In 1974, Ideda et al. [159] measured urinary metabolites of styrene in six workers who were exposed to styrene during the production of electric motor parts in a Japanese factory. On the day the workers were studied, they were exposed at 50-200 ppm for two 80-minute periods with a 200-minute nonexposure interval. One week later, five of these same workers and an additional worker were studied. The workers were exposed that day to styrene at 4-60 ppm for 120 minutes. Styrene concentrations in the workplace were determined by colorimetric detector tubes and by gas-liquid chromatography.

Urinary hippuric acid concentrations following the first exposure (50-200 ppm) reached a maximum a few hours after urinary excretion of mandelic and phenylglyoxylic acids had returned to normal. At the lower level of exposure (i.e., 4-60 ppm) a week later, no increase in hippuric acid above control values could be detected. The reason for the delayed hippuric acid excretion was considered by Ikeda et al. [159] to be due to a slow step in the conversion of mandelic and phenylglyoxylic acids to hippuric acid. The half-lives of mandelic and phenylglyoxylic acids, and therefore styrene, were estimated to be about 8 hours [159].

In 1981, Pfaffli et al. [160] verified by gas chromatography/mass spectrometry the presence of 4-vinylphenol in the urine of workers in two reinforced plastics factories where average airborne styrene concentrations were about 130 ppm. The correlation between mandelic acid and 4-vinylphenol was good (r=0.93); increasing excretion of mandelic acid was also accompanied by increasing amounts of 4-vinylphenol in the urine. The presence of 4-vinylphenol was not detected in urine of unexposed individuals. The presence of 4-vinylphenol in the urine of styrene workers suggested to the investigators [160] that styrene was also metabolized via arene oxidation, with styrene-3,4-oxide probably functioning as an intermediate. Styrene-3,4-oxide was found in 1982 by Watabe et al. [161] to have potent mutagenicity and cytogenicity toward Salmonella typhimurium strain TA 100. However, other pathways to 4-vinylphenol are possibly present which do not involve arene oxides. The metabolic pathway via the oxidation of the vinyl group is at least quantitatively the more important route as compared to arene oxidation, since the amount of 4-vinylphenol was only about 0.3% of the amount of mandelic acid.

Analytical methods for determining mandelic acid and relationships between urinary mandelic acid concentrations and TWA exposures of workers to styrene are discussed further in Chapter V and in Appendix II.

EFFECTS ON ANIMALS

Toxicity

Effects of styrene vapor exposures at 1,300-10,000 ppm on rats, guinea pigs, rabbits, and monkeys were reported in 1942 by Spencer et al. [53]. The styrene contained 0.01% 4-tert-butylcatechol. Rats (405) and guinea

pigs (410) were exposed to a series of concentrations during single exposures of various lengths of time. After exposure, the animals were observed for 2-4 weeks, after which some were sacrificed for microscopic study. The responses of animals during exposure to styrene are presented in Table IV-20.

TABLE IV-20

EFFECTS ON RATS AND GUINEA PIGS DURING INHALATION OF STYRENE

Styrene Exposures			Observation During Exposure
ppm	Hours to 100% Mortality		
	Rats	Guinea Pigs	
1,300	>40	40	Rubbing of eyes and nose, violent scratching of face, lacrimation, nasal discharge, salivation, general weakness, unsteadiness after 12-30 hours
2,000	>30	30	As above, but becoming marked after 24-30 hours. Some animals lost consciousness
2,500	21	14	Weakness and stupor followed by incoordination, loss of equilibrium, tremors, unconsciousness after 10-12 hours
5,000	8	8	Weakness, immediate loss of equilibrium, tremors, clonic convulsions, unconsciousness after <1 hour
10,000	3	3	Same as above, with more rapid onset

Taken from Spencer et al. [53]

Local irritation of the eye, nose, and throat and CNS depression manifested by incoordination, loss of equilibrium, tremors, and loss of consciousness were observed during exposure [53]. Findings from examination of the lungs included congestion, frequent hemorrhage, edema, exudation, and varying degrees of leukocytic infiltration. The severity of these effects was greater in guinea pigs than in rats and depended on styrene concentration and duration of exposure. Mild injury to the liver, particularly to the parenchymal cells, and to the kidneys was frequently

observed in rats. Most deaths that occurred during single exposures were attributed to the action of styrene on the central nervous system; delayed deaths were attributed to pneumonia secondary to lung irritation.

The authors [53] concluded that because of the irritating properties of styrene at 1,300 ppm, it was unlikely that humans would tolerate this concentration very long, and that the irritation would serve as an adequate warning of hazardous concentrations.

Guinea pigs, rats, rabbits, and monkeys were exposed to styrene for 7-8 hours a day, 5 days a week for up to 264 exposures by Spencer et al. [53] (see Table IV-21).

TABLE IV-21

EFFECTS OF CHRONIC STYRENE INHALATION ON LABORATORY ANIMALS

Animal		Styrene	No. of	Effects
Species	No.	(ppm)	Exposures	
Rat	28	2,000	105	Marked eye and nose irritation, listlessness, poor weight gain
Guinea pig	12	2,000	98	Marked eye and nose irritation, listlessness, poor weight gain
Rabbit	1	2,000	126	None
Rat	50	1,300	130-139	Eye and nose irritation, increased kidney and liver weight
Guinea pig	94	1,300	130-139	Pronounced lung irritation with congestion, hemorrhage, edema, exudation, and a general acute inflammation; 10% mortality after first few exposures; poor weight gain in survivors
Rabbit	12	1,300	45-264	None
Monkey	4	1,300	262-264	None
Guinea pig	24	650	23-130	None

Taken from Spencer et al. [53]

The authors [53] found no signs of intoxication in rabbits and monkeys after repeated inhalation of styrene at 1,300 ppm. Rats exposed at this concentration for similar periods of time exhibited eye and nose irritation. Guinea pigs exposed at 1,300 ppm exhibited marked lung irritation but were unaffected at 650 ppm.

In a group of 59 rats, Spencer et al. [53] found that those administered styrene orally at 8.0 g/kg all died and those given styrene at 1.6 g/kg all survived. The styrene was emulsified in olive oil with gum arabic. Repeated gastric intubation of styrene at 2.0, 1.0, 0.5, and 0.1 g/kg, given 5 d/wk for 28 days, was also performed. The number of rats given each dose was not given. At 2.0 g/kg, rats died after only a "very few" doses, and pronounced stomach and esophageal irritation was found. Similar irritation and some deaths resulted at 1.0 g/kg. Slight local irritation of the esophagus and stomach and poor weight gains were found in five male rats that survived 20 oral doses of 0.5 g styrene/kg. No differences in unspecified blood components were found between the rats given 0.5 g/kg and the five control rats. Rats that were administered 20 doses of 0.1 g/kg survived and were found to be generally in good health.

After one application of undiluted styrene to the ear of a rabbit, Spencer et al. [53] found "no appreciable reaction." However, after 20 applications over a 4-week period, moderate irritation with blistering and hair loss was observed. Two applications of styrene to the shaved abdomens of rabbits caused marked irritation and some necrosis similar to the effect caused by benzene or toluene [53].

