

Mutagenicity of industrial compounds

VII. Styrene and styrene oxide: II. Point mutations, chromosome aberrations and DNA repair induction analyses

by N. LOPRIENO,¹ S. PRESCIUTTINI,¹ I. SBRANA,¹ G. STRETTI,¹ L. ZACCARO,¹
A. ABBONDANDOLO,² S. BONATTI,² R. FIORIO² and A. MAZZACCARO²

LOPRIENO, N., PRESCIUTTINI, S., SBRANA, I., STRETTI, G., ZACCARO, L., ABBONDANDOLO, A., BONATTI, S., FIORIO, R. and MAZZACCARO, 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. The possible genetic effects produced by styrene have been investigated by means of different methodologies in several biological organisms: (a) the induction of point mutation has been investigated in *Salmonella typhimurium* (reverse mutation), in the yeast *Schizosaccharomyces pombe* (forward mutation), both in vitro and in vivo, in the host-mediated assay of mice, and in the Chinese hamster cell line grown in vitro (V-79) (forward mutation); (b) the induction of chromosome mutation has been investigated in vivo, in mice, through the analysis of the presence of chromosome aberrations in bone marrow cells of treated animals; (c) the production of DNA (deoxyribonucleic acid) damage and the stimulation of DNA repair synthesis have been evaluated from measurements of unscheduled DNA synthesis in a heteroploid human cell line (EUE) and gene-conversion produced in the yeast *Saccharomyces cerevisiae* treated in vitro and in vivo (host-mediated assay). All the in vitro studies have been developed by the testing of the styrene in the presence of a metabolic activating system obtained with a mouse liver microsomal preparation. Styrene oxide, one of the in vivo metabolites of styrene with electrophilic properties towards DNA molecules, have also been tested in similar systems. Styrene was not mutagenic in all the systems tested; styrene oxide, on the contrary, was shown to be an active mutagen, independently of the genetic system under evaluation.

Key words: chromosome aberrations, DNA repair, induction, point mutations, styrene, styrene oxide.

In a previous paper (8) we analyzed the ability of styrene (C.A. no. 100-42-5) and styrene oxide (C.A. no. 96-09-3) to produce point mutation in the yeast *Schizosaccharomyces pombe*, strain P₁, in a V-79 Chinese hamster cell line growth in vitro

and mitotic gene conversion in the yeast *Saccharomyces cerevisiae*, strain D₁. Both yeast systems were analyzed with and without metabolic activation provided by purified mouse liver microsomes or by intraperitoneal host-mediated assay; V-79 Chinese hamster cells were treated without metabolic activation. In the present paper we report the results of studies developed on the mutagenicity of these two compounds on: (a) *Salmonella ty-*

¹ Laboratorio di Genetica, Istituto di Antropologia dell Università, Pisa, Italy.

² Laboratorio di Mutagenesi e Differenziamen-to del C.N.R., Pisa, Italy.

phimurium (point mutational assay), (b) cultured human cells [unscheduled DNA (deoxyribonucleic acid) synthesis: UDS], (c) mouse bone marrow cells (chromosomal mutational assay), and (d) V-79 Chinese hamster cells with and without metabolic activation (point mutational assay).

Preliminary results on UDS assay were reported at the Second International Conference on Environmental Mutagens (2). The results of the characterization of styrene oxide-induced V-79 Chinese hamster mutants have been submitted for publication. Other results aimed at the investigation of the molecular nature of mutant clones induced by styrene oxide in V-79 Chinese hamster cells are reported in the present paper.

MATERIAL AND METHODS

Salmonella reversion analyses were carried out according to the method of Ames et al. (3). Strain TA 1535 was chosen on the basis of the results of other investigations (4, 5, 11, 14, 16). Metabolic activation was provided by S-9 mix obtained from mouse liver; the animals (CD-1 males) were pretreated with phenobarbital (80 mg/kg $\times$ 5 d).

UDS repair studies were developed on human EUE cells in the presence of an inhibitor (hydroxyurea) of semiconservative DNA synthesis. Cells were treated in single layers for 1 h at maximum tolerable

doses and subsequently incubated for 4 h with ^3H -TdR (10 $\mu\text{Ci/ml}$) in the presence or absence of a 5 mM solution of hydroxyurea. The 10,000 $\times g$ supernatant from mouse liver homogenate and cofactors (S-10) were added in some experiments. The ratio of ^3H -TdR incorporation (evaluated by liquid scintillation counting) in the presence or absence of hydroxyurea (HU) is reported as "HU resistant incorporation," which gives a quantitative value of the unscheduled DNA synthesis observed after treatment with a genotoxic compound.

