed to styrene in the reinforced plastics industry show a significant increase of chromosome aberrations, mainly chromosome breaks, in peripheral blood lymphocytes. The high incidence of aberrant lymphocytes (mean 15.1 ± 4.8 ‰; referents 2.0 ± 1.3 ‰) was retained when the same men were reexamined one year later (mean 16.2 ± 2.9 ‰). However, the frequency of sister chromatid exchanges (SCE), a newly developed sensitive parameter for the detection of recombinational exchanges linked with DNA repair, was not significantly increased (mean 5.3 ± 1.0 SCE/cell) in comparison to the referents (mean 4.4 ± 0.6 SCE/cell). This finding suggests a specific role of styrene or its metabolites in inducing genetic lesions mainly manifesting themselves as chromosomal breaks.

Key words: chromosome aberrations, sister chromatid exchange, styrene exposure.

During recent years evidence has accumulated to support the role of genetic lesions in the etiology of cancer. Various methods have been developed to screen for risk chemicals with mutagenic/carcinogenic potentials. At present, the possibilities of detecting mutagenesis in exposed persons are still quite limited. It is a hopeless task to detect mutations in the human germ line. More easily observable data, and at the same time the most relevant information from the point of view of human genetic health, are obtainable from the somatic tissues of exposed persons if clastogenic and other cytogenetic parameters are studied in peripheral blood lymphocytes.

This report confirms our earlier findings (7) of a significant increase of chromosome aberrations in workers exposed to styrene in the reinforced plastics industry.

MATERIAL AND METHODS

Exposed persons

In 1976 blood samples for chromosome analysis were taken from 16 men employed in two plants manufacturing polyester plastic products. As estimated by determinations of atmospheric styrene and mandelic acid in urine (2), the exposure levels may occasionally rise to 200—300 ppm of styrene in air, especially during hand lamination. The subjects, all men aged 21 to 51 years, had been employed mainly in laminating work for 1 to 15 years (table 1).

One year later, in the fall of 1977, new blood samples were taken from the same men so that we could confirm the results and determine whether changes had occurred in the frequency of aberrant lymphocytes after prolonged exposure. At present, 10 subjects have been reexamined for chromosome aberrations.

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Table 1. Incidence of chromosome aberrations and sister chromatid exchange (SCE) in exposed persons and referents.

Persons	Age (years)	Smoking habit ^a	Years of exposure (1976)	Degree of exposure in lamination work	Mandelic acid (mg/g creatinine)		Percentage of aberrant lymphocytes		SCE (Mean/cell)
					1976	1977	1976	1977	
<i>Plant 1</i>									
L. M. (4)	21	—	1	Full-time	3257	1646	26	21	4.2
L. R. (5)	37	++	8	Part-time	23	580	25	19	4.6
R. L. (6)	41	—	2.5	Part-time	219	124	13	—	4.3
L. A. (7)	27	—	2	Full-time	1452	172	15	20	5.2
K. R. (8)	21	—	1	Full-time	422	691	16	16	4.4
H. E. (20)	51	—	13	Full-time	731	—	12	—	—
<i>Plant 2</i>									
L. S. (9)	31	+	9	Full-time	267	73	12	15	6.5
V. T. (10)	41	+++	15	Part-time	85	52	15	14	4.8
K. S. (11)	30	+	10	Full-time	203	118	10	16	5.8
J. M. (12)	32	++	8.5	Part-time	75	56	17	12	7.3
K. I. (13)	23	++	3	8 months out 1 month back	645	57	17	14	—
L. E. (14)	28	—	4	Part-time	55	53	15	15	5.4
V. A. (27)	24	++	6	Full-time	512	—	10	—	—
K. E. (33)	26	—	8	Part-time	305	—	12	—	—
L. A. (36)	31	++	3	Full-time	229	—	10	—	—
V. A. (25)	41	—	7.5	Part-time	636	—	17	—	5.3
Total	16				$\bar{x}=15.1 \pm 4.8$ $t=6.57^{***}$		$\bar{x}=16.2 \pm 2.9$ $t=7.89^{***}$	$\bar{x}=5.3 \pm 1.0$ $t=NS$	
<i>Referents</i>									
K. R.	32	—	—		—		2		—
K. A.	35	++	—		—		4		—
S. R.	33	—	—		—		1		5.0
S. A.	30	+++	—		—		1		4.0
S. H.	30	—	—		—		1		—
J. J.	29	—	—		—		3		4.1
Total	6				$\bar{x}=2.0 \pm 1.3$		$\bar{x}=4.4 \pm 0.6$		

^a +++ = heavy smoker; ++ = moderate smoker; + = light smoker; — = nonsmoker.