In 1956, Wolf et al. [162] applied undiluted styrene 10-20 times to the ears and shaved abdomens of an unspecified number of rabbits over 2-4 weeks. Skin effects from styrene were characterized by definite erythema of the skin and development of a thin layer of necrotic tissue that resulted in exfoliation. Under the conditions of this experiment, there was no indication that styrene was absorbed through the skin in acutely toxic amounts.

Wolf et al. [162] also placed two drops of liquid styrene on the right eyes of an unspecified number of rabbits. Visual observations of the degree of irritation were made at 3 minutes, 1 hour, 2 days, and 7 days after administration. External corneal injury was visualized with the aid of 5% fluorescein in water. Ocular injury from styrene was manifested by moderate conjunctival irritation, inflammation and slight swelling of the eyelids, and slight transient corneal injury (perceptible superficial necrosis involving less than 50% of the cornea).

Wolf et al. [162] also reported a series of studies on alkylated benzenes, including styrene. After single-dose intubations of 37 rats with 7 ml or less of styrene (undiluted or emulsified in olive oil or corn oil with 5-10% gum arabic), data suggesting an approximate LD50 of 5.0 g/kg were obtained. Upon necropsy, slight liver changes and occasional kidney involvement were observed. Repeated administration of styrene by intubation

(2-3 ml in olive oil, emulsified with gum arabic) to groups of 10 female rats was performed once a day, 5 days per week for 6 months. A group of 20 rats fed only 2.5 ml of olive oil with gum arabic served as controls. One hundred and thirty-two intubations were given at each of the following doses: 66.7, 133, 400, and 667 mg/kg/d. Counts of formed elements of the blood were performed on selected animals from each group after 20, 40, 80, and 130 doses. The examinations included total and differential WBC, total RBC, and hemoglobin content. No effect on rats given styrene was found at 66.7 or 133 mg/kg/d; however, at 400 mg/kg/d, a slight general growth depression and slight liver and kidney weight depressions were reported. These effects were more pronounced when 667 mg/kg/d was administered [162].

In 1968, Gut [163] reported studies of the behavioral effects of styrene on rats. Using spontaneous motor activity as an indicator of behavioral effects, the activity of rats after single exposures to styrene for about 8 hours at 315-1,000 ppm and after repeated exposure concentrations of 228-575 ppm 8 hours per day, 5 days per week for 4-7 weeks was observed. Spontaneous motor activity was measured by the number of movements of the rats in a cage with partitions and doors. All testing was done 8 hours after styrene exposure.

Spontaneous motor activity decreased after a single exposure at 325 ppm. Activity initially decreased after repeated styrene exposures at 345 ppm, but, with further repeated exposures, the effects of styrene weakened and the concentration required to decrease activity rose to more than 985 ppm. Gut [163] stated that the decreased spontaneous motor activity may have been caused by the irritating effects of styrene on the rats, because they often spent time rubbing their eyes and nose rather than moving about in the cage. In 1968, Gut [164] reported further results from this experiment. After 4 weeks, styrene was found to be significantly related ($p<0.001$) to increased relative liver weight in the rats exposed to 325 ppm. However, after 7 weeks of inhalation exposure, no significant change was found in relative liver weight which Gut [164] suggested was due to apparent adaptation by the liver parenchyma.

In 1969, Shugaev [165] reported LC50's of 2,800 ppm for rats exposed to styrene for 4 hours and 4,900 ppm for mice exposed for 2 hours. Styrene content was measured in the tissues of animals that died during exposure at the LC50 to estimate lethal tissue concentrations. Tissue samples (300-500 mg) were extracted with 10 ml of solvent for analysis of styrene by gas chromatography. Concentrations of styrene found in liver, kidney, spleen, perirenal fat, and brain, expressed as milligrams percent of tissue extract, were about 20, 15, 19, 133, and 25 milligrams percent, respectively. The concentration of styrene at the LC50 level in the brains of mice that died during exposure was 18 mg/100 ml.

In 1977, Szulinska et al. [166] exposed 2 groups of 8 male Wistar rats to styrene vapor for 24 hours a day for 172 days. Exposure concentrations were 0.02 and 0.16 ppm as measured using a spectrophotometric method. Blood counts, blood cholinesterase activities, and weights of selected organs

(kidneys, spleen, and liver), expressed as percentages (probably of body weights) were studied, and lungs, liver, spleen, heart, and kidneys were examined microscopically. The investigators [166] described an increased erythrocyte count and a decreased hemoglobin level in the blood (neither significant) in both groups of exposed rats. There were also increases in liver and spleen weight percentages (not significant) for the rats exposed at 0.02 ppm as compared to the controls. The rats exposed at 0.16 ppm had an increased spleen weight percentage and a decreased liver weight percentage (neither significant) as compared to controls. No pathologic changes were found except for an increase in peribronchial tissue in two rats exposed at the higher concentration.

In 1979, Quast et al. [167] administered styrene to Beagle hounds by stomach tube at 200, 400, or 600 mg/kg/day for up to 561 days. Erythrocyte Heinz bodies were found in males dosed with 400 and 600 mg/kg/day, and sporadically in females administered 200 mg/kg/day. Other changes found occasionally were decreased packed cell volume, erythrocyte counts, erythrocyte sedimentation rate, and hemoglobin levels; an increased incidence of anisocytosis and hypochromia of erythrocytes, hemosiderin in reticuloendothelial cells of the liver; and an increased number of hepatocellular intranuclear acidophilic crystalline inclusions. Other blood elements examined were not affected by the administration of styrene at these doses. The blood changes were readily reversed after the administration of styrene was stopped.

Body weight, food consumption, clinical chemistry results, and organ weights (brain, heart, liver, kidneys, and testes) were not affected by the styrene absorption. There were no significant macroscopic or microscopic changes except for the hepatic cells mentioned above, which were attributed to the removal of altered red blood cells. Electron microscopy of peripheral blood and fixed liver tissue confirmed some of the changes detected by light microscopy [167].

In 1978, Seppalainen [168] conducted an experimental study with rats on the peripheral nervous system effects of styrene. Twenty young adult rats were exposed to 300 ppm of styrene, and 15 littermate control rats were sham-exposed in a similar chamber with air circulation. The rats were exposed for 6 hours a day, 5 days per week for up to 11 weeks. A transient increase in the motor conduction velocity of the tail nerve was noted after 6 weeks, but in the comparison to control rats no significant differences were found in measurements taken on rats exposed for 8 and 11 weeks.

In 1979, in another Finnish study, Vainio et al. [169] reported on styrene inhalation in rats. Forty adult male Wistar rats were intermittently exposed for 11 weeks to 300 ppm of styrene 6 hours daily, 5 days per week in a dynamic exposure chamber of 1 cubic meter. The styrene concentration in the chamber air was continuously monitored by infrared analysis. The exposed animals exhibited microscopic liver alterations after 2 weeks, consisting of parenchymal hydropic degeneration, intracellular vacuolization (steatosis), and congestion.

