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Metabolism and Toxicity of Styrene

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The absorption, blood levels, distribution, excretion, and biotransformation of styrene in man and experimental animals are briefly reviewed. The acute toxicity of styrene appears to be unrelated to its biotransformation. Reports of organ toxicity upon chronic exposure to styrene are rare; however, since the chief intermediate in styrene metabolism is an epoxide, hepatotoxicity due to covalent binding at the site of formation appears to be a possibility.

Styrene (phenylethylene, vinylbenzene) is an important intermediate in chemical synthesis and monomer for plastics manufacture. It is a colorless, refractive, oily liquid of density $d_4^{20} = 0.909$. Its melting and boiling points are —33 and 146°C, respectively. It is sparingly soluble in water, but soluble in most organic solvents. Its vapor has a characteristic, penetrating odor, the perception of which diminishes as exposure is continued (*1*).

Styrene may be absorbed into the bloodstream by all routes: on peroral administration or inhalation, by percutaneous absorption, or after subcutaneous or intraperitoneal administration. The most common routes of absorption in industrial exposure are pulmonary and percutaneous. The ACGIH recommended threshold limit value (TLV) is 100 ppm (420 mg/m³ of air).

Blood levels of styrene that have been reported (*1*) in man after exposure for varying periods to air containing different concentrations of styrene are shown in Table 1. Exposures were continuous for the indicated times, except for that of 410-min duration, which was scheduled to simulate exposure for a full working day to the TLV concentration; subjects were exposed for 3.5 hr, given a 30-min lunch period, and again exposed for 3.5 hr, and blood samples were drawn 10 min before the end of the second period.

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As would be expected from its high lipid solubility, the rate of percutaneous absorption of styrene is high (*2*). The absorption rate of liquid styrene through the skin of the hand is 9–15 mg/cm²-hr. That for aqueous solutions (66–269 mg/l.) is 40–180 μg/cm²-hr; the rate was found to be linear with concentration. A few minutes' exposure of the hands to liquid styrene, or exposure to a saturated aqueous solution for about 1 hr, may result in the absorption of as much styrene as does breathing air containing 50 mg/m³ for 8 hr.

Concentrations of styrene found in rodent organs after exposure of the animals to the LC_{50} (*3*) are shown in Table 2. There appears to be a fairly uniform distribution of styrene among the aqueous compartments of the body, as would be expected from the high lipid solubility of the compound; the latter property also leads to extensive sequestration of styrene in the fat.

After subcutaneous injection of [β-¹⁴C] styrene in oil (*4*), the blood was essentially cleared of radioactivity within 24 hr (Table 3). By that time, about 85% of the radioactivity had been excreted. Most of the radioactivity was

Table 1. Human blood concentrations of styrene.

Exposure, ppm	Time, min	Blood concentration, mg/l.
51	55	0.2–0.7
99	410	0.9–1.4
117	55	1.7
117	115	2.7

excreted via the urine, but about 12% of the β-carbon was oxidized to CO_2. There is a considerable excretion of unchanged styrene in the expired air as well. Thus, in the experiment referred to above in which a day's exposure to the TLV concentration was simulated, the concentration of styrene in the expired air was about 2.5 ppm shortly after cessation of exposure and was still 0.3 ppm 6 hr later (*1*).

The metabolites of styrene found in the urine of rats, rabbits, and man are shown in Table 4. A major metabolite in rodents is hippuric acid; in man, however, the level of urinary hippuric acid after exposure to styrene is generally in the normal range of excretion of this compound, which is quite variable (*1,12,13*). Mandelic and phenylglyoxylic acids are prominent metabolites; these are the major metabolites that have been demonstrated in man, and they have been proposed as indices of exposure to styrene (*12,13*). Although the ratio of mandelic to phenylglyoxylic acid excreted after experimental human exposure to styrene was reported by Bardodej and Bardodejova (*7*) to be 8.5, the concentration ratio in urine samples taken at 3 P.M. from factory workers exposed to 10–13 ppm during the day was found by Ohtsuji and Ikeda to be about 2 (*12*). The glucuronide excreted by rats was shown to be that of phenylethylene glycol (*8*).

The half-lives of urinary excretion of mandelic and phenylglyoxylic acids in man after exposure to 50–200 ppm of styrene for 160 min were 7.8 and 8.5 hr, respectively (*13*). In contrast, the half-life of pulmonary excretion of unchanged styrene after the exposures shown in Table 1 varied from 1 to 5 hr (*1*).