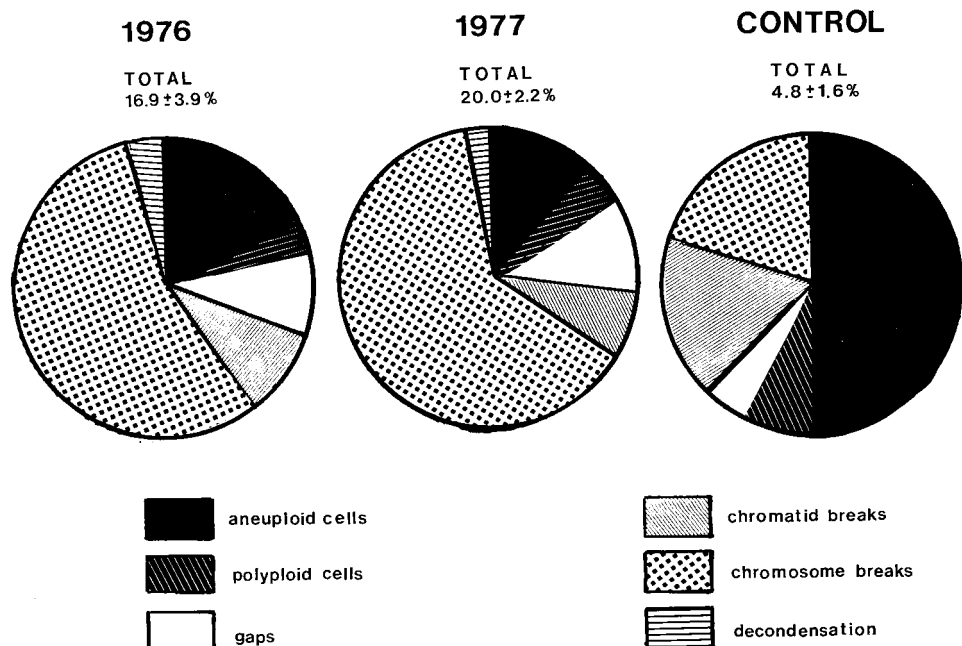


Fig. 1. Frequency of different aberration types in persons exposed to styrene for two consecutive years and in referents.

The occupational and health histories of the subjects, as well as the referents (men outside the factory environment) were recorded at the time when the blood samples were taken. A complete hematological count was also done simultaneously. According to this information the subjects were all in good health and had no previous exposure to any known clastogenic agents.

Cytogenetic methods

The chromosome studies were performed according to conventional micro methods (7). The cells were harvested after a 66- to 68-h culture period and stained with a 10 % solution of Giemsa. A total of 100 metaphases for each subject was analyzed on coded slides.

The analysis for sister chromatid exchange (SCE) was carried out in harlequin-stained second metaphase chromosomes according to the conventional Fluorescent Plus Giemsa technique (8). A total of 15–20 metaphases was counted in each subject for the frequency of exchanges between the two differently stained chromatids. At present, 10 subjects and four referents have been analyzed for the sister chromatid exchange frequency.

RESULTS

Frequencies of aberrations in lymphocytes

The results of the chromosome analysis and the determination of sister chromatid exchange are given in table 1. The frequencies of aberrant lymphocytes varied from 10 to 26 % in the exposed group and from 1 to 4 % in the referents. The aberration frequency determined in healthy Finnish males of the age group 20–40 years by similar methods is in the range of 3 %, according to a personal communication from Dr. P. Aula. The present material is too limited for any

conclusions about the degree and duration of exposure in relation to the frequency of chromosome aberrations. However, the highest aberration frequency observed in 1976 in subject 4 was detected after only 1 year of employment in the plant. This man also happened to have the highest urinary mandelic acid concentration, which may hint to his high exposure during the processing of the glass fiber plastic products. On the other hand, an employment time of 15 years for subject 10 does not seem to correlate with especially high aberration frequencies.

