Mutagenicity Testing of Styrene and Styrene Epoxide in *Salmonella typhimurium*

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Styrene is used in the manufacture of polystyrene plastics and as an organic solvent. Human exposure to styrene may occur in industrial environments or as a result of the leaching of residual styrene monomer from polystyrene food containers. A recent communication (WITHEY, 1976) revealed that styrene monomer was present in dairy products, which were packaged in polystyrene containers, at up to 150 ppb (wt/vol) and that it was also rapidly leached from drinking cups to give similar concentrations in 50% ethanol:water mixtures. Polystyrene containers, which were intended for food use, were found to retain up to 4,000 ppm (wt/wt) of the monomer.

Styrene is thought to be metabolized via the epoxide and the glycol to mandelic acid and hence to phenylglyoxylic acid or hippuric acid (OHTSUJI and MASAVUKI, 1971). Styrene epoxide was therefore considered a candidate for mutagenicity testing on the basis of 1) an early report of carcinogenic activity (VAN DUUREN et al., 1963) and 2) its probable derivation after the ingestion of styrene monomer by humans.

Materials and Methods

The mutagenicity of styrene and styrene epoxide was examined in Salmonella typhimurium, an assay which has proved effective in detecting the mutagenic activity of a variety of carcinogens (MCCANN et al., 1975a). Styrene and the epoxide were examined in spot tests and plate incorporation assays for activity against strains TA1535, 1537, 1538, 98 and 100 as described by AMES et al. (1975). Assays were performed with and without liver metabolizing systems from Arochlor 1254-pretreated rats and hamsters (AMES et al., 1975).

Styrene and styrene expoxide (Aldrich) were dissolved in ethanol which was subsequently diluted to 20% EtOH:H_2O. A positive control, β-naphthylamine (Sigma), was dissolved in spectrophotometric grade dimethylsulfoxide (Aldrich). 0.1 ml aliquots were added to the top agar.

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Results

Styrene was not mutagenic for any of the tester strains in spot or plate incorporation tests at concentrations up to 1 mg/plate even in the presence of liver metabolizing systems (Table 1). The epoxide was mutagenic for strains TA1535 and TA100 in both spot and plate incorporation assays. The liver extracts had little or no effect on the mutagenicity of the epoxide (Fig. 1). The mutagenic specificity of styrene epoxide for strains TA1535 and TA100 indicates that styrene epoxide acts as a base substitution mutagen. Similar specificity has been reported for vinyl chloride and its metabolites (MCCANN et al., 1975b).

TABLE 1

Lack of reverison of S. typhimurium TA1535 by styrene in the presence of different amounts of fortified liver homogenates from Arochlor 1254-pretreated rats and hamsters.

Treatment	mg microsomal protein/plate	Revertants/plate Rat	Hamster
20% EtOH	0	12.3	16.3
	1	14.0	15.6
	2	16.0	11.0
	3	12.3	12.0
styrene 500 µg/plate	0	8.3	9.7
	1	9.3	10.7
	2	15.0	12.7
	3	15.7	10.0
DMSO	0	10.7	12.0
	1	5.7	6.0
	2	6.7	5.0
	3	7.7	7.3
β-naphthylamine 2 µg/plate	0	13.3	11.3
	1	226.3	137.3
	2	284.7	75.7
	3	168.0	56.3

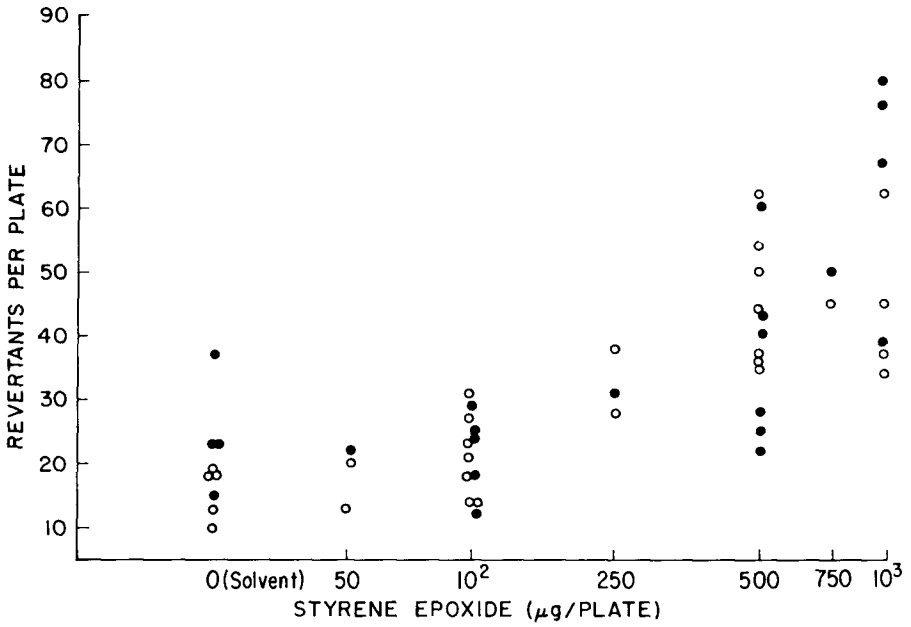

FIGURE 1. Induction of reverse mutations in S. typhimurium TA1535 by styrene epoxide in the plate incorporation assay with (o) and without (●) fortified Arochlor 1254-induced rat liver homogenates.

Discussion

The lack of a demonstrated mutagenic activity of styrene may be explained on the basis of poor conversion to the epoxide by the liver metabolizing system and/or rapid detoxification of styrene epoxide by metabolism to the glycol by epoxide hydrase and conjugation with glutathione catalyzed by glutathione-S-epoxide transferase. Reports that a) the rate of conversion of styrene to the glycol is low (LEIBMAN and ORTIZ, 1970), b) the affinity of the epoxide for the hydrase is about twice that of styrene for the monoxygenase (BELVEDERE et al., 1976), and c) glutathione-S-epoxide transferase may play a major role in detoxification of styrene epoxide (JAMES et al., 1976) suggest both poor conversion and efficient removal of the epoxide by liver enzymes.

Our results confirm and extend recent observations on the mutagenicity of styrene and styrene epoxide. MILVY and GARRO (1976) determined the

mutagencity of styrene and eight metabolites, including the epoxide, in five strains of S. typhimurium without metabolic activation. Only the epoxide was active, reverting strains TA1535 and TA100.

It is impossible to estimate the potential cancer threat of styrene on the basis of the mutagenic activity of styrene epoxide in the absence of more specific and quantitative data on human metabolism of styrene and of a quantitative relationship between mutagenic potency and carcinogencity.

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