The production of mandelic, phenylglyoxylic, and hippuric acids and of glucuronide conjugates has been shown to be increased in rats after pretreatment with phenobarbital (*5,14*), and to be inhibited by coadministration of SKF 525–A, an inhibitor of microsomal drug-metabolizing enzymes (*5*). Coadministration of toluene, which is known to be oxidized by the hepatic microsomal enzyme system, also reduced the formation of the three acids (*14*). It would thus appear that a primary step in styrene metabolism is catalyzed by liver microsomal enzymes.

The most probable pathways of mammalian styrene metabolism are shown in Figure 1. The first steps of the chief pathway have been

Table 2. Styrene concentrations in rodent organs after exposure to LC_{50}.

Organ	Rat, mg/100 g[a]	Mouse, mg/100 g[b]
Brain	25.0	18.0
Liver	20.0	
Kidney	14.7	
Spleen	19.1	
Perirenal fat	133	

[a] LC_{50}: 11.8 mg/l., 4-hr exposure.
[b] LC_{50}: 21.0 mg/l., 2-hr exposure.

Table 3. Disposition of radioactivity after subcutaneous injection of [β—^{14}C] styrene in rats.

	1 hr	6 hr	24 hr
Radioactivity in organs, % of dose			
Site of injection	57.2	28.0	2.1
Blood	1.0	0.8	0.02
Liver	4.6	1.0	0.1
Kidneys	1.8	0.5	0.01
Other[a]	2.7	0.9	0.2
Radioactivity in excreta, cumulative % of dose			
Urine	4.0	37.2	71.0
Feces	0.0	0.4	2.6
Expired CO_2	0.0	6.0	11.8

[a] Pancreas, lungs, stomach, intestine, heart, spleen, adrenals, bone, brain, thymus.

Table 4. Urinary metabolites of styrene.

Metabolite	Rat, % of dose	Rabbit, % of dose	Man, % of retained dose
Mandelic acid	9[a]	32[b]	85[c]
Phenylglyoxylic acid	11[a]		10[c]
Hippuric acid	10[a]	40[d]	+
Glucuronide	8[a]	6[d]	
Sulfur compounds			
"Neutral" sulfur	+[e]	+[e]	+[f]
Hydroxymercapturic acid	9[g]	5[b]	
4-Vinylphenol	0.1[h]		
1-Phenylethanol	+[h]		
2-Phenylethanol	Trace[h]		

[a] Dose, 4.4 mmole/kg IP; 10-hr collection (*5*).
[b] Dose, 1.4 mmole/kg PO; 24-hr collection (*6*).
[c] Inhalation exposure to 22 ppm for 8 hr; collection period unspecified (*7*).
[d] Dose, 5 mmole/kg PO; 24-hr collection (*8*).
[e] Repeated doses PO or by inhalation (*9*).
[f] Inhalation exposure to 800 ppm for 4 hr; 24-hr collection (*10*).
[g] Dose, 2.0 mmole/kg PO; 24-hr collection (*6*).
[h] Dose, 1.0 mmole/kg PO; 48-hr collection (*11*).

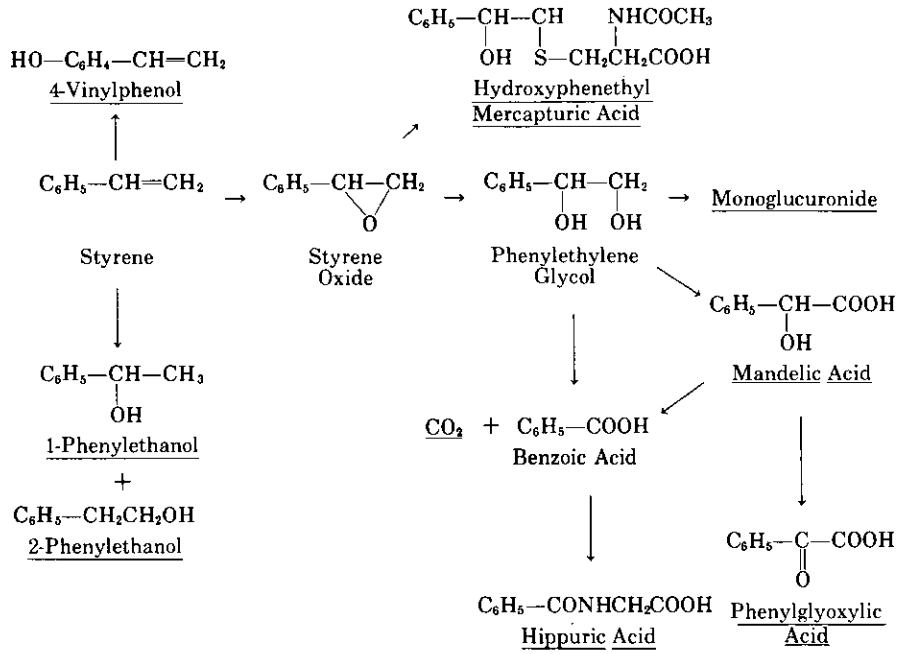

FIGURE 1. Pathways of biotransformation of styrene. Underlined metabolites are those that are excreted in urine (or in expired air in the case of CO_2).

