

Metabolism and mutagenicity of styrene

by TADASHI WATABE,¹ MASAKAZU ISOBE,¹ TADASHI SAWAHATA,¹ KUNIE YOSHIKAWA,² SHINGO YAMADA³ and EIGO TAKABATAKE³

WATABE, T., ISOBE, M., SAWAHATA, T., YOSHIKAWA, K., YAMADA, S. and TAKABATAKE, E. Metabolism and mutagenicity of styrene. *Scand. j. work environ. & health* 4 (1978): suppl. 2, 142—155. Distilled styrene showed mutagenic activity towards *Salmonella typhimurium* TA 100 after activation with hepatic 9,000 × g supernatant fractions (S-9). The S-9 fractions had been obtained from rats treated with 3-methylcholanthrene or phenobarbital and fortified with an NADPH-generating system in the presence of 3,3,3-trichloropropene oxide (TCPO), an hepatic microsomal epoxide hydratase inhibitor. No mutagenic activity was observed when either the TCPO was omitted or rats were treated with a polychlorinated biphenyl mixture (PCBs). The S-9 fraction from 3-methylcholanthrene-treated rats activated styrene more effectively than the liver homogenate from phenobarbital-treated animals. As has already been pointed out, phenyloxirane, an intermediate in the hepatic microsomal biotransformation of styrene, induced the mutagenesis of TA 100 cells in the absence of S-9 and TCPO, but the mutagenesis was totally abolished by the addition of S-9. A further addition of TCPO partially restored the mutagenetic activity of phenyloxirane. Gas chromatographic data on phenyloxirane and 1-phenyl-1,2-ethanediol in the testing system for styrene indicated that phenyloxirane could not account for the mutagenesis induced by the hepatic activation of styrene since sums of phenyloxirane and its hydrolytic product, 1-phenyl-1,2-ethanediol (formed from styrene during incubation with S-9 in the presence and absence of TCPO) were much smaller than the amount which induced mutagenesis; they also showed that PCBs were the most potent inducers for hepatic monooxygenase, which catalyzes the conversion of styrene to phenyloxirane. PCBs induced hepatic monooxygenase approximately 1.4 times as effectively as 3-methylcholanthrene, in spite of its inability to increase the hepatic activation system for the mutagenicity of styrene. There is therefore a possibility that at least one more unknown active metabolite with an epoxide structure is present. The arene oxide 1-vinylbenzene 3,4-oxide, which was synthesized in our laboratory and was assumed to be a precursor to 4-vinylphenol (a previously isolated urinary metabolite of styrene), had a potent killing effect but no mutagenic activity towards TA 100 cells. A search for the ultimate mutagen is now in progress in our laboratory. ¹⁴C-styrene bound covalently to microsomal protein of rat liver when incubated in the presence of an NADPH-generating system. The absence of the cofactor decreased the binding remarkably. The NADPH-dependent protein-binding was increased by TCPO in vitro and by the phenobarbital pretreatment of rats in vivo. Pretreatment of rats with carbon tetrachloride decreased the amount of radioactivity bound to hepatic microsomal protein.

Key words: epoxide, epoxide hydratase, liver S-9, monooxygenase, mutagenesis, 1-phenyl-1,2-ethanediol, phenyloxirane, *Salmonella typhimurium*, styrene, 3,3,3-trichloropropene oxide.

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During the past few decades, it has become increasingly evident that chemically inert foreign compounds can be converted in the body to chemically reactive metabolites that combine with tissue macromolecules through covalent bonding and thereby cause various adverse biological effects, including carcinogenesis and mu-

tagenesis (37). The recent development of a rapid and simple assay, in which mammalian metabolism is taken into account (1, 19, 21), has raised the question of the validity of data from such tests for the toxicologic evaluation of compounds in terms of human risk (3, 21, 37), as they offer a promising tool with which to detect, remove and prevent carcinogenic chemical hazards to man since it is recognized that environmental factors play an essential role in the etiology of human cancer (22, 26).