The Chemical Manufacturers Association sponsored a two-year study [170] that was finalized in 1980 on styrene administered in the drinking water of albino rats. Styrene was provided continuously in the drinking water at intended concentrations of 125 and 250 ppm. The approximate daily styrene consumption was estimated to have been, in mg/kg of body weight, 7.7 and 14 for the low- and high-dose males, respectively, and 12 and 21 for the low-and high-dose females, respectively. Initially, there were 50 males and 70 females in each group receiving styrene and 76 males and 106 females as untreated controls. The study [170] concluded that the two-year administration of styrene in the drinking water of rats at these dosage levels resulted in no gross or histological changes. This conclusion was based on evaluation of data on mortality, body weight gain, food and water consumption, hemograms, clinical chemistry, urinalysis, clinical signs (including ophthalmologic observations), gross necropsy findings (including organ weights), and histopathological examination of tissues taken from rats at interim and terminal sacrifice, as well as rats that died on study or were sacrificed in a moribund condition. All tumors encountered among rats dying during the course of the study or at the terminal sacrifice were common spontaneously-occurring tumors of Sprague-Dawley rats, or uncommon tumors that affected only individual rats without regard to treatment group. There were no apparent treatment-related increases in tumor incidence. Also there were no apparent differences between treated and control rats with respect to nontumorous lesions encountered in the study [170].

Mutagenicity

Many studies of the potential mutagenicity of styrene have been performed. Most of these have been tests with microorganisms or mammalian cell cultures *in vitro*, but there have also been some investigations in mammals *in vivo*. Because of the possibility that styrene oxide was an intermediary metabolite of styrene and the likelihood that this epoxide could be mutagenic or carcinogenic, it was also tested for mutagenicity in most of these studies.

In 1976, Vainio et al. [171] studied the mutagenic properties of styrene, styrene oxide, and phenylglycol. These substances were dissolved in absolute ethanol, mixed with agar, and applied to petri dishes. Liver microsomes from rats that had received a polychlorinated biphenyl (PCB) mixture 5 days earlier to stimulate microsomal enzyme activity, and cofactors for generation of reduced nicotinamide adenine dinucleotide phosphate (NADPH), were included in the agar mixture added to some petri dishes to test for indirect mutagenic activity. *Salmonella typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98, and TA 100 were incubated on the petri dishes at 37°C for 2 days, and revertant colonies were counted. Reversion of *S. typhimurium* from histidine auxotrophy to prototrophy was used to indicate mutagenic activity. Phenylglycol was not found to be mutagenic under these conditions. Styrene oxide, however, caused mutations in strains TA 1535 and TA 100, the rate of which was not increased by adding

the microsomal preparation. Therefore, styrene oxide was considered a direct mutagen with the effect due to base substitution. The data on styrene were difficult to interpret because of styrene's toxicity to the organisms, although it seemed to be mutagenic to TA 1535 and TA 100 after metabolic activation [171].

When diethyl maleate (DEM), which depletes cells of cytoplasmic glutathione, or trichloropropane oxide (TCPO), an inhibitor of epoxide hydratase activity, were added to the culture media of TA 1535, only a slight increase in the rate of mutation with styrene in the presence of the enzyme preparation was found. If styrene oxide were the first metabolite of styrene, addition of DEM or TCPO should have resulted in an increase in the concentration of styrene oxide and consequently the rate of mutation, because glutathione depletion or hydratase inhibition should reduce the rate at which the epoxide is inactivated. The authors [171] concluded that a more detailed investigation was warranted. (Epoxide hydratase is often referred to in various reports as epoxide hydrase; this document will uniformly use the former name).

Other investigators [172,173,174,175] have confirmed the mutagenicity of styrene oxide, but have found little evidence that styrene is mutagenic. In 1976, Milvy and Garro [172] tested styrene, styrene oxide, styrene glycol, benzyl alcohol, and mandelic, phenylglyoxylic, benzoic, and hippuric acids by the Ames test using S. typhimurium TA 1535, TA 1537, TA 1538, TA 98, and TA 100. Only styrene oxide was found to be mutagenic under the experimental conditions used [172].

In 1977, Stoltz and Withey [173] also investigated the mutagenicity of styrene and styrene oxide by the Ames test. Using S. typhimurium TA 1535, TA 1537, TA 1538, TA 98, and TA 100 and a liver microsomal preparation from rats or hamsters that had been pretreated with a PCB, styrene was not found to be mutagenic, but there was a dose-dependent increase in the rate of mutations of both TA 1535 and TA 100 due to styrene oxide. The authors [173] concluded that styrene and styrene oxide results differed because of a slow rate of conversion of styrene to styrene oxide and a very rapid rate of removal of styrene oxide due to the action of epoxide hydratase and glutathione-S-transferase.

In 1977, Greim et al. [174] used a modified Ames test using S. typhimurium TA 1535 and TA 1538 and E. coli K12, incubated with and without a liver microsomal preparation that included a NADPH-generating system, to assay the mutagenicity of styrene and styrene oxide. The microsomal fraction was prepared from rat livers that had been pretreated with phenobarbital for 10 days (0.1% in drinking water) or by a single injection (500 mg/kg) of a PCB 4 days before sacrificing. The authors [174] concluded that styrene oxide was mutagenic but styrene was not.

In 1976, Loprieno et al. [175] studied the ability of styrene or styrene oxide to produce forward mutations at 5 loci in the Schizosaccharomyces pombe P1 strain of yeast and in cultured V79 Chinese hamster cells,

and to produce gene conversions at 2 loci in Saccharomyces cerevisiae diploid strain D4 yeast. Host-mediated assays were also performed with the two yeasts. For the in vitro studies, the yeasts were incubated with a purified mouse liver microsome preparation together with NADPH and glucose-6-phosphate dehydrogenase. In the host-mediated assay, male Swiss albino mice were injected intraperitoneally (ip) with yeast cells, and the animals were then treated by gavage with 1 ml of styrene or styrene oxide. Styrene was not active in producing forward mutations in S. pombe or in Chinese hamster V79 cells or in producing gene conversions in S. cerevisiae. In the host-mediated assay, styrene produced gene conversions in S. cerevisiae, but was unable to increase spontaneous forward mutation frequency in S. pombe. As the investigators [175] pointed out, this in vivo mutagenic effect of styrene was produced with a very high dose of styrene, i.e., 1 g/kg. Styrene oxide was active, i.e., mutagenic, in all systems tested except with S. pombe in the host-mediated assay.