The induction of chromosome aberrations in mouse bone marrow cells after treatment with styrene or styrene oxide was evaluated on male and female, 10- to 14-week-old, CD-1 mice. The animals were treated with a single dose by gavage with 0.5 ml of solution 66 % in olive oil (control animals were treated with olive oil). The animals were killed 24 h after treatment. They were injected intraperitoneally with 0.2 ml of a Colcemid solution at a concentration of 0.06 % 1 h before the sacrifice. The preparation of bone marrow cells was made according to the method reported by Schmid and Staiger (12). Four to six animals were used for each treatment. The evaluation of chromosome damage included chromatid gaps and breaks, fragments, terminal deletions, chromatid interchanges, dicentric chromosomes, and ring chromosomes.

Mutation studies with styrene on V-79 Chinese hamster cells were made in the presence of an S-10 metabolic activation system. Due to the toxic effect of styrene

Table 1. Mutagenicity of styrene and styrene oxide on the TA 1535 strain of *Salmonella typhimurium* with and without metabolic activation (S-9 mix).

Styrene					Styrene oxide				
μ moles/ plate	Survival %		His ⁺ /plate $\pm$ SD		μ moles/ plate	Survival %		His ⁺ /plate $\pm$ SD	
	—	+(S-9)	—	+(S-9)		—	+(S-9)	—	+(S-9)
Control	100	100	41.3 $\pm$ 3.0	26.4 $\pm$ 3.2	Control	100	100	41.3 $\pm$ 3.0	26.4 $\pm$ 3.2
1 $\times$ 10 ⁻³	100	100	31.0 $\pm$ 5.0	30.7 $\pm$ 3.8	1	84.7	96.5	98.5 $\pm$ 9.6	62.1 $\pm$ 8.7
5 $\times$ 10 ⁻¹	100	100	35.7 $\pm$ 2.5	49.7 $\pm$ 10.8	5	50.6	57.7	286.0 $\pm$ 9.8	204.7 $\pm$ 3.7
1	61	100	33.0 $\pm$ 2.6	36.0 $\pm$ 4.1	10	44.3	41.6	436.7 $\pm$ 68.9	381.0 $\pm$ 91.0
5	3	61	35.5 $\pm$ 6.2	39.8 $\pm$ 7.7	50	12.6	21.5	477.3 $\pm$ 52.5	782.6 $\pm$ 50.7
7.5	0	7	57.0 $\pm$ 6.2	38.6 $\pm$ 14.5	100	0.1	1.0	toxic	470.0 $\pm$ 158.8
10	0	0.8	40.7 $\pm$ 11.0	35.0 $\pm$ 2.0					

on cells, only a 1-mM concentration was tested. Lower concentrations were found to be inactive when tested without metabolic activation (8). Reversion studies with styrene oxide-induced 8-azaguanine or 6-thioguanine resistant V-79 Chinese hamster cell mutants have been developed with three well-known mutagens, namely, ethylmethane sulfonate (EMS), ICR-170³, and ultraviolet light. Treatment and scoring of the results were performed according to Abbondandolo et al. (1).

Styrene and styrene oxide (Aldrich, U.S.A., or Merck-Schuchardt, Federal Republic of Germany) were obtained by the International Agency for Research on Cancer (IARC), Lyon (France). Mitomycin C Kyowa (Japan) was obtained from Dr. U. Ehling, Munich (Federal Republic of Germany). Methylmethane sulfonate (MMS), EMS and dimethyl nitrosamine (DMN) were all from Merck-Schuchardt, (Federal Republic of Germany). ICR-170 was obtained from Dr. H. Bartsch, Lyon (France, IARC).

RESULTS

Data obtained from styrene and styrene oxide mutagenicity tests on *Salmonella* TA 1535 are reported in table 1. Styrene, tested at concentrations from 1×10^{-3} to $10 \mu\text{mol}$ per plate, was inactive with and without S-9 mix. The toxicity under these conditions ranged from 0.8 to 100 % survival. Styrene oxide, on the contrary, was found to be mutagenic for TA 1535. The spontaneous reversion frequency was increased 10-fold in the direct test, and 30-fold in the presence of S-9 mix due to the reduced toxic effect observed at the high doses employed in the presence of the S-9 mix. The ability of the S-9 mix preparation to activate a promutagen was tested on benzo(a)pyrene; $5 \mu\text{g}/\text{plate}$ resulted in mutagenic activity (fivefold increase over the spontaneous reversion number) in the presence of S-9 mix.