Styrene has been known to produce various oxygenated urinary metabolites (1-phenyl-1,2-ethanediol, mandelic acid, phenylglyoxylic acid, hippuric acid, and 1-phenyl-2-hydroxyethylmercapturic acid) when inhaled by animals (7, 8, 14) and man (5, 11, 12, 28). Among them, mandelic, phenylglyoxylic, and hippuric acids are with no doubt further metabolites of 1-phenyl-1,2-ethanediol since they have been demonstrated to be excreted in urine when 1-phenyl-1,2-ethanediol was administered to rabbits (13). In addition mercapturic acid is a possible metabolite of phenylloxirane, a hypothetical intermediate to 1-phenyl-1,2-ethanediol *in vivo*. The epoxide phenylloxirane, which is assumed to be a possible key intermediate to all the urinary phenyl metabolites of styrene, has been tentatively detected, together with the hydrolytic product 1-phenyl-1,2-ethanediol, by Leibman and Ortiz (15) in a hepatic microsomal reaction mixture con-

taining styrene as the substrate. This phenomenon was confirmed by Watabe et al. (36) by means of gas chromatography-mass spectrometry. Oesch et al. (24) also obtained evidence for the enzymatic hydrolysis of phenylloxirane to 1-phenyl-1,2-ethanediol in hepatic microsomes. And very recently Ryan and Bend (29) demonstrated the formation of the glutathione conjugate 1-phenyl-2-hydroxyethyl-S-glutathione (which could be a precursor to the urinary mercapturic acid) from the epoxide and glutathione in hepatic soluble supernatant and concluded that all of the already isolated phenyl-substituted metabolites arise from the epoxide in animal body (fig. 1). Another interesting metabolite, 4-vinylphenol (which could be a spontaneous rearrangement product of 1-vinylbenzene 3,4-oxide formed by the catalytic action of a microsomal monooxygenase on styrene) has been isolated from rat urine (4).

The toxicity of styrene has been approached in many ways with respect to health control against industrial poisoning (14). The finding of the intermediacy of phenylloxirane in the major metabolism of styrene in animal body and the recently accumulated information about the role of metabolically formed epoxides as cause materials in chemical carcinogenesis (38) have raised the question of whether or not the hydrocarbon is carcinogenic or mutagenic, as has been seen in the case of carcinogenic chlorinated ethylenes,

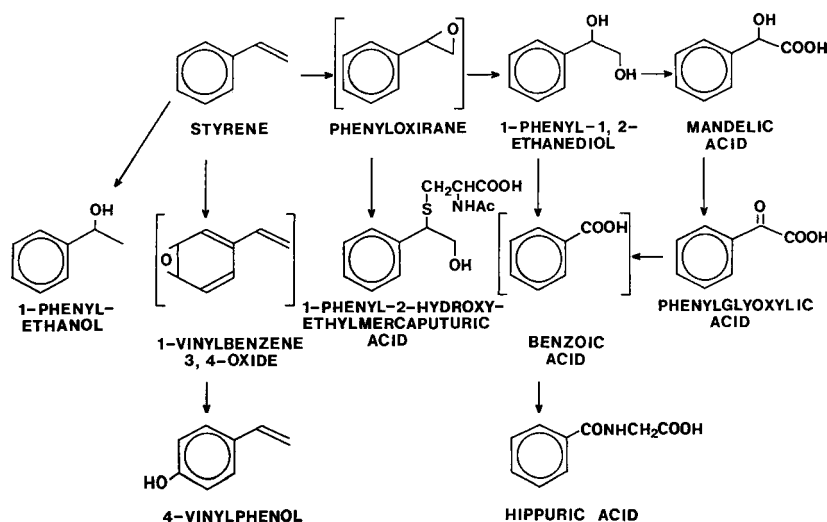


Fig. 1. Metabolism of styrene *in vivo*.

including vinyl chloride (17), and carcinogenic polycyclic aromatic hydrocarbons (9), all of which afford highly reactive epoxides with cellular macromolecules that cause malignant transformations of cells in culture. In this connection, phenyloxirane has been suggested to be a skin carcinogen in mice (33) and also a mutagen in the *Salmonella typhimurium* strains used in the Ames testing system (6, 20, 31, 32). Moreover, styrene itself has been shown to be a mutagen in a host-mediated system involving mice and yeast (16). Covalent binding of phenyloxirane to rat liver protein has been demonstrated in vivo and in vitro (18). However, Parkki and Marniemi (27) have claimed that the covalent binding of radioactive styrene to liver protein does not require metabolism.