Later in 1978, Loprieno et al. [176] investigated the ability of styrene or styrene oxide to induce (1) point mutations in S. typhimurium TA 1535 and in Chinese hamster cells with and without metabolic activation, (2) unscheduled DNA synthesis in heteroploid human cells, and (3) chromosomal changes in mouse bone marrow cells. Styrene was not mutagenic with TA 1535, with or without activation with an S9 mix. Styrene oxide, however, was mutagenic and after activation it was even more so. Styrene was similarly ineffective in the induction of 8-azaguanine resistant mutants in V79 Chinese hamster cells, whether or not activated by an S10 metabolic activation system from mouse liver. Only styrene oxide, and not styrene, each activated by an S10 mix, was able to increase incorporation of tritiated thymidine above background into the DNA of the heteroploid human cells in the presence of an inhibitor of DNA synthesis (hydroxyurea); thus, by this test of unscheduled DNA synthesis, styrene oxide but not styrene was found to be mutagenic. Styrene or styrene oxide was intubated into male and female mice and, after the mice were sacrificed 24 hours later, bone marrow cell preparations were made. Styrene oxide but not styrene caused a statistically significant increase in chromosomal aberrations. Positive controls used in these tests of mutagenicity showed the test systems to be effective. Thus, in this investigation of mutagenicity in several test systems, styrene oxide was consistently found by Loprieno et al. [176] to be mutagenic, while styrene was not.

In 1978, a report by Roberfroid et al. [177] contained data which demonstrated that styrene lowered the Michaelis constant of the enzyme aryl hydrocarbon hydroxylase in Wistar rats in a manner similar to that of benzo(a)pyrene and 3-methylcholanthrene, both of which the investigators considered to be mutagenic and carcinogenic. Styrene caused only about 50 reversions per plate among S. typhimurium TA 1538, while there were about 20 spontaneous reversions. In comparison, benzo(a)pyrene caused about 180 reversions among the same strain, thus suggesting once again that styrene is not more than weakly mutagenic [177]. Earlier, investigators from this laboratory [178] had found styrene not to be mutagenic to S. typhimurium strains TA 98, TA 100, TA 1535, TA 1537, or TA 1538, except that, in the

presence of S9 mix (microsomal enzymes), a reversion to histidine prototrophy was observed with TA 1535 only, suggestive of a base-substitution type of mutation. Styrene oxide caused histidine prototrophy in TA 100 and TA 1535 with or without S9 mix, but did not cause mutations in TA 98, TA 1537, or TA 1538.

In 1978, Linnainmaa et al. [179] studied the effect of styrene and styrene oxide on chromosomes from human lymphocytes. Styrene was incubated with a lymphocyte preparation for 72 hours, and styrene oxide for a shorter time. A total of 100 metaphases or 100 anaphases were examined at each concentration of styrene or styrene oxide for cytogenetic aberrations, and 1,000 interphase nuclei were examined for the presence of micronuclei and nuclear bridges.

Styrene mainly caused chromosome breaks (19 breaks/100 metaphases, $p<0.001$); styrene oxide was primarily responsible for the formation of micronuclei and nuclear bridges (38 micronuclei/1,000 interphase cells, $p<0.001$). Linnainmaa et al. [179] suggested that impurities in the styrene preparation might have been responsible for the results that differed from those of styrene oxide. They reported that the styrene contained 0.60 ppm ethylbenzene, 0.75 ppm alpha-methyl styrene, and 0.50 ppm styrene oxide, but did not test all of these contaminants for mutagenicity. The investigators [179] speculated that the chromosome breaks they observed were due to formation of styrene oxide or possibly to the presence of the alleged metabolite [160,180] styrene-3,4-oxide.

In a later study by this same group in 1980, Norppa et al. [181] found that styrene and styrene oxide showed a clear dose-response relationship in the induction of sister chromatid exchanges (SCE) in human whole blood lymphocyte cultures. To find out whether the potential of styrene to induce SCE was accompanied by metabolic conversion to styrene oxide, gas chromatographic analyses were carried out in human lymphocyte cultures after 0.5, 2, 6, and 24 hours after treatment with styrene and styrene oxide. In the styrene-treated cultures, the ratio of styrene oxide to styrene was progressively increased with incubation time. In styrene-treated control cultures without blood, there was no increase of styrene oxide. In the styrene oxide-treated cultures, the amount of styrene oxide gradually decreased with incubation time. The investigators [181] concluded that styrene oxide could be formed from styrene in human cells but that it was not clear which cells in human blood were responsible for the metabolic activation of styrene.

In 1978, De Raat [182] tested the ability of styrene and its presumed metabolite styrene oxide to induce SCE in Chinese hamster ovarian cells with and without metabolic activation. Styrene oxide appeared to be a potent inducer of SCE. Styrene in doses up to 1.0 ml/l did not increase the number of SCE per metaphase, even with metabolic activation. Induction of SCE by styrene in the presence of metabolic activation only occurred when cyclohexene oxide was used as an inhibitor of the enzyme epoxide hydratase. De Raat [182] interpreted the lack of induction of SCE by styrene in the

presence of metabolic activation as being caused by a very rapid biotransformation of styrene oxide, clearly a compound that induces SCE, rather than styrene not being converted to its oxide.

There is some in vivo experimental evidence in rodents and flies that styrene itself can cause mutations. In 1980, Conner et al. [183] exposed 3-4 mice for 6 hours per day to styrene vapor concentrations ranging from 104-922 ppm for 4 days and at 920 ppm for 1 and 2 days. An increased SCE frequency was found in regenerating liver cells, in bone marrow cells, and in alveolar macrophages. Some of the mice had two-thirds of their liver removed surgically a day prior to the first exposure. On the last day of styrene exposure, the mice were injected with 5-bromodeoxyuridine; on the following morning, they were injected with colchicine and sacrificed several hours later. The SCE frequencies in regenerating liver cells, bone marrow cells, and alveolar macrophages of hepatectomized mice exposed to styrene at 387, 591, and 922 ppm for 4 days and in bone marrow cells and alveolar macrophages from nonhepatectomized mice exposed to styrene at 591 and 922 ppm for 4 days were statistically greater ($p<0.05$) than those in the controls. There was also a significant increase ($p<0.001$) in cell frequency in regenerating liver, bone marrow, and alveolar macrophage cells from both hepatectomized and nonhepatectomized mice exposed for 2 days at 922 ppm but not in those exposed for 1 day at that concentration.

In 1980, Meretoja et al. [184] found clastogenic effects in bone marrow cells of rats. Male rats were exposed to styrene vapor at 300 ppm for 6 hours per day, 5 days per week, for 2-11 weeks. Animals were sacrificed weekly, and bone marrow tissue was taken from the femur. A total of 100 metaphases per animal were examined for aneuploidy and chromosomal aberrations. A significant increase in chromosomal aberrations appeared after 9 weeks of exposure. Most of the aberrations were chromosomal breaks, but there were also a few chromatid breaks. The incidence of aberrant cells was 8-12% in exposed animals and 1-6% in controls. Polyploid cells were found in every sample preparation from the rats exposed to styrene for 11 weeks.

However, in another investigation by these researchers, published in 1980 by Norppa et al. [185], styrene was not found to cause clastogenic effects in bone marrow cells of hamsters. Male Chinese hamsters, 3-4 months old, were exposed to styrene vapor at 300 ppm for 6 hours per day. One group of 4 hamsters was exposed for 4 days, the other group of 4 was exposed 5 days per week for 3 weeks. The investigators [185] analyzed 100 bone marrow metaphases from each animal for chromosome or chromatid aberrations and gaps. No significant difference from controls was found in either styrene-exposed group. The effect of ethanol, alone and with styrene, was also studied. Animals receiving both 300 ppm of styrene and ethanol (15% v/v in drinking water, ad libitum) for 4 days had significantly more chromosomal aberrations, not including gaps, than did controls or hamsters administered only styrene, but not more than hamsters given only ethanol. The incidence of gaps was not significantly different among the groups. No significant differences among the several groups treated for 3 weeks were found, either in aberrations or in gaps [185].