Results on DNA repair stimulation in human cells are reported in table 2. The stimulation of ³H-TdR incorporation into

Table 2. Stimulation of DNA repair synthesis by styrene and styrene oxide in a human heteroploid cell line (EUE).

Compound	S-10 mix	% HU ^a resistant- ³ H-TdR incorporation
Styrene		
0.0 mM	+	2.99
14.3 mM	+	4.16
28.5 mM	+	3.93
42.8 mM	+	toxic
Styrene oxide		
0.0 mM	—	3.00
4.4 mM	—	28.07
8.7 mM	—	toxic
Positive controls		
Methylmethane sulfonate		
0.0 mM	—	2.82
2.0 mM	—	19.10
Dimethyl nitrosamine		
0.0 mM	+	3.29
400.0 mM	+	36.45

^a HU = hydroxyurea.

DNA of EUE cells in the presence of an inhibitor of semiconservative DNA synthesis (hydroxyurea) by S-10 mix metabolically activated styrene and by styrene oxide is shown. Only styrene oxide was able to increase thymidine incorporation above the background. Results obtained with a direct alkylating agent (MMS) and a compound requiring metabolic activation (DMN) are reported for comparison.

Results of the chromosome breaking activity of styrene and styrene oxide in mouse bone marrow cells are reported in table 3; for a comparison results of a similar analysis done with mitomycin C are reported. Styrene oxide induced a statistically significant increase in chromosome aberrations at doses from 50 to 1,000 mg/kg, although the highest tested dose was found to be partly toxic to the animals (fig. 1a and b). The induction of chromosome aberrations was dose-dependent (fig. 2). Cytogenetic analyses on animals treated with styrene demonstrated that this compound was not active (fig. 3a and b). Mitomycin tested at two concentrations (3.5 mg/kg and 14 mg/kg) was shown to be extremely positive for the

³ ICR-170: 2-methoxy-6-chloro-9-[3-(ethyl-2-chloroethyl) aminopropyl-amino] acridine dihydrochloride.

Table 3. Chromosome aberrations induced in bone marrow cells of CD-1 mice by styrene (St) styrene oxide (St O) and mitomycin (Mit C).

Dose (mg/kg)	Number of animals	Cells			Gaps	Chro- matid breaks	Others	Chro- matid ex- changes	Dicen- tric	Total number of aberra- tions	Aberra- tions/ damaged cell	Chi- square
		Ana- lyzed N	Damaged N	%								
Control	9	807	27	3.35	5	1	27	0	0	33	1.22	—
St 500	4	400	6	1.50	3	0	4	0	0	7	1.17	Negative
St 1,000	2 ^a	88	4	4.54	1	3	0	0	0	4	1.00	0.06
St O 50	4	317	13	4.10	4	0	11	0	0	15	1.15	5.83*
St O 500	12	718	51	7.10	13	4	34	4	0	54	1.05	9.24***
St O 1,000	8 ^b	573	54	9.42	14	7	37	0	0	58	0.07	18.73***
Mit C 3.5	6	617	141	22.85	63	65	44	38	0	210	1.49	59.36***
14.0	3	228	143	62.72	66	85	55	140	1	347	2.43	135 ***

^a Four animals treated, two of which died.

^b Twelve animals treated, four of which died.

* $P < 0.05$; *** $P < 0.001$.