The present paper deals with the mutagenic activity of styrene towards *S. typhimurium* in the presence of $9,000 \times g$ supernatant fractions (S-9) obtained from rats pretreated with various inducers, of an NADPH-generating⁴ system and of 3,3,3-trichloropropene oxide (TCPO), as the microsomal epoxide hydratase inhibitor and also with an inherent mutagenic activity of phenyloxirane to the bacteria. Evidence has also been presented for the NADPH-dependent covalent binding of styrene to rat liver microsomal protein.

MATERIALS AND METHODS

Materials

Styrene and phenyloxirane were purchased from the Wako Pure Chemical Co., Tokyo, purified by distillation at a boiling point of 48°C (20 mm Hg) and 85°C (15 mm Hg), respectively, and stored at -5°C. The purified samples were used within two weeks in the biological experiments. 3-Methylcholanthrene and glutathione were also purchased from the Wako Pure Chemical Co.; and sodium phenobarbiturate

from the Sanko Pharmaceutical Co., Tokyo, Japan; 3,3,3-trichloropropene oxide (TCPO) from the Aldrich Chemical Co., Milwaukee, Wis., U.S.A.; NADPH and glucose 6-phosphate from the Oriental Yeast Co., Tokyo, Japan. A polychlorinated biphenyl mixture KC-500 (PCBs) was the product of the Kanegafuchi Chemical Co., Osaka, Japan. Ring-¹⁴C-styrene was obtained from the New England Nuclear, Boston, Mass., U.S.A. All other reagents used were reagent grades.

Preparation of liver $9,000 \times g$ supernatant fractions from rats for mutagenicity assay

Young adult male Wistar rats (90–110 g) were used, and treatments with monooxygenase inducers were carried out as follows: rats were injected intraperitoneally once with 3-methylcholanthrene dissolved in olive oil (dose: 50 mg/kg) 2 d before the sacrifice or once daily with phenobarbital dissolved in saline (single dose: 80 mg/kg) for 2 d before the sacrifice or once with PCBs diluted with olive oil (dose: 500 mg/kg) 5 d before the sacrifice. The liver $9,000 \times g$ supernatant fraction (S-9) was prepared according to the method of Ames et al. (2) and stored at -80°C. The preparations were used within two weeks.

Mutagenicity assay

Salmonella typhimurium strains TA 100, TA 1535, TA 98, TA 1537, and TA 1538 were kindly provided by Dr. B. N. Ames.

The mutagenicity test was carried out in triplicate in airtight petri dishes according to the method of Ames et al. (2). Styrene and phenyloxirane dissolved in dimethylsulfoxide were added to the bacterial overnight culture, and the mixture was incubated with and without the liver $9,000 \times g$ supernatant fraction fortified with an NADPH-generating system (S-9 mix) for 20 min at 37°C prior to the addition of the top agar.

Mutation frequency was assayed by the some modified method with *S. typhimurium* TA 100. The S-9 mix (0.15 ml), containing 16 μ mol of magnesium chloride, was preincubated in the presence (when required) of 1 mM TCPO at 37°C for

⁴ NADPH: reduced nicotinamide adenine dinucleotide phosphate.

20 min prior to the addition of 0.2 M phosphate buffer (pH 7.4, 0.2 ml, containing 2.3 μ mol of NADPH and glucose 6-phosphate), the bacterial overnight culture (0.15 ml, containing about 1.7×10^9 cells/ml), and the proper amount of the dimethylsulfoxide solution of tested chemicals (less than 0.05 ml). After the mixture had been incubated for 20 min at 37°C with shaking, a 0.1-ml aliquot was removed and assayed for the bacterial survival as follows. The mixture (diluted appropriately with 1/30 M phosphate buffer, pH 6.8) was placed on a Vogel-Bonner E medium-agar plate supplemented with 160 μ g/ml of the nutrient broth and 100 μ g/ml of casein hydrolyzate. After the incubation of the plate for 48 h at 37°C, the numbers of viable colonies were counted. The remaining 0.4-ml aliquot was centrifuged at $6,000 \times g$, 5°C for 10 min and the precipitate, the bacterial cells free from chemicals, was suspended well in 2 ml of 0.45 % soft agar containing 1 μ mol of L-histidine and D-biotin. The suspension was poured on a Vogel-Bonner E medium-agar plate and the His⁺ revertants were scored after incubation for 48 h at 37°C. We obtained the value of the mutation frequency by dividing the number of induced mutants per milliliter by the number of surviving cells per milliliter.