demonstrated by Leibman and Ortiz (*15–17*) to occur *in vitro* in rat and rabbit liver microsomal preparations. The major product detected after incubation of styrene with liver microsomes in the presence of an NADPH-generating system was phenylethylene glycol (*15*). After short incubation periods, the production of small amounts of styrene oxide could be demonstrated (*16*); epoxide levels rapidly fell thereafter, as the hydration of the oxide, mediated by microsomal epoxide hydrase (*17*), proceeded. However, the incorporation of substantial radioactivity from [β-^{14}C]styrene into a pool of unlabeled styrene oxide was demonstrated (*16*).

The conversion of both styrene and styrene oxide to hydroxyphenethylmercapturic acid has been shown to occur *in vivo* in rats and rabbits by James and White (*6*). The first step in this process, conjugation of the epoxide with glutathione, has been demonstrated *in vitro* in rat liver cytosol (*18*) and in a purified enzyme preparation (*19*).

Glucuronide and mandelic, phenylglyoxylic, and hippuric acids have all been found to be excreted after administration of styrene, styrene oxide, or phenylethylene glycol to rats and rabbits (*5,6,8*), which demonstrates that the epoxide-diol pathway is intermediate in the production of the major metabolites of styrene. Hippuric acid is excreted after administration of mandelic, but not of phenylglyoxylic acid, while mandelic acid gives rise to phenylglyoxylic acid, but the reverse apparently does not occur (*5*). Since more hippuric acid is produced *in vivo* from phenylethylene glycol than from mandelic acid (*5*), it would appear that two pathways exist for formation of this compound.

Minor metabolic pathways of styrene in rats include ring hydroxylation and formation of 1- and 2-phenylethanol (*8*). Whether the production of the latter metabolites involves styrene oxide is not known.

The urinary metabolites of styrene all have a lower order of toxicity than does styrene itself, and therefore do not contribute to its acute toxicity. The LD_{50} of styrene in rats is about 2.5 g/kg after intraperitoneal injection (*5*) and about 5 g/kg after oral administration (*20*). The acute toxicity of styrene oxide is about four times that of styrene (*5*).

The acute toxicity of styrene appears to be unrelated to its biotransformation, but is similar to that of other hydrocarbons. The most common toxic actions after exposure to styrene vapors or liquid are irritation of the skin, eyes, and respiratory tract, and depression of the central nervous system. At or below the

TLV, there are no subjective symptoms or objective signs in man on short exposure. With continued exposure, mild eye and throat irritation develops, and there is slight impairment of coordination and balance. At higher air concentrations, nasal, eye, throat, and skin irritation becomes more pronounced. There is a decrease in coordination, balance, and manual dexterity. Nausea, headache, fatigue, and a feeling of drunkenness have been reported.

As with other hydrocarbons, chemical pneumonitis is a great hazard if aspiration occurs after ingestion of styrene.

Organ toxicity has been reported for styrene, but appears to be rare. In one experiment (*9*), rats, guinea pigs, rabbits, and monkeys were exposed to 1300 ppm of styrene for 7–8 hr/day, 5 days/week for 6 months. The only deaths due to styrene occurred among the guinea pigs, 10% of which developed acute lung inflammation, edema and hemorrhage. All surviving animals had normal weight gains, and at the end of the experimental period, no abnormal microscopic tissue changes or alterations in the blood picture could be found. After oral administration of 667 mg/kg-day, 5 days/week for 6 months, however, depression of growth was noted, together with a moderate increase in liver weight and a slight increase in kidney weight (*20*).

Epoxide intermediates have been implicated in hepatotoxicity (*21*) and carcinogenicity (*22*). Styrene oxide has been found to have little or no carcinogenic potency after long-term skin-painting in mice (*23,24*), but was reported to cause malignant lymphomas in mice after administration by an unspecified route (*25*). Covalent binding of styrene oxide at the site of formation in liver, with resultant hepatotoxicity, analogous to that postulated for bromobenzene epoxide (*26*), appears a distinct possibility, especially under conditions where glutathione has been depleted or the activity of epoxide hydrase has been reduced. Clinical hepatotoxicity has apparently been reported very rarely after styrene exposure. Two reports have appeared in the Russian literature in which liver enlargement and decrease in liver function were found in workers exposed to styrene (*27,28*), but it is likely that these workers were exposed to a number of other chemicals as well as to styrene. It would appear, however, that an investigation of possible covalent binding of styrene metabolites in liver would be a worthwhile project.

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