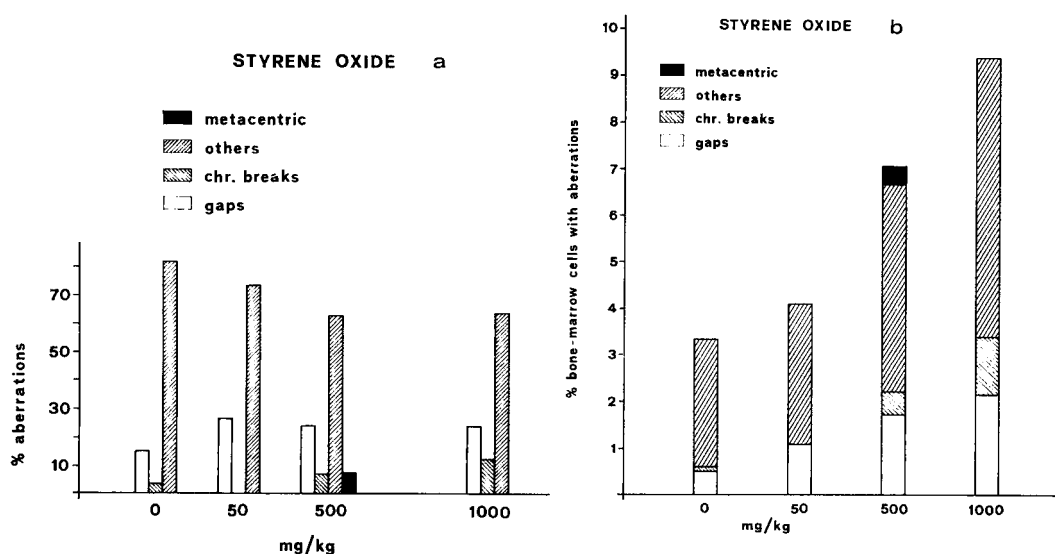


Fig. 1. Induction of chromosome aberrations in bone marrow cells of CD-1 mice treated by gavage with styrene oxide: (a) frequency of single effects; (b) frequency of total chromosome aberrations observed.

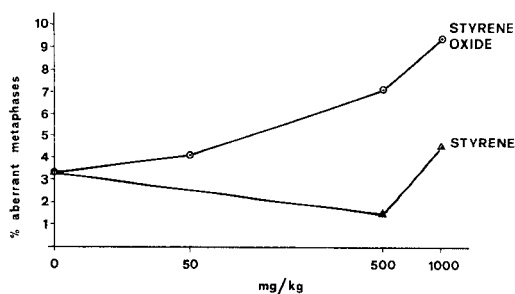


Fig. 2. Dose-response relationships of chromosome aberrations in bone marrow cells of CD-1 mice obtained after treatment by gavage with styrene oxide and styrene.

induction of chromosome aberrations (fig. 4a and b). A linear dose response effect was found (fig. 5).

Preliminary data on the dose-dependent induction of 8-azaguanine resistant mutants in V-79 Chinese hamster cells by styrene oxide have already been published (8). Styrene, tested at concentrations of 8.5 and 17.0 mM in a direct test, was ineffective (8). In table 4 we report the results of an experiment performed with styrene in the presence of an S-10 metabolic system. Different expression times for the mutant expression demonstrated

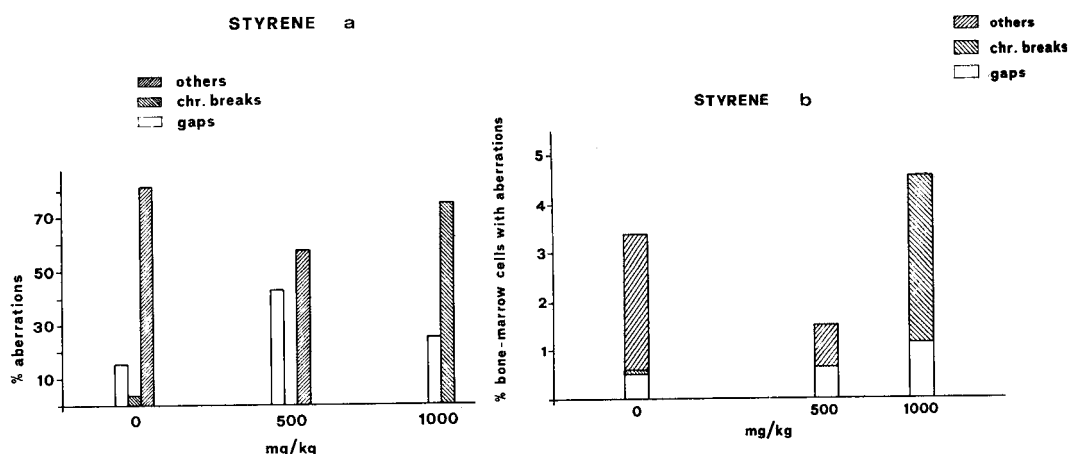


Fig. 3. Induction of chromosome aberrations in bone marrow cells of CD-1 mice treated by gavage with styrene: (a) frequency of single effects; (b) frequency of total chromosome aberrations observed.