Assay of phenyloxirane and 1-phenyl-1,2-ethanediol

Phenyloxirane and 1-phenyl-1,2-ethanediol, formed from styrene (4 mM) during the incubation with S-9 mix under the same conditions as the mutation frequency assay system without bacteria, were assayed by gas chromatography according to the method described in a previous paper (36).

Assay of epoxide hydratase activity

The activity of epoxide hydratase in rat liver S-9 was assayed photometrically by the method of Watabe and Akamatsu (35); safrole oxide was used as the substrate.

Assay of protein binding

Wistar rats and ddY mice were used. Their livers were removed after perfusion with physiological saline. Microsomes were isolated by the usual centrifugation method from a 25 % liver homogenate in 1.15 % KCl-0.05 M Tris-HCl buffer (KCl-Tris), pH 7.4, and suspended in KCl-Tris at a protein concentration of 8 mg/ml. Radioactive styrene was diluted with distilled unlabeled styrene and dissolved in acetone to make a solution of 0.1 μ Ci/1.5 μ mol/20 μ l. The radioactive substrate solution was added to a mixture of 0.5 ml of the microsomal suspension, 0.3 ml of an NADPH-generating system consisting of 1.5 μ mol of NADP, 15 μ mol each of magnesium chloride and glucose 6-phosphate, 2 I.U. of glucose 6-phosphate dehydrogenase, and 1.2 ml of KCl-Tris. After the preincubation of the mixture for 5 min at 37°C the substrate solution (20 μ l) containing TCPO was added, if necessary, and incubation was continued for an additional 30 min. The NADPH-generating system was replaced by water when incubations were carried out in the absence of NADPH. The biological reaction was terminated by the addition of 6 ml of ethanol, and the precipitate was filtered through a membrane filter (Whatman 1 μ m). The precipitate collected on the filter was washed successively with 3 ml each of 70 % ethanol (once), KCl-Tris (twice), acetone-chloroform (4:1, twice), methanol-ether (1:1, once), and ether alone (once). After drying, it was put in a vial containing 10 ml of a dioxane scintillator, consisting of 0.4 g of POPOP, 4 g of DPO and 100 g of naphthalene in 1 l of dioxane. The radioactivity was measured on an Aloka Model 653R liquid scintillation counter with an external standard.

RESULTS

Mutagenic activity of styrene and phenyloxirane in the Ames testing system

Styrene, previously reported to be non-mutagenic (20, 31), was examined for

Table 1. Mutagenicity test of styrene and phenyloxirane with *Salmonella typhimurium* strains.

Chemical ($\mu\text{g}/\text{plate}$)	Number of His ⁺ revertant colonies/plate in tested strains				
	TA 100	TA 1535	TA 98	TA 1537	TA 1538
Styrene ^a					
0	92	26	37	23	32
10	87	25	41	47	37
50	91	29	34	58	29
100	90	23	40	42	31
500	91	14	24	4	20
Phenyloxirane ^b					
0	86	26	43	10	47
10	84	34	27	14	41
50	92	39	23	23	42
100	130	115	32	36	26
500	268	195	55	22	32

Assay was carried out (a) with S-9 mix prepared from rats pretreated with polychlorinated biphenyls and (b) without S-9 mix.