In vivo inhalation exposure of male Chinese hamsters to styrene oxide (25, 50, 75, and 100 ppm) was reported in 1979 by these same investigators (Norppa et al. [186]) to have no effects on chromosomal aberration rates or frequencies of SCE in bone marrow cells. The only positive response in chromosome aberration frequency was obtained when styrene was injected ip in a lethal concentration (500 mg/kg body weight); 1 of 6 hamsters showed slightly elevated values of SCE after this high dose.

In 1979, Donner et al. [187] reported that styrene and styrene oxide caused recessive lethal mutations in fruit flies. Drosophila melanogaster were exposed to styrene oxide vapor at 200 ppm for 6 hours per day for 4 days, or to styrene by being fed for 24 hours on tissue paper moistened with 10 ml of a solution containing styrene at 200 ppm in 1% aqueous sucrose. The fruit flies were pretreated either with phenobarbital, to induce metabolizing enzymes, or, in the case of flies exposed to styrene oxide, with trichloropropane oxide (TCPO), to inhibit epoxide hydratase. Both styrene and styrene oxide produced a significant increase in recessive lethal mutations. Neither pretreatment showed mutagenic potency, but the frequency of recessive lethal mutations from styrene or styrene oxide was doubled by phenobarbital pretreatment; also, in fruit flies exposed to styrene oxide, pretreatment by an epoxide hydratase inhibitor (TCPO) increased the mutation rate about two-fold over that caused by styrene oxide alone [187].

Reproductive Effects

There have been a number of experimental animal studies investigating possible reproductive effects of exposure to styrene. In 1977, Vainio et al. [188] investigated the toxicity of styrene and styrene oxide toward chicken embryos from White-Leghorn SK12 chickens. Various amounts of the compounds in 50 µl of an olive oil-ethanol mixture were injected into the air sacs of the eggs. For control purposes, 50 µl of the olive oil-ethanol vehicle were injected into other eggs.

After incubation for 14 days at 37°C, 80-90% of the control embryos were alive, but no embryos survived that had received 100 µmol of styrene. Of those embryos that received 50 µmol of styrene, only 30% survived; with 2 µmol, 90% survived. Malformations were found in about 15% of the embryos that had received styrene and in about 7% of those that had received styrene oxide. Vainio et al. [188] determined that the embryos were most susceptible to the toxic effects of styrene and styrene oxide on the day of, and the day after, the beginning of incubation.

In 1974, Zlobina et al. [189] reported studies of styrene concentrations in rat maternal and fetal blood and in amniotic fluid after a 2-hour exposure to styrene vapor (3.6 or 10 ppm). Placental styrene concentrations were not determined. The results presented in Table Iv-22 demonstrate that styrene can cross the placenta.

TABLE IV-22

CONCENTRATION OF STYRENE IN MATERNAL BLOOD, FETAL BLOOD, AND AMNIOTIC FLUID IN 18- TO 21-DAY RAT FETUSES

Styrene Exposure	Styrene Concentration, µg/ml		
	Maternal Blood	Fetal Blood	Amniotic Fluid
10 ppm	10.95-12.25	8-8.8	2.35-2.8
3.6 ppm	1.07-2.62	0.9-1.65	1.25-1.8

Adapted from Zlobina et al. [189]

In 1974, Ragul'ye [190] reported the effects of maternal styrene inhalation exposures on the development of the embryo. In one study, 23 pregnant albino rats were exposed at 1.2 or 12 ppm of styrene for 4 hours per day throughout gestation (21 days); there was a control group of 15 rats. Numbers of live embryos, stillbirths, resorptions, and implantation sites, together with embryo mortality, pre- and post-implantation deaths, and embryo weight and size were studied. In addition, RBC, WBC, hemoglobin, oxygen consumption, and respiratory rate of the dams were measured on the 15th to 17th day of gestation and compared to pre-exposure findings.

Maternal exposure to styrene at 12 ppm significantly increased pre-implantation loss (20.7 vs. 3.6 in controls) as well as total embryo mortality (25.2 vs. 15.5 in controls); no significant changes were found in the other indices. No significant changes in any indices studied were found in the rats exposed at 1.2 ppm. There was no pathological embryo development observed in either experimental group. Ragul'ye [190] also reported a significant increase in deaths/litter during the first 2 weeks of life (1.66 and 0.6 in the rats exposed at 12 and 1.2 ppm, respectively, vs. 0 in the controls).

Ragul'ye [190] also exposed 10 pregnant rats to styrene at 1.2 ppm and 10 other pregnant rats at 0.4 ppm for 4 hours per day for 21 days (the entire gestation period). At the same concentrations, two other groups of 10 pregnant rats each were exposed, but only during the first 7 days of gestation. Twenty pregnant rats served as controls. All rats were sacrificed on the 21st day of gestation.

In the rats exposed for the entire gestation period, the rate of resorptions/dam was 0.2 for those exposed at 0.4 ppm, 1.3 for those exposed at 1.2 ppm, and 0 for the control group. There was also a higher rate of resorptions/dam (0.66) found in rats exposed at 1.2 ppm for the first 7 days of gestation, with none in the control group. Other significant results included an increased embryonic mortality in litters of dams exposed at 1.2 ppm for the entire gestation period (12.8% vs. 2.5%), an increased number of pre-implantation deaths at exposures to 1.2 and 0.4 ppm for the entire gestation period and during the first 7 days, an increased number of post-implantation deaths in both groups exposed for the entire gestation period, and a decreased embryo size and weight among rats exposed at 1.2 ppm for the entire gestation period [190]. All litters examined appeared to have been first litters.

Ragul'ye [190], who did not state the levels of statistical significance, gave no data other than means and an undescribed index of variation. The description of how pre-implantation and total embryo losses were measured was not given; however, observation of corpora lutea, resorption sites, and total births seems the obvious method. Had the data or a description of methods been given, it would be possible to be more certain of the meaning of total embryo mortality; most likely, this term was used to mean a total of pre- and post-implantation losses. (The word translated as embryo is interpreted to have been applied to all stages of prenatal development, from blastocyst through fetus.)

In 1979, Vergiyeva et al. [191] studied embryotoxicity in rats in an investigation designed at least in part to check on the results of Ragul'ye [190]. Three groups of pregnant rats were exposed to styrene vapor 4 hours per day, 5 days per week. Groups I and II were exposed at 47 ppm on days 2-21 of gestation; group III was exposed at 165 ppm on days 2-16 of gestation. Progeny from dams of groups I and III were observed for 90 days after birth. Dams from group II were sacrificed on the 21st day; maternal weights, numbers of corpora lutea, resorptions and autolysis, the number and weights of live pups, and structural anomalies were recorded.