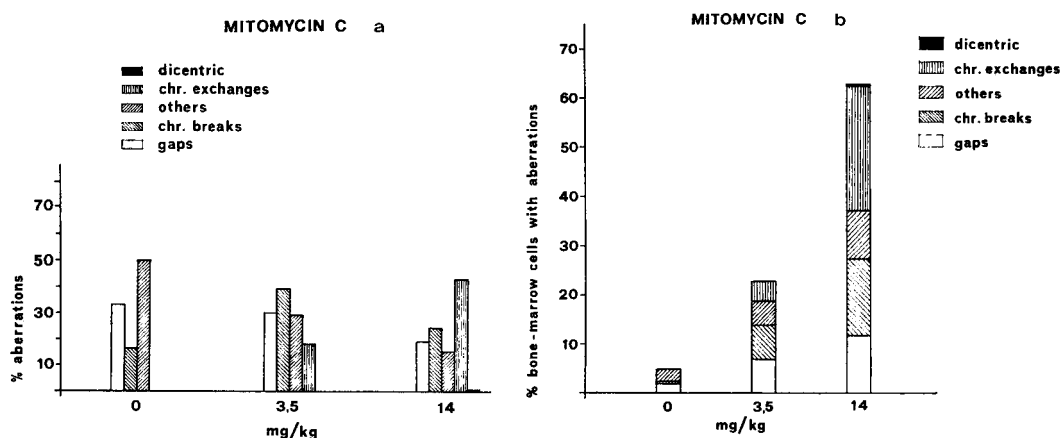


Fig. 4. Induction of chromosome aberrations in bone marrow cells of CD-1 mice, treated by intraperitoneal injection with mitomycin C: (a) frequency of single effects; (b) frequency of total chromosome aberrations observed.

that styrene at a dose of 51 mM was not converted to a mutagenic metabolite for V-79 cells by the S-10 mix preparation from mouse liver.

Further experiments carried out with styrene oxide allowed a better evaluation of the dose-effect relationship observed with treated cells from V-79 Chinese hamsters. This evaluation (table 5) was based on the definition of the expression curves of *azg^r* mutants induced by different concentrations of styrene oxide. As it resulted, the frequency of mutants decreased after 90 h when the maximum of mutation frequency was attained. Similar results were found when 6-thioguanine was used

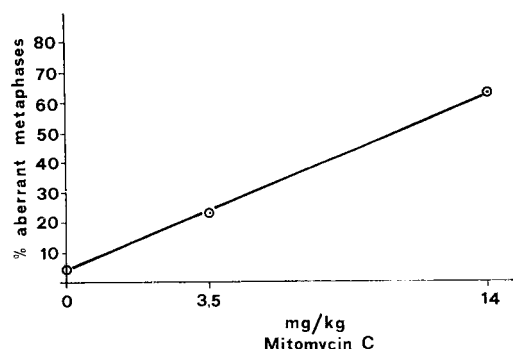


Fig. 5. Dose-response relationship of chromosome aberrations in bone marrow cells of CD-1 mice obtained after treatment by gavage with mitomycin C.

Table 4. Induction of 6-thioguanine resistant mutants by styrene in V-79 Chinese hamster cells.

Styrene (mM)	S-10 mix	Expression time (h)	Plating efficiency (%)	Number of viable cells $\times 10^{-3}$	Number of mutants	Mutants/ 10^6 viable cells
0.0	+	0	85.0	1,275.0	3	2.3
		90	66.5	997.5	3	3.0
		114	91.7	1,375.5	4	2.9
		138	75.0	1,125.0	2	1.8
51.0	—	0	0.0	—	—	(toxic)
51.0	+	0	86.2	1,293.0	5	3.9
		90	80.0	800.0	1	1.2
		114	82.0	1,230.0	9	7.3
		138	70.0	1,050.0	7	6.7

Table 5. Induction of 8-azaguanine resistant mutants by styrene oxide in V-79 Chinese hamster cells.

Styrene oxide (mM)	Number of viable cells $\times 10^{-3}$	Number of mutants	Mutants/ 10^6 viable cells $\pm$ SD	Number of experiments
0.0	2,588	12	5.80 $\pm$ 2.3	4
4.2	777	46	59.16	1
8.5	239	20	82.84 $\pm$ 5.1	2
17.0	365	52	155.37 $\pm$ 15.4	3

for the selection of mutants. Using the maximum expression values, we obtained a linear dose-response relationship with a slope of 8.52×10^{-6} mutants per mmole per hour. A preliminary characterization of styrene oxide-induced mutants is shown in table 6. Six clones were originally isolated on 6-thioguanine (5 $\mu\text{g/ml}$), and five clones on 8-azaguanine (20 $\mu\text{g/ml}$). They were all cross-resistant and showed a residual Hypoxanthine guanine phosphoribosyl transferase activity ranging from 0.3 to 25 % of the wild type levels. Four clones were reverted by mutagens and caused base-pair substitutions in prokaryotes (EMS and ultraviolet light) and one by the frameshift mutagen ICR-170.

