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A study on the mutagenic activity of styrene and styrene oxide¹

by HARRI VAINIO, M.D.,² RAIJA PÄÄKKÖNEN, M.Sc.,³ KAI RÖNNHOLM, B.M.,³ VEIJO RAUNIO, M.D.,³ and OLAVI PELKONEN, M.D.⁴

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* 3 (1976) 147—151. Styrene oxide is mutagenic, without metabolic activation, to *S. typhimurium* strains TA 1535 and TA 100, which have been devised to detect mutagens causing base-pair substitutions. Styrene seems to be mutagenic toward the same strains, but only after metabolic activation. The toxicity of both styrene and styrene oxide make the construction of reliable dose-response curves rather difficult. Diethylmaleate and 3,3,3-trichloropropene oxide enhanced the mutagenicity of styrene oxide in the presence of homogenate; this result indicates the participation of epoxide hydratase and glutathione S-oxide transferase in the metabolism of styrene oxide. These two chemicals did not influence the mutagenic activity of styrene. Styrene glycol and 4-tert.-butyl-brenzcatechin were not mutagenic to any of the strains studied. Results show that further, more detailed experimental and, possibly, epidemiologic studies are warranted.

Key words: mutations, styrene, metabolic activation, microsomes.

Styrene is widely used in the plastic industry. Styrene is converted by liver microsomes to its epoxide, styrene oxide, which is further metabolized by epoxide hydratase to styrene glycol (5).

Structurally, styrene, i.e., vinylbenzene, is related to vinyl chloride, a compound known for its carcinogenic potential in man

and animals (4). In in vitro experiments vinyl chloride is metabolized by liver microsomes to a compound which is mutagenic to *Salmonella typhimurium* bacteria (histidine auxotrophs) strains (9) developed by Ames and coworkers for the detection of mutagenic compounds (1, 2).

Because of the wide use of styrene and its resemblance to vinyl chloride we decided to study the mutagenic potential of styrene and its metabolites in the *Salmonella* system, both in the presence and in the absence of liver microsomal enzymes.

MATERIALS AND METHODS

Mutagenicity testing

Specially constructed mutants of *Salmonella typhimurium*, selected for their sen-

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Table 1. Mutagenicity of styrene oxide to *Salmonella typhimurium*. (— indicates the absence and + the presence of 0.1 ml of S-9 per plate)

Concentration of styrene oxide moles/plate	TA 1535		TA 1537		TA 1538		TA 100		Ta 98	
	—	+	—	+	—	+	—	+	—	+
10 ⁻⁴		toxic		toxic		toxic		toxic		toxic
10 ⁻⁵	357	484	8	8	11	32	1111	1165	25	26
10 ⁻⁶	425	468	6	8	7	35	443	639	26	16
10 ⁻⁷	249	—	—	—	9	16	217	146	16	16
10 ⁻⁸	124	—	—	—	5	12	240	142	8	19
10 ⁻⁹	19	18	—	—	7	9	115	108	11	13
0	20	45	7	4	9	7	84	135	26	21

Table 2. Mutagenicity of styrene to *Salmonella typhimurium*. (— indicates the absence and + the presence of 0.1 ml of S-9 per plate)

Concentration of styrene moles/plate	TA 1535		TA 1537		TA 1538		TA 100		Ta 98	
	—	+	—	+	—	+	—	+	—	+
10 ⁻⁴		toxic		toxic		toxic		toxic		toxic
10 ⁻⁵										
10 ⁻⁶	13	16	7	11	8	10	197	206	21	24
10 ⁻⁷	20	84	12	12	—	—	56	78	—	—
10 ⁻⁸	22	112	—	—	—	—	115	163	—	—
0	17	16	7	12	9	7	83	97	24	13

Fig. 1. Mutagenic effect of styrene, styrene oxide, and styrene glycol on strain TA 1535. The mutagenicity of styrene and styrene glycol were tested in the presence of and styrene oxide in the absence of S-9 fraction (0.1 ml/plate).

sitivity and specificity in being reverted from a histidine requirement back to prototrophy by a wide variety of mutagens, were used in the study (1, 2, 6). Strains TA 1535, TA 1537, TA 1538, TA 98, and TA 100 were kindly provided by Dr. Bruce N. Ames (Berkeley, Calif.). Diagnostic tests were those described by Ames et al. (3).

Styrene or styrene oxide (both obtained from Koch-Light Laboratories Ltd., Colnbrook England) was dissolved in absolute ethanol, put with a top agar, enzyme preparation and a cofactor mixture when appropriate into petri plates, and incubated for 2 days at +37°C, after which the number of revertant colonies were calculated. Both direct (without enzyme preparation) and indirect mutagenesis (with enzyme and cofactors) assays were performed. Spot tests were also performed after the addition of the compound to a small filter paper disc in the center of a petri plate and bacteria with other constituents. This test could not be per-

formed with styrene because obviously styrene does not diffuse from the filter paper, or from the spot if added without filter paper.

Enzyme preparation

Male rats (200 to 300 g) of the Sprague-Dawley strain were injected with a polychlorinated biphenyl compound, Clophen C (200 mg/kg, obtained from Bayer AG, Leverkusen) in sesame oil 5 days before they were killed. Livers were removed, homogenized in four volumes of 0.15M KCl, and centrifuged at $10,000 \times g$ for 10 min. The supernatant fraction ("S-9") was divided into 2-ml fractions and stored at -80°C until used.