When the lymphocytes were retested one year later (table 1), the aberration frequencies were still observed to be at the same high level, ranging from 12 to 21 %. The means for aberration frequencies in the same subjects were almost identical for the two consecutive years, 16.8 ± 5.1 % in 1976 and 16.2 ± 2.9 % in 1977. Minor increases or decreases, not exceeding 6 %, were detected individually, but without any obvious correlation to recorded changes in exposure. However, the slight decrease of 3 % in the aberration frequency of subject 13, who was in the military service for eight months between the two tests, could relate to his absence from exposure. On the basis of the present material, we did not find any correlations between the habit of smoking and the aberration frequencies, nor the SCE frequency.

Types of aberrations

When both the “unstable” changes (breaks, gaps, etc.) and the “stable” aberrations (aneuploidy, polyploidy) are combined, the total frequencies for abnormal lymphocytes were 16.9 % in 1976, 20.0 % in 1977, and 4.8 %, respectively, for the referents. By far the most frequent class of aberrations in the exposed persons was chromosome type breaks, as compared to aneuploidy among the referents (fig. 1). A typical finding in the lymphocyte chromosomes of styrene-exposed persons was decondensation of the metaphase chromosome, often occurring in the constriction regions, but occasionally resulting in more or less pronounced decondensation of the

2

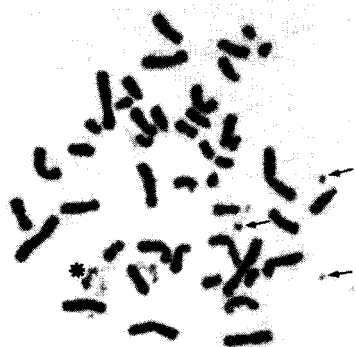


Fig. 2. A typical damaged metaphase with fragments (arrows) and one disrupted chromosome.

3



Fig. 3. Harlequin-stained chromosomes revealing several exchanges between chromatids (arrows).

whole metaphase chromosome structure in an otherwise normal karyotype (fig. 2).

Sister chromatid exchange

The incidence of SCE is generally considered a sensitive indicator of DNA (deoxyribonucleic acid) damage in proliferating mammalian cells (3). The SCE technique is less tedious than the metaphase aberration analysis, and the exchanges are easily scorable in harlequin-stained chromosomes (fig. 3). Even though our material was quite limited at the time of the study, it seems evident that the frequency of SCE among styrene-exposed persons is not significantly higher than in referents (table 1).

DISCUSSION

In recent years, occupational exposure to various chemicals has become recognized as a potential risk for the induction of genetic changes in exposed workers.

In this report we confirm our earlier results (7) of a significantly increased frequency of aberrations in the cultured lymphocytes of workers exposed to styrene in the reinforced plastics industry. Furthermore, this frequency seems to stay in the same high range with prolonged exposure. The incidence of aberrant lymphocytes, about 15 % on the average, is among the highest ever reported in human populations exposed to chemicals in a work environment; it is higher than reported in benzene exposure (4) and in the same range with subjects exposed to vinyl chloride (1).

The characteristic types of aberration in the lymphocytes of the exposed group are chromosome type changes, fragments and rarely dicentrics, which occur even in a shorter culture time (48 h) and should thus represent original double strand breaks. The problem with peripheral blood lymphocyte analysis, however, is that we cannot date back the occurrence of the mutational event due to the long life of lymphocytes.

The views about molecular mechanism in the induction of sister chromatid ex-

changes are still controversial. Our results on the induction of SCE in persons exposed to styrene have thus far been negative. These negative results may partly be due to the limited material, but they may also suggest different molecular events behind the manifestation of chromosome aberrations and of SCE (3).