DISCUSSION

Table 7 summarizes the results obtained in our mutagenicity tests developed with styrene and styrene oxide. Some of them

have been published elsewhere (8). In our analysis styrene was nonmutagenic in vitro, in the presence of a metabolic activation system, in all tested organisms, namely, *Salmonella*, *S.pombe* and V-79 Chinese hamster cells (point mutations), *S.cerevisiae* (gene conversions), EUE human heteroploid cells (UDS), and for the production of chromosome aberrations, in vivo, in mice. The only slightly positive (mutagenic) response was obtained when a host-mediated assay was developed from mice treated with a styrene concentration of 1,000 mg/kg for 6–12 h and assayed with the forward mutation system of *S.pombe* (P_1 strain) or with the mitotic gene-conversion system of *S.cerevisiae* (D_1 strain) (8). In the former case we obtained, on the average, a slightly positive effect in different experiments, the results of which were always lower than those observed with other olefins, such as vinyl chloride and trichloroethylene (fig. 6); in this case, however, a very toxic dose of styrene was used. On the contrary, styrene oxide pro-

Table 6. Hypoxanthine guanine phosphoribosyl transferase (HGPRT) activity and reversion pattern of styrene oxide-induced 8-azaguanine or 6-thioguanine resistant. HAT sensitive clones in V-79 Chinese hamster cells.^a

Clone number and origin	HGPRT (% of wild type)	Reversion to HAT resistance			
		Spontaneous	Induced by		
			EMS	UV	ICR-170
34 thg ^r	24.9	< 0.8	6.8 (1.8—17.4)	61.0 ^{oo}	8.1 (3.5—13.5)
36 thg ^r	3.9	10.6 (4.3—21.8)	< 3.4	1.3 (0.03—7.3)	458.0 ^{oo}
44 thg ^r	6.2	< 1.7	27.0 ^o (9.0—58.8)	5.5 (0.7—19.9)	13.3 (2.7—38.9)
46 thg ^r	7.3	< 1.9	5.6 (0.14—31.0)	18.5 ^o (8.9—34.0)	
48 thg ^r	3.5	5.0 (1.0—14.6)	3.3 (0.08—18.4)	5.1 (1.7—12.0)	11.6 (5.0—22.8)
50 thg ^r	8.7	35.7 (4.3—128.9)	31.8 (3.8—114.6)	22.2 (4.6—65.0)	
39 azg ^r	2.7	< 1.8	3.0 (0.08—16.7)	3.1 (0.08—17.0)	2.2 (0.3—7.9)
45 azg ^r	12.8	< 4.1	< 2.1	< 2.0	
49 azg ^r	3.5	< 1.2	125.0 ^{oo}	< 1.1	< 1.0
53 azg ^r	0.3	< 37.2	< 13.3	41.7 (16.7—85.8)	
61 azg ^r	7.9	< 10.5	< 3.7	< 5.1	

^a Cells were treated for 1 h and incubated after 90 h in HAT medium (10⁻⁴M hypoxanthine, 3 × 10⁻⁷ M aminopterin, 10⁻⁵ M thymidine) for 10 d. Ninety-five percent confidence limits, calculated from the tables in Fischer and Yates, are given in parentheses whenever reversion frequencies were based on a number of mutants ≤ 10. Significant increases are indicated by ^o.

Table 7. Summary of the mutagenic data developed with styrene and styrene oxide treatments with different test systems.

Biological system	Mutational assay	Styrene	Styrene oxide
1. <i>Salmonella typhimurium</i> (TA 1535)	Point mutation (reverse mutation) ± Metabolic activity	—	+
2. <i>Schizosaccharomyces pombe</i> (P ₁)	Point mutation (forward mutation) A. ± Metabolic activity B. Host-mediated assay	— (+)	+ (+)
3. Chinese hamster cell line (V-79)	Point mutation (forward mutation) ± Metabolic activity	—	+
4. Human cell line (EUE)	Unscheduled DNA synthesis ± Metabolic activity	—	+
5. <i>Saccharomyces cerevisiae</i> (D ₄)	Mitotic gene recombination A. ± Metabolic activity B. Host-mediated assay	— (+)	+ (+)
6. Mouse CD-1	Chromosome aberrations in bone marrow cells	—	+

duced positive results in all the mutagenicity tests performed. It induced point mutations in three different systems (*Sal-*

monella, yeast *S.pombe*, and V-79 Chinese hamster cells), mitotic gene conversions in the yeast *S.cerevisiae*, unscheduled DNA