RESULTS

Tables 1 and 2 show the preliminary screening of the mutagenic potential of styrene oxide and styrene over a wide range of concentrations. As can be seen, both styrene oxide and styrene were toxic to all strains at high concentrations, styrene oxide at about 10^{-4} moles/plate and above and styrene at about 10^{-6} moles/plate and above. This high toxicity made the mutagenicity determinations at this range of concentration difficult. However, as table 1 shows, styrene oxide was clearly mutagenic to two of the five strains, namely, to TA 1535 and TA 100. It was not mutagenic to TA 1537, 1538, or 98. The enzyme preparation did not remarkably affect the mutation rate, and this finding suggests that we were dealing with a direct mutagen. On the other hand, the preliminary screening with styrene showed (table 2) that it may be mutagenic to TA 1535 and TA 100. However, a more detailed dose-response study with TA 1535 revealed that styrene may be both an indirect and direct mutagen, although results suggest that the considerable toxicity of styrene disturbs the interpretation of results obtained from concentrations as low as about 10^{-7} moles/plate (fig. 1). A similar study with styrene and TA 100

Table 3. Effect of diethylmaleate (DEM) and 3,3,3-trichloropropene oxide (TCPO) (both at 1 mmol/l concentration) on the mutagenicity of styrene oxide and styrene. The amount of S-9 is 0.1 ml/plate when present.

	Revertant colonies (TA 1535)			
	—S-9	+S-9	+S-9 +TCPO	+S-9 +DEM
Styrene oxide				
10^{-5} mol/plate	233	116	231	244
control	22	14	19	16
Styrene				
5×10^{-8}				
mole/plate	52	112	146	132
control	36	41	21	34

gave more equivocal results. A dose-response study with styrene oxide and TA 100 revealed that styrene oxide is a direct mutagen. Spot tests demonstrated that styrene oxide is mutagenic to TA 1535 and TA 100, but not to TA 1537, TA 1538 and TA 98, and thus confirmed the results of the plate incorporation assays. Styrene could not be studied with spot tests, because of its solubility and diffusion characteristics in agar.

Diethylmaleate, a compound which is known to deplete cytoplasmic glutathione, and 3,3,3-trichloropropene oxide, an inhibitor of epoxide hydratase (8), slightly increased the mutation rate caused by styrene (table 3). At the concentration used and in the presence of the S-9 fraction, diethylmaleate and trichloropropene oxide had no effect on the mutation rate of the strains studied.

The ratio styrene oxide/homogenate, when added, affects greatly the data obtained. Larger amounts of the liver homogenate seem to protect the bacteria, perhaps due to the presence of the enzymes glutathione S-transferase and epoxide hydratase, which metabolize styrene oxide. The protective effect could be abolished by diethylmaleate and trichloropropene oxide (table 3).

Styrene glycol did not exhibit mutagenicity towards TA 1535 and TA 100 in the concentration range from 10^{-4} to 10^{-10} moles/plate. In addition the preservation agent, 4-tert-butyl-brenzcatechin, was not mutagenic to any of the five strains used.

DISCUSSION

Earlier studies have demonstrated that styrene is converted by microsomal monooxygenase systems to styrene oxide, which is metabolized further by epoxide hydrolase to styrene glycol (5). Because styrene is structurally similar to vinyl chloride, a known mutagen and carcinogen, one can suspect that styrene is potentially mutagenic and possibly carcinogenic. This study demonstrates clearly that styrene oxide is mutagenic to *S. typhimurium* strains that have been especially constructed to detect mutagens, namely, to TA 1535 and TA 100. These two strains can be used to detect mutagens causing base-pair substitutions, and consequently styrene oxide seems to be a base-pair substitution mutagen. As small an amount as about 10^{-8} moles of styrene oxide per plate causes a significant increase in the mutation rate. Styrene oxide seems to be a directly acting mutagen, because the presence of an enzyme preparation does not enhance its mutagenicity.

Styrene seems to be an indirectly acting mutagen to TA 1535 and TA 100, the same strains which are also sensitive to styrene oxide. One can speculate that styrene oxide, formed by the monooxygenase, is the active principle in the enhanced mutagenesis caused by styrene. However, in tests with TA 100, styrene also seemed to be a direct mutagen, but, because styrene is rather toxic to *Salmonella*, the results of certain doses are difficult to interpret.

In the presence of liver homogenate, styrene appears to be even more mutagenic to strain TA 1535 than styrene oxide. This phenomenon is not necessarily in nonagreement with the metabolic activation theory of styrene to corresponding epoxide, but it may be due to the complex factors of chemical/homogenate association. The difference at very low concentrations may be also explained by the presence of an impurity in the styrene preparation. The main impurity, 4-tert-butyl-benzocatechin, is not, however, mutagenic at all. There is then a possibility that vinylphenol forms perhaps via an epoxide, and it might be the toxic and mutagenic metabolite in bacteria.

While this work was in progress, Milvy and Garro (7) reported the mutagenicity of styrene oxide in *S. typhimurium*. They found that styrene was unable to increase the mutation rate in the *Salmonella* strains. They have not, however, tested the mutagenic activity in the presence of liver homogenate, which transforms styrene to its active metabolites. In addition they reported a high spontaneous mutation rate, especially with strains TA 1535 and TA 100 (7). This high rate may perhaps have been a result of exposure to styrene monomers in the petri dishes used, since we had similar problems with a certain set of petri dishes. After we changed to another type, the spontaneous mutation rate diminished to the normal level.

In conclusion, this study suggests that further, more detailed investigations on the mutagenic and carcinogenic potential of styrene and styrene oxide are warranted and it points to the possible need of an epidemiologic study on workers in industry.

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