The crucial question still remains of whether it is the styrene monomer, one of its metabolites, some impurities in the chemical, or some unknown synergistic effect caused by multiple exposure to chemicals in the work environment which is responsible for the high incidence of chromosome aberrations in styrene workers. Our results from in vitro studies (5) speak in favor of the specific clastogenic potentiality of either styrene or one of its metabolites.

Furthermore, our knowledge of the biological significance of high aberration rates in healthy human subjects is still vague. According to the current evidence on the close correlation between genetic lesions and carcinogenesis (6), it seems warranted to state that the workers exposed to styrene in the reinforced plastics industry represent a "population at risk." Future efforts should be directed toward evaluating the degree of risk involved.

REFERENCES

1. DUCATMAN, A., HIRSCHHORN, K. and SELIKOFF, I. J. Vinyl chloride exposure and human chromosome aberrations. *Mutat. res.* 31 (1975) 163—168.
2. ENGSTRÖM, K., HÄRKÖNEN, H., KALLIOKOSKI, P. and RANTANEN, J. Urinary mandelic acid concentration after occupational exposure to styrene and its use as a biological exposure test. *Scand. j. work environ. & health* 2 (1976) 21—26.
3. EVANS, H. J. What are sister chromatid exchanges? In: A. DE LA CHAPELLE and M. SORSA (eds.), *Chromosomes today* (vol. 6). Elsevier/North-Holland, Amsterdam 1977, pp. 315—326.
4. FORNI, A. M., PACIFICO, E. and LIMONATA, A. Chromosome studies in workers exposed to benzene or toluene or both. *Arch. environ. health* 22 (1971) 373—378.
5. LINNAINMAA, K., MERETOJA, T., SORSA, M. and VAINIO, H. Cytogenetic effects of styrene and styrene oxide. *Scand. j. work environ. & health* 4 (1978): suppl. 2, 156—162.
6. MAGEE, P. N. The relationship between mutagenesis, carcinogenesis and teratogenesis. In: D. SCOTT, B. A. BRIDGES and F. H. SOBELS (eds.), *Progress in genetic toxicology*. Elsevier/North-Holland, Amsterdam 1977, pp. 15—27.
7. MERETOJA, T., VAINIO, H., SORSA, M. and HÄRKÖNEN, H. Occupational styrene exposure and chromosomal aberrations. *Mutat. res.* 56 (1977) 193—197.
8. PERRY, P. and WOLFF, S. New giemsa method for the differential staining of sister chromatids. *Nature* 251 (1974) 156—158.

QUESTIONS AND ANSWERS

Questions to: Dr. SORSA

Dr. HERNBERG:

Do you know of any study showing cancer patients to have an abnormally high prevalence of chromosome aberrations?

Dr. SORSA:

Yes, I remember a study of about 50 cancer patients showing an increase of lymphocyte chromosome aberrations in 72 % of the patients. The problem is, of course, to avoid the role of induced clastogeny by chemotherapeutic cytostatic drugs.

- Dr. RANTANEN: The average life span for lymphocytes is about 5,000 days. It could be possible to calculate the "natural" loss of lymphocytes in peripheral blood. One of your subjects had been off work for eight months; during this period the aberration frequency had decreased by 3 %. Is this "natural" loss of damaged lymphocytes, or does it depend on the repair of chromosomes after the elimination of the exposure?
- Dr. SORSA: The life span of lymphocytes in human blood circulation is a matter of much controversy. Some authors claim that it may be as long as 20 years. This material, and this one case, is too little for the analysis of possible elimination or repair of lymphocytes, which is a difficult task, because it is not known whether the damage has occurred in the bone marrow in the stem cells. However, we are planning to study the effect of time out of exposure and its correlation to the aberration frequency.
- Dr. RINZEMA: Did you check for the presence/use of (aromatic) amines in the laminating industries included in your study as the use of amines as catalysts are quite common in such applications?
- Dr. SORSA: I shall pass this question to Dr. Härkönen who knows the situations in the plants better.
- Dr. HÄRKÖNEN: No, I am not aware of aromatic amines being used, and I do not believe that they are used in Finnish laminating plants.