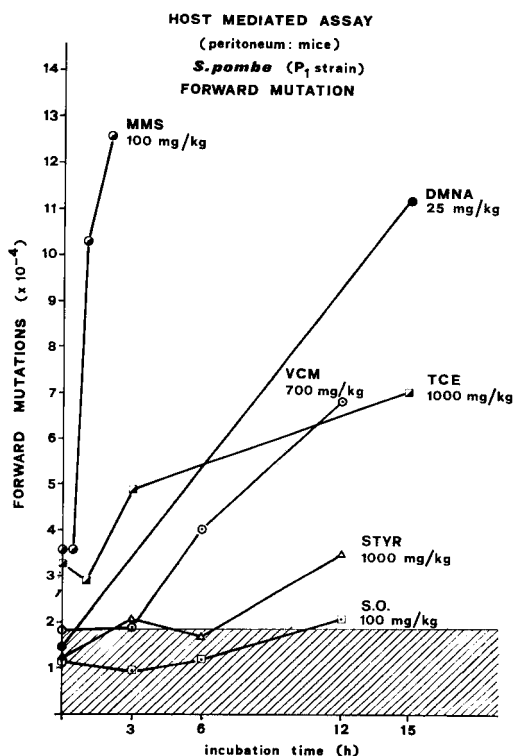


Fig. 6. Forward mutation frequencies induced by different mutagens on *Schizosaccharomyces pombe*, P₁ strain, tested in the host-mediated assay. (MMS = methylmethane sulfonate; DMNA = dimethyl nitrosamine; VCM = vinyl chloride monomer; TCE = trichloroethylene; STYR = styrene; S.O. = styrene oxide)

synthesis in EUE human cells (DNA repair test), and chromosome aberrations, in vivo, in mouse bone marrow cells. Styrene oxide has been also found to be slightly positive in the host-mediated assay for the production of both point mutations and gene conversions in yeast inoculated in mice treated with 100 mg/kg of the chemical (8).

In *Salmonella* styrene has been reported to induce reverse mutations in the presence of an S-9 mix metabolic system (5, 16). Analyses done by other investigators have not confirmed such a result (4, 14); in all these studies styrene oxide gave positive results. In *Drosophila*, styrene and styrene oxide were not able to induce recessive lethals in the F₂ progeny of treated animals (13).

Recent investigations have shown that styrene-exposed men (occupational expo-

sure) presented an increase in the frequency of chromosome aberrations over the value observed in a reference group (10).

In a group of 12 occupationally exposed workers, whose exposure to styrene vapor was estimated to be an atmosphere concentration of about 1 ppm, the frequency of chromosome aberrations was not different from the one found in the unexposed group (15).

Styrene is known to be biotransformed in animals and in man to hippuric acid, which is excreted in urine. According to Leibman (7) styrene is metabolically converted to styrene oxide and subsequently to phenylethyleneglycol by microsomal epoxide hydrase from the liver, kidney, intestine, lung and skin of several mammalian species (6). The present results confirm the unspecific genotoxic activity of styrene oxide.

These results indicate that styrene might represent a genetic hazard for man, through exposure to styrene vapors or residues in food, owing to the metabolic conversion of this compound into its genotoxic metabolite, styrene oxide.

Thus far, however, biological data, even those obtained in the presence of high concentrations of styrene, have not supported this potentiality, since styrene has given negative results in most genetic systems, in vitro and in vivo. The lack of mutagenic activity of styrene may be due to the rapid conversion of its active metabolite, styrene oxide, to phenylethyleneglycol. It is known that the conversion of styrene oxide to glycol is faster than that of styrene to styrene oxide activated by microsomal monooxygenase (6). It is also possible that the glutathione-S-epoxide transferase may detoxify styrene oxide by converting it to N-acetyl-S-(2-hydroxyphenethyl)-L-cysteine (6).

In conclusion, human exposure to styrene oxide might present a clear genetic risk, well-documented by the experimental mutagenic data so far available. Exposure to styrene is still questionable, however, and requires more research, mainly in vivo in mammalian systems.

The negative results obtained in long-term carcinogenic studies with animals treated with styrene through feeding

might be correlated with the nonmutagenicity of styrene; in this case a negative carcinogenic result, together with negative mutagenic data in different short-term tests, may demonstrate the absence of any genetic risk.

ACKNOWLEDGMENTS

Part of the present work was supported by contract 267-77-1-ENV of the Commission of European Communities (Environmental and Raw Material Research Programs), and by Contract 2005/RB of the International Atomic Energy Agency.

We are thankful to Dr. S. Baroncelli for his advice concerning the statistical analyses of the data.