There were no changes from controls in the experimental animals as to the course, duration, and outcome of pregnancies or in indices such as implantations, resorptions, and live and still births. Dams and progenies kept for 90 days after birth did not differ significantly from controls in hemoglobin and RBC, in hexobarbital sleeping time one month after birth, or in behavioral tests consisting of an open field test and a measured reaction to loud noise. Methods of studying structural anomalies were not described, but the investigators [191] stated none were found in either controls or Group II progeny.

Vergiyeva et al. [191] gave a few more details of methods and results than did Ragul'ye [190] and, especially because it described exposures of rats at higher concentrations without evidence of embryotoxicity, this study is taken as refuting the implications of embryotoxicity found at low concentrations of styrene in the earlier report [190].

In 1978, Murray et al. [192] investigated possible styrene teratogenicity in rats and rabbits. Groups of 29 or 30 Sprague-Dawley rats and 20 New Zealand white rabbits were exposed to styrene vapor for 7 hours per day from day 6 of pregnancy through day 15 (rats) or day 18 (rabbits). Exposure levels were 0, 300, or 600 ppm styrene. In addition, other rats were intubated with styrene in peanut oil at 0, 90, or 150 mg/kg twice daily on days 6 through 15 of gestation. There were no significant changes ($p<0.05$) in the exposed animals as compared with controls during gestation except for a reduced weight gain on days 6 through 9 of gestation in rats (inhalation and gavage treatments) associated with a decreased food consumption, and increased water consumption on days 9 through 20 of gestation by rats (inhalation treatment). Dams were sacrificed just prior to expected delivery, and fetuses were examined. There was a statistically significant increase ($p<0.05$) in fetal crown-rump length in litters from rats exposed at 300 ppm but not at 600 ppm. Mean crown-rump length of intubated rats was similar to that of controls. External, visceral, and skeletal malformations did not significantly differ in incidence from matched or historical controls. There were no significant changes ($p<0.05$) seen in rabbits except for the incidence of unossified fifth sternebrae among litters from rabbits exposed at 600 ppm; however, this incidence was similar to that seen in control groups from other recent studies. Because of this and because the change in rat fetal size at 300 ppm was not dose-related and the incidence of skeletal changes in rats were not different from controls, it seems inappropriate, from the evidence in this study, to draw conclusions that styrene is teratogenic or otherwise embryotoxic at these levels.

In 1980, Kankaanpaa et al. [193] exposed pregnant BMR/T6T6 mice and Chinese hamsters to styrene vapor and examined possible changes in fetal development. Pregnant mice were exposed at 250 ppm for 6 hours per day on days 6 through 16 of gestation, and were sacrificed for examination of fetuses after the day 16 exposure. Chinese hamsters were exposed 6 hours per day on days 6 through 18 of gestation, but the exposure concentrations were 300, 500, 750, or 1,000 ppm. There were no significant differences in ratios of live fetuses to total litter size, in numbers of dead or resorbed fetuses, or in numbers of malformed fetuses in either species, except that there was a significant excess ($p<0.001$) of dead or resorbed fetuses from hamsters exposed at 1,000 ppm and there was a nonsignificant excess of dead or resorbed fetuses from mice. There were also more malformations (rib fusion and extra ribs) in exposed mice, but the statistical significance was not described. This study [193] suggests embryotoxicity at high exposure levels, but is consistent with results of other studies [190,191,192] in failing to find a significant excess of terata from styrene exposure.

A report [170] of a three-generation reproductive study on styrene administered in the drinking water of rats was finalized in 1980. It was part of the study sponsored by the Chemical Manufacturers Association that was discussed earlier in the Toxicity Section. At least 10 males and 20 females (F0 generation) from each group of the chronic study (those dosed at 125 or 250 ppm, and controls) were mated to produce F1 pups. At breeding

age, the F1 rats were mated within their respective dose groups to produce F2 litters, and these rats in turn produced F3 pups. Styrene treatment in drinking water was maintained throughout.

The report of the study [170] concluded that styrene administered in the drinking water had no deleterious effects on the reproductive capacity of rats through three generations. The conclusion was based on evaluation (for each generation of each dose group) of fertility indices, mean litter size, live-to-total pup ratios, pup survival indices at intervals from birth to weaning, liver and kidney weights of representative pups necropsied at weaning, cytogenetic evaluation of bone marrow samples of other weanlings, and gross necropsy of F1 and F2 parents, including organ weights. In addition, histopathologic examinations were made of liver and kidneys of weanlings and of tissues of representative F1 and F2 parents.

In 1981, Sikov et al. [194] assessed the teratogenic effects of styrene oxide inhalation on rats and rabbits. Rats were exposed to 100 or 300 ppm for 7 hours per day, 5 days per week for 3 weeks before being mated and exposed daily through 19 days of gestation. Rabbits were artificially inseminated and exposed for 7 hours daily through the 24th day of gestation. The rats were sacrificed at the 21st day of gestation and the rabbits at the 30th day of gestation. Pregnant animals were examined for toxic changes including altered tissue weights and histopathological effects. Litters were examined for embryotoxicity and live fetuses were examined for external, visceral, and skeletal malformations. There was extensive mortality in rats that received prolonged exposure to 100 ppm styrene oxide, and 300 ppm was so rapidly lethal that exposures were terminated. Lower concentrations (15 and 50 ppm) were used for exposure of the rabbits; 50 ppm styrene oxide produced 79% mortality. Gestational exposure appeared to decrease fecundity by increasing loss of embryos before implantation in rats, and tended to increase the incidence of resorptions in rabbits. In both species, fetal weights and lengths were reduced by gestational exposure. The incidence of ossification defects of the sternebrae and occipital bones was increased by gestational exposure of rats to styrene oxide [194].

Carcinogenicity

There have a been a number of experimental animal studies investigating the long-term effects of styrene. In 1978, Jersey et al. [195] conducted an inhalation study of male and female Sprague-Dawley rats exposed at 600 or 1,200 ppm, the higher concentration being reduced to 1,000 ppm after 2 months because of excessive toxicity in the male rats. Male rats were exposed for more than 18 months and female rats for almost 21 months. Exposure periods were 6 hours per day, 5 days per week. All survivors were sacrificed for necropsy at 24 months. There were reduced weight gains in all exposed groups of rats except in females exposed at 600 ppm. Female rats at the higher concentration had increased liver weights and a higher incidence of alveolar histiocytosis in their lungs. Male rats in the control group and those exposed to styrene at the higher concentration had excessive mortality from an intercurrent disease, chronic murine pneumonia. There was no evidence of tumors in the male rats attributable to the exposure regimen. The female rats in both exposure groups had an increase (though not significant) in the combined frequency rate of tumors in the leukemia and lymphosarcoma classifications. The investigators [195] believed that the exposure concentrations used overwhelmed the ability of the rats to detoxify styrene; because of this, the high incidence of spontaneous disease, and the equivocal results, they recommended that a new study be initiated.

In 1978, Ponomarkov and Tomatis [196] investigated the long-term effects of styrene in 020 and C57 B1 mice and BD IV rats. Styrene dissolved in olive oil was administered by stomach tube; other groups of mice and rats were given only olive oil, and others were not treated. All animals were fed a commercial food preparation that had been analyzed for aflatoxins, which were absent, and nitrosamines, which were present at 0.2-0.6 ppb.