mutagenicity towards *S. typhimurium* TA 100, TA 1535, TA 98, TA 1537, and TA 1538 by the method of Ames et al. (2) after preincubation with S-9 mix, which consisted of (a) the 9,000 $\times$ g supernatant fraction of the liver of rats pretreated with PCBs and (b) an NADPH-generating system. After the incubation of agar plates for 48 h, His⁺ revertant colonies were counted. No appreciable change was observed in the number of His⁺ revertant colonies of all the *S. typhimurium* TA strains examined on the agar plates containing styrene at the dose range of up to 500 $\mu\text{g}/\text{plate}$ (table 1). The mutagenicity test of phenyloxirane, an inherent mutagen and primary metabolite of styrene in rat liver S-9 mix, was also carried out by the method of Ames et al. (2) in the absence of S-9 mix. It showed selective mutagenesis towards strains TA 100 and TA 1535 over the dose of 100 $\mu\text{g}/\text{plate}$ but not towards strains TA 98 and TA 1538 even at a dose of 500 $\mu\text{g}/\text{plate}$, and therefore it is indicated it to be a base-pair change type of mutagen (table 1). However, the mutagenicity of phenyloxirane was considerably weak when compared with that of benzo[a]pyrene 4,5-oxide, a well-known potent mutagen (38). (With the TA 100 strain as the testing bacteria,

575 induced colonies at 10 $\mu\text{g}/\text{plate}$ were found for the latter and 182 colonies at 500 $\mu\text{g}/\text{plate}$ for the former.)

Mutagenic activity of styrene towards Salmonella typhimurium TA 100 by its metabolic activation with rat liver S-9 mix

In order to confirm whether or not styrene has a mutagenic activity towards bacteria by metabolic activation, we preincubated it with S-9 mixes prepared from the liver of rats pretreated with various inducers in the presence of TCPO, a potent epoxide hydratase inhibitor (25) which has been demonstrated to prolong the biological half-life of phenyloxirane formed from styrene in vitro (36). For the mutation assay *S. typhimurium* TA 100, the most sensitive tester strain to phenyloxirane as shown in table 1, and the mutation frequency method were used so that it might permit a more accurate determination of bacterial mutation. Two dosages of styrene, 3 and 6 mM, were preincubated with the bacteria in the presence of TCPO and S-9 mixes from rats untreated and pretreated with PCBs, phenobarbital, and 3-methylcholanthrene. Higher con-

centrations of the hydrocarbon had a more potent killing effect on the bacteria and resulted in a significant decrease in the survival percentage, especially when liver

S-9 mixes from rats pretreated with phenobarbital and 3-methylcholanthrene were used for the metabolic activation. Only a slight difference was observed in the

Table 2. Mutagenicity of styrene towards *Salmonella typhimurium* TA 100 by its metabolic activation with S-9 mix prepared from liver of rats pretreated with various inducers in the presence of trichloropropene oxide (TCPO).^a

Inducer	Styrene (mM)	Survival ^b (%)	Revertant colonies (per plate)	Induced mutation frequency ^c
None	0	100	85	—
	3	88	84	—
	6	14	70	—
Polychlorinated biphenyls	0	100	92	—
	3	100	79	—
	6	23	71	—
Phenobarbital	0	100	86	—
	3	67	87	2
	6	9	91	83
3-Methylcholanthrene	0	100	81	—
	3	68	100	40
	6	6	102	477

^a The liver S-9 fractions prepared from rats pretreated with various inducers were preincubated with TCPO for 20 min at 37°C prior to 20 min of incubation with the bacteria and styrene in the presence of an NADPH-generating system. The final concentration of TCPO was 0.3 mM.

^b The value, 100 %, represents 1.7×10^9 of surviving bacterial cells per milliliter of incubation medium.

^c Values are ratios of number of induced mutants to 10^8 of surviving bacterial cells. For instance, the induced mutation frequency 40 at 3 mM styrene in 3-methylcholanthrene pretreated rat liver S-9 mix was obtained from the following calculation: $(100-81)/0.4/0.38 \times 1.7 \times 10^9$.

Table 3. Mutagenicity of styrene and phenyloxirane towards *Salmonella typhimurium* TA 100 in the presence and absence of S-9 mix.

Chemical (4 mM)	Addition ^a		Survival (%)	Revertant colonies (per plate)	Induced mutation frequency
	S-9 mix	Trichloropropene oxide			
None	—	—	100	73	—
	+	—	100	80	—
	+	+	100	86	—
Styrene	—	—	5	66	—
	+	—	34	79	—
	+	+	30	105	91
Phenyloxirane	—	—	51	156	235
	+	—	87	90	17
	+	+	84	118	54

^a The S-9 prepared from liver of rats pretreated with 3-methylcholanthrene was incubated with trichloropropene, when added, for 20 min at 37°C prior to the addition of chemicals, cofactors, and bacteria. Other conditions for the assay were the same as described in table 2.