REFERENCES

1. ABBONDANDOLO, A., BONATTI, S., COLELLA, C., CORTI, G., MATTEUCCI, F., MAZZACCARO, A. and RAINALDI, G. A comparative study of different experimental protocols for mutagenesis assays with the 8-azaguanine resistance system in cultured Chinese hamster cells. *Mutat. res.* 37 (1976) 293—306.
2. ABBONDANDOLO, A., FIORIO, R., DURANTE, M. and BONATTI, S. Evaluation of a short-term test for the detection of genetic damage in human cultured cells. *Mutat. res.* 53 (1978) 141.
3. AMES, B. N., MC CANN, J. and YAMASAKI, E. Methods for detecting carcinogens and mutagens with the *Salmonella* mammalian microsomal mutagenicity test. *Mutat. res.* 31 (1975) 347—364.
4. BUSK, L. Mutagenic effects of styrene and styrene oxide. *Mutation res.* (in press)
5. DE MEESTER, C., PONCELET, F., ROBERFROID, M., RONDELET, J. and MERCIER, M. Mutagenicity of styrene and styrene oxide. *Mutat. res.* 56 (1977) 147—152.
6. INTERNATIONAL AGENCY FOR RESEARCH ON CANCER. *Monograph on the evaluation of carcinogenic risk of chemicals to man: Epoxides and some other chemicals of industrial importance* (no. 11). Lyon 1976 pp. 201—208.
7. LEIBMAN, K. C. Metabolism and toxicity of styrene. *Environ. health perspect.* 11 (1975) 115—119.
8. LOPRIENO, N., ABBONDANDOLO, A., BARALE, R., BARONCELLI, S., BONATTI, S., BRONZETTI, G., CAMMELLINI, A., CORSI, C., CORTI, G., FREZZA, D., LEPORINI, C., MAZZACCARO, A., NIERI, R., ROSELLINI, D. and ROSSI, A. M. Mutagenicity of industrial compounds: Styrene and its possible metabolite styrene oxide. *Mutat. res.* 40 (1976) 317—324.
9. LOPRIENO, N., BARALE, R., BARONCELLI, S., BAUER, C., BRONZETTI, G., CAMMELLINI, A., CERCIGNANI, G., CORSI, C., GERVASI, G., LEPORINI, C., NIERI, R., ROSSI, A. M., STRETTI, G. and TURCHI, G. Evaluation of the genetic effects induced by vinyl chloride monomer (VCM) under mammalian metabolic activation: Studies *in vitro* and *in vivo*. *Mutat. res.* 40 (1976) 85—96.
10. MERETOJA, T., VAINIO, H., SORSA, M. and HÄRKÖNEN, H. Occupational styrene exposure and chromosomal aberrations. *Mutat. res.* 56 (1977) 193—197.
11. MILVY, P. and GARRO, A. J. Mutagenic activity of styrene oxide (1,2-Epoxyethylbenzene), a presumed styrene metabolite. *Mutat. res.* 40 (1976) 15—18.
12. SCHMID, W. and STAIGER, G. R. Chromosome studies on bone marrow from Chinese hamsters treated with benzodiazepine tranquilizers and cyclophosphamide. *Mutat. res.* 7 (1969) 99—108.
13. SORSA, M., LAAMANEN, I. and VAINIO, H. Mutagenicity of styrene and styrene oxide in *Drosophila* test. *Mutat. res.* 53 (1978) 266—267.
14. STOLTZ, D. R. and WITHEY, R. J. Mutagenicity testing of styrene and styrene epoxide in *Salmonella typhimurium*. *Bull. environ. contam. toxicol.* 17 (1977) 739—742.
15. THIESS, A. M. and FLEIG, I. Chromosomes investigations on workers exposed to styrene/polystyrene, ACS Meeting, Health aspects of polymers, April 7, 1976.
16. VAINIO, H., PÄÄKKÖNEN, R., RÖNNHOLM, K., RAUNIO, V. and PELKONEN, O. A study of the mutagenic activity of styrene and styrene oxide. *Scand. j. work environ. & health* 2 (1976) 147—151.

QUESTIONS AND ANSWERS

Question to Dr. LOPRIENO

Dr. SORSA:

I can report to you the findings of Dr. Meretoja which may explain your negative results of induction of chromosomal aberration in mice in 1-d styrene exposure. The results in an 11-week exposure of rats at a styrene vapor concentration of 300 ppm showed very clearly that only after 9 weeks of treatment, 6 h a day, does the chromosomal aberration frequency rise significantly; it stayed at an average aberration level of 12 % until the end of the experiment.

Dr. LOPRIENO:

I agree that an acute exposure experiment is not a good one to detect chromosomal aberrations, and it may very well be that a longer exposure would have induced chromosomal aberrations also in mice.