Each of 29 pregnant 020 mice was administered 1,350 mg styrene/kg on the 17th day of gestation. Nine other pregnant females were given 0.1 ml of olive oil only on the same day. After weaning, their progeny received the same dose once a week; treatment with styrene was stopped after 16 weeks because of the toxicity of styrene. Offspring (54 male and 47 female mice) of untreated dams received no treatment [196].

For 020 mice, pre-weaning mortality was significantly greater among the styrene-treated animals than among olive oil-treated animals (43% vs. 22%). Higher mortality continued even after treatment with styrene had ended. The average age of styrene-treated mice at death was 32 weeks for males and 49 weeks for females, compared with 88 and 85 weeks for olive oil-treated male and female mice and 94 and 99 weeks for untreated male and female mice, respectively [196].

Multiple centrilobular necrosis of the liver, hypoplasia of the spleen, and severe congestion of the lungs were the most frequently observed lesions

in styrene-treated 020 mice which died within the first 20 weeks [196]. Extensive inflammation around necrotic foci was often observed in the livers of styrene-treated mice that died up to the 45th week of treatment; bronchitis and peribronchitis were also frequently observed. Animals that died after 45 weeks had what the authors [196] described as multiple abscess-like round cavities in the liver that were filled with polymorphonuclear leukocytes and were surrounded by connective tissue capsules. Calcium deposits were also observed.

The percentage of tumor-bearing, singly-dosed, styrene-treated dams did not differ from the group treated only with olive oil. Among their offspring, however, 89% of the males and 100% of the females treated with styrene had lung tumors compared with 42% of the males and 67% of the females of the olive oil-treated controls, both statistically significant differences ($p<0.01$). When styrene-treated 020 mice were compared with untreated animals, the incidence of lung tumors was significantly greater ($p<0.001$) only in styrene-treated females.

Average age at death of the lung tumor-bearing 020 mice was 49 weeks for male and 58 weeks for female styrene-treated mice, 84 and 85 weeks for olive oil-treated males and females, and 87 and 91 weeks for untreated male and females, respectively. The lung tumors were histologically identified as either adenomas or adenocarcinomas. The incidences of lung tumors in olive oil-treated and untreated animals at sites other than the lungs were greater than the incidences in styrene-treated mice, perhaps, as the authors [196] commented, because of the longer survival of the controls.

Styrene (300 mg/kg in 0.1 ml olive oil) was also given to each of 15 pregnant C57 Bl mice on the 17th day of gestation; their progeny received the same dose after weaning weekly for life, up to 120 weeks. For controls, 0.1 ml olive oil was administered after weaning weekly to the offspring (12 males and 13 females) of five pregnant mice given 0.1 ml olive oil on the 17th day of pregnancy; 51 males and 49 females served as untreated controls [196].

For C57 Bl mice there was an increase, though not statistically significant, in the incidence of tumors, mainly lymphomas, in dams singly dosed with styrene. There were three hepatocellular carcinomas in the styrene-treated mice and one hepatocellular adenoma each in an olive oil-treated mouse and in an untreated mouse. Unlike the 020 mice, there were no significant differences in mortality, life-span, or body weights between the C57 Bl mice treated with styrene and those treated with olive oil.

On the 17th day of gestation, each of 21 pregnant BD IV rats were administered 1,350 mg styrene/kg in olive oil. Their progeny, 73 males and 71 females, each received 500 mg styrene/kg weekly for 120 weeks after weaning. Ten pregnant BD IV rats received only olive oil (0.3 ml) and their progeny (36 males and 39 females) were given 0.3 ml olive oil weekly after weaning. Untreated controls were not used [196].

Average litter sizes of the BD IV rats were similar, but offspring of styrene-treated dams had a greater pre-weaning mortality than did the olive oil-treated dams (10% vs. 2.5%); the difference was not significant. In some styrene-treated animals that died between weeks 50 and 60, the investigators [196] found moderate congestion of the lungs and kidneys and small necrotic foci in liver parenchyma. Although liver damage was not observed in animals that died between weeks 80 and 90, lesions of the forestomach (atrophy or desquamation of epithelium, and necrotic areas with inflammation) and of the kidney (hyperplasia of the pelvis epithelium) were frequently noted.

The percentage of tumor-bearing BD IV dams given a single dose of styrene during pregnancy was greater than that in the olive oil-treated group (65% vs. 50%); this difference was not statistically significant. The percentage of tumor-bearing, styrene-treated progeny was not significantly different from that of the olive oil-treated controls (24% vs. 39%). There were, however, a few tumors in styrene-treated rats which were not observed in the control animals, namely three neurogenic and three stomach tumors. Ponomarkov and Tomatis [196] concluded that their results provided weak evidence of the carcinogenicity of styrene in one of the two strains of mice tested, when it was given at a high dose level.

In 1979, under the program of the National Cancer Institute (NCI) for testing possible carcinogenicity, Fischer 344 rats and B6C3F1 mice were given styrene in corn oil by stomach tube [197]. The animals were intubated 5 days a week for up to 103 weeks in the case of rats administered the lowest dose and up to 78 weeks in all other groups. Surviving rats were sacrificed for necropsy at 104-105 weeks, and mice at 91 weeks. There were 50 rats of each sex at each of 3 doses (500, 1,000, and 2,000 mg/kg) and 40 of each sex given only the corn oil vehicle. There were 50 mice of each sex at each of two doses (150 and 300 mg/kg) and 20 of each sex given only the corn oil vehicle.

In male mice, there was an excess incidence of lung adenomas and carcinomas compared with vehicle controls but not compared with historical controls at NCI. There was no significant excess of tumors in rats, but so few male and female rats survived on the highest dose that there were limited numbers of these animals at risk of developing neoplasms. NCI [197] concluded that under the conditions of the bioassay the data (i.e., excesses of lung adenomas and carcinomas) provided "suggestive" evidence for the carcinogenicity of styrene in male B6C3F1 mice, but did not provide "convincing" evidence for the carcinogenicity of styrene in Fischer 344 rats or B6C3F1 mice of either sex.

In 1979, in a companion experiment by NCI [198], a solution of beta-nitrostyrene, consisting of 30% nitrostyrene and 70% styrene, was dissolved in corn oil and intubated into Fischer 344 rats and B6C3F1 mice. The beta-nitrostyrene doses were 150 and 300 mg/kg for male rats; 75 and 150 mg/kg for female rats; and 87.5 and 175 mg/kg for mice of both sexes. Doses of styrene, then, in addition, were 350 and 700 mg/kg for the male rats; 175

and 350 mg/kg for the female rats; and 204 and 408 mg/kg for the mice. The doses were administered 3 days a week for 78-79 weeks, after which mice were held an additional 14 weeks and the rats an additional 29 weeks before survivors were sacrificed for necropsy.