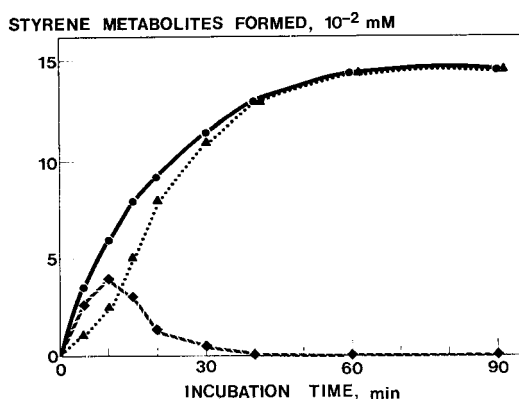


Fig. 2. A time course study of styrene metabolism by rat liver microsomes in the presence of an NADPH-generating system. Styrene (1 mM) was incubated aerobically with the microsomes (5.6 mg protein/ml) in airtight flasks (36). (■ : phenyloxirane; ▲ : 1-phenyl-1,2-ethanediol; ● : phenyloxirane + 1-phenyl-1,2-ethanediol)

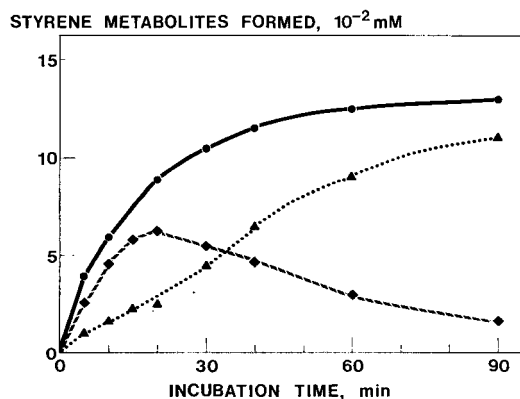


Fig. 3. A time course study of styrene metabolism by rat liver microsomes in the presence of an NADPH-generating system and 3,3,3-trichloropropene oxide as the epoxide hydratase inhibitor. Styrene was incubated aerobically with the microsomes (5.6 mg protein/ml) in airtight flasks (36) in the presence of the inhibitor (1 mM). (■ : phenyloxirane; ▲ : 1-phenyl-1,2-ethanediol; ● : phenyloxirane + 1-phenyl-1,2-ethanediol)

number of revertant colonies which appeared on the agar plates, while the pretreatment of the rat with 3-methylcholanthrene or phenobarbital increased the His⁺ revertant colonies obviously to a small extent. Therefore, the mutation

frequencies calculated were apparently positive when the S-9 mix was from the liver of animals pretreated with either 3-methylcholanthrene or phenobarbital but negative when the liver was from untreated animals or those pretreated with PCBs. 3-Methylcholanthrene was the most effective inducer for the hepatic activation of styrene.

The mutagenicity of styrene towards *S. typhimurium* TA 100 was compared with that of phenyloxirane in the presence and absence of S-9 mix prepared from the livers of rats pretreated with 3-methylcholanthrene in connection with the effect of the hepatic epoxide hydratase inhibitor TCPO. The mutagenic activity of styrene, which was exerted in the presence of both S-9 mix and TCPO, disappeared when either of them were omitted from the preincubation media; this result strongly suggests that styrene was biologically transformed to certain active metabolites, including phenyloxirane. The mutagenic activity of phenyloxirane was remarkably decreased by S-9 mix, and the decrease was partially inhibited by TCPO. The bacterial mutation caused by styrene in the metabolic activation system including TCPO was almost twice as high as that caused by phenyloxirane when examined under the same conditions. It is apparent from the data on survival that styrene has a more potent killing effect on bacteria than phenyloxirane has. However the effect was diminished by the addition of S-9 mix in the presence or absence of TCPO.

Biotransformation of styrene to phenyloxirane and 1-phenyl-1,2-ethanediol in the mutation assay system

Phenyloxirane formed from styrene by liver microsomal monooxygenase was rapidly inactivated to 1-phenyl-1,2-ethanediol by microsomal epoxide hydratase as shown in fig. 2. The briefness of the biological half-life of the epoxide in the reaction medium was found to be due to decreasing ratios of monooxygenase to epoxide hydratase activities during aerobic incubations of the microsomes in the presence of NADPH. The hydratase activity was still unchanged even 60 min

after incubation. But the activity of monooxygenase was rapidly decreased, as previously shown by Watabe and Akamatsu (34), who have attributed this phenomenon to the loss of monooxygenase activities, caused by microsomal lipid peroxidation. However, the addition of the hydratase inhibitor TCPO to the microsomal reaction medium prolonged the biological half-life of the epoxide (fig. 3).

In order to elucidate whether the mutagenicity of styrene can be ascribed to the biologically formed phenyloxirane, we incubated styrene with S-9 mixes from rat liver in the absence of *S. typhimurium* but in the presence of TCPO under the same conditions as were used for the mutation frequency assay. The phenyloxirane and 1-phenyl-1,2-ethanediol formed were assayed by gas chromatography as previously reported (36). Under the incubation conditions used, the absence of the microsomal epoxide hydratase inhibitor TCPO led to the disappearance of the intermediate phenyloxirane at the end of the 20-min incubation and only to the formation of a larger amount of the diol, which corresponded to the sum of the two metabolites formed in the presence of TCPO. The S-9 mix from the rat liver pretreated with PCBs yielded an abundant amount of the oxygenated metabolites. Pretreatment with 3-methylcholanthrene and phenobarbital also increased the formation of both metabolites approximately

to a level three times higher than that of the control but to a less extent than the PCBs pretreatment (table 4). The inhibition of the hydratase by TCPO caused the highest accumulation of phenyloxirane in the incubation media in the liver preparations from the 3-methylcholanthrene pretreated animals and the lowest such accumulation in animals pretreated with PCBs. The hepatic hydratase activity was induced maximally by pretreatment with PCBs and minimally by 3-methylcholanthrene. The relative ratio of the hepatic monooxygenase activity for the oxidation of the vinyl group of styrene, which is represented by the sum of the phenyloxirane and 1-phenyl-1,2-ethanediol formed, to the activity of epoxide hydratase was almost the same for PCBs as for the control and was the least among the inducers. The pretreatment with 3-methylcholanthrene showed the highest monooxygenase to hydratase ratio, and this ratio strongly suggested why 3-methylcholanthrene pretreatment induced the mutation in *S. typhimurium* TA 100 by styrene the most effectively (table 2). However, the sum of phenyloxirane and 1-phenyl-1,2-ethanediol, 0.307 mM (table 4), formed from 4 mM styrene by the S-9 mix prepared from the liver of rats pretreated with 3-methylcholanthrene could not completely account for the mutagenicity of 4 mM styrene, which showed a small but larger magnitude than that exerted by the epoxide used at 4 mM under the same conditions (table 3).

Table 4. Biotransformation of styrene to phenyloxirane (PO) and 1-phenyl-1,2-ethanediol (PE) by S-9 mixes prepared from liver of rats pretreated with various inducers.^a

Inducer	Metabolites formed			Relative EHase activity ^b	Relative ratio of monooxygenase to EHase activities
	PO (mM)	PE (mM)	PO + PE (mM) (relative ratio)		
None	0.026	0.093	0.119 (1.0)	1.0	1.0
Polychlorinated biphenyls	0.020	0.413	0.433 (3.6)	3.1	1.2
Phenobarbital	0.036	0.304	0.340 (2.9)	1.6	1.8
3-Methylcholanthrene	0.053	0.254	0.307 (2.6)	1.3	2.0

^a Styrene (4 mM) was incubated with S-9 mixes containing the epoxide hydratase inhibitor, trichloropropene, in the absence of bacteria under the same condition as described in table 2.

^b The activity of epoxide hydratase (EHase) was shown as the relative ratio to that of untreated S-9 (3.9 μ mol of safrole oxide hydrolyzed per minute per milliliter of S-9).