When the incidences of alveolar and bronchiolar adenomas and carcinomas were combined, there was a significant excess in low-dose male mice compared to the corn oil vehicle controls by the Fisher exact comparison ($p=0.016$), but not by the Cochran-Armitage test. There was no significant excess in high-dose male mice found by either test. No other significant excess in tumors was found. NCI [198] concluded that under the conditions of the bioassay, there was no "convincing" evidence for the carcinogenicity of a solution of beta-nitrostyrene and styrene in Fischer 344 rats or B6C3F1 mice.

Studies of the potential carcinogenicity of styrene oxide were reported by Kotin and Falk [199], Van Duuren et al. [200], Weil et al. [201], and by Maltoni et al. [202]. In 1963, Kotin and Falk [199] reported that, 11 months after administering 20 μmoles of styrene oxide by an unspecified route to 30 C3H mice, there were 3 malignant lymphomas (16%) among the 19 survivors. The cause of death in these mice was not stated, nor was the time when they died. The use of controls was also not reported.

In 1963, Van Duuren et al. [200] studied possible tumor formation from styrene oxide in male Swiss-Millerton mice. Thirty animals were tested with 10% styrene oxide in benzene that was topically applied. The mice, 8 weeks old at the onset of the experiment, had their backs painted three times a week with approximately 100 mg of solution/application. After a median survival time of 431 days, three styrene oxide treated mice (10%) were found to have tumors (type not specified), one of which was considered cancerous. Control groups were painted with either benzene or acetone. Positive controls received 100 mg of benzo(a)pyrene in benzene or acetone; negative controls received no treatment. Incidence of tumors in the controls was: (a) benzene group, 11/150 mice (7%), 10 had papillomas, and 1 developed cancer; (b) acetone group, 7/120 mice (6%) had papillomas; (c) benzo(a)pyrene in benzene (positive control), 49/90 mice (54%) developed tumors, 26 of which were cancerous; and (d) benzo(a)pyrene in acetone (positive control), 83/120 mice (69%) developed tumors, 49 of which were cancerous. Of the 267 untreated controls, 5% developed tumors, 1 of which was a squamous cell cancer, as compared with the long-term incidence of tumors in untreated Swiss-Millerton mice in the laboratory of 1.4%, (all squamous papillomas). Because the incidence of tumors in mice administered styrene oxide in benzene was not significantly greater than the incidence due to benzene application alone, this experiment does not give evidence that styrene oxide is tumorigenic.

In 1963, Weil et al. [201] studied the possible carcinogenicity of various epoxides in mice. Styrene oxide dissolved in acetone was applied by brush three times a week to the shaved backs of two groups of C3H mice that were 90 days old when the experiment began. One group was treated with a solution containing 10% styrene oxide and the other with a solution

containing 5%. The number of mice initially in the group that received 10% styrene oxide was not reported, but 18 were in the group at 12 months and 2 were still alive at 24 months. The maximum number of months the mice in the 10% group were painted was 18. No tumors were found in the group treated with 10% styrene oxide. Forty mice were initially present in the group treated with 5% styrene oxide; 35 were alive at 17 months and 17 were still alive at 24 months. Administration of styrene oxide continued through this period, and no tumors were found [201]. This experiment provides some evidence, albeit in small groups of mice, that styrene oxide is not tumorigenic by topical application.

In 1979, Maltoni et al. [202] reported that styrene oxide caused stomach epithelial tumors in rats. Styrene oxide was administered in olive oil by stomach tube at two dose levels, 50 and 250 mg/kg, to Sprague-Dawley rats once per day, 4-5 days per week, for 52 weeks, after which the animals were allowed to live until their natural deaths. Controls were intubated with olive oil alone. Papillomas and carcinomas, both in situ and invasive, were observed in the forestomachs of the rats at both dose levels at an incidence significantly greater than controls and with an evident dose-response relationship. Many of the carcinomas had metastasized to the liver. Rats with or without tumors of the forestomach often had precursor lesions. Maltoni et al. [202] commented that epithelial tumors of the forestomach were rare in this strain of rat, and concluded that styrene oxide was carcinogenic, affecting the organ "most directly exposed."

Uptake, Metabolism, and Elimination

In 1954, Danishefsky and Willhite [203] reported a study of the metabolism of styrene in Wistar rats. Styrene labeled with carbon-14 at the 8 position (8-14C styrene) was dissolved in peanut oil to make a 20% solution. Each rat was injected subcutaneously with 0.1 ml of the radioactive solution. Distribution of the isotope in tissues was determined 1, 6, and 24 hours after injection. Exhaled carbon dioxide, urine, feces, and expired styrene were collected and analyzed for carbon-14. After 1 hour, the liver, kidneys, blood, and urine had the highest radioactivity. Styrene was rapidly metabolized and excreted primarily in the urine. Six hours after administration, 37% of the initial radioactivity was found in the urine and 6% in expired carbon dioxide. After 24 hours, 71.0% of the radioactivity had been excreted in the urine, 11.8% in expired carbon dioxide, 2.6% in the feces, and 2.9% as unchanged expired styrene. Although the finding of labeled carbon dioxide indicates cleavage of the vinyl moiety of styrene, it does not distinguish initial cleavage of the terminal carbon from that of the entire vinyl group.

In 1969, Shugaev [165] exposed rats to styrene for 1 hour at 2,800 ppm (the LC50 for 4 hours). The animals were sacrificed at various times after exposure and brains and livers were removed and extracted for determination of styrene levels. Data are presented in Table IV-23.

TABLE IV-23

AVERAGE CONCENTRATION OF STYRENE IN THE BRAIN AND LIVER OF RATS AFTER EXPOSURE TO STYRENE AT 2,800 PPM FOR ONE HOUR

Time After Removal From Exposure (min)	AVERAGE STYRENE CONCENTRATION Brain mg/100 ml Extract	Liver mg/100 ml Extract
0.1	21.8	20.2
15	22.2	23.5
30	17.7	19.1
60	8.6	12.8
90	Trace-4.4	6.8

Taken from Shugaev [165]

Shugaev [165] found that styrene was more slowly removed from the organs than butadiene or hexene and that the anesthetic effects of styrene lasted longer than similar effects from the other compounds. Shugaev [165] also found that near-lethal concentrations of styrene were absorbed when tails of rats were immersed in liquid styrene for 1 hour (precautions had been taken to avoid styrene inhalation). Styrene concentrations of 11.1-17.3 mg/100 ml extract in the brain were found in rats sacrificed immediately after the exposure ended.

In 1977, Savolainen and Vainio [204] conducted an investigation of 14C-styrene and tritiated(3H)-styrene oxide injected ip into adult male Sprague-Dawley rats. Ten rats each received 460 µmol of styrene oxide and 15 each received 577 µmol of styrene. Each compound was dissolved in 1 ml of olive oil for administration. Three and six hours after injection of styrene oxide, 5 rats each were sacrificed. The same procedure was followed after injection of styrene, and in addition, five rats were sacrificed 24 hours after injection. The blood, brain, spinal cord, liver, duodenum, lungs, and kidneys were removed for analysis of radioactivity and separation into protein, water-soluble, and lipid (brain only) fractions. Radioactivity (14C) in blood after styrene injection increased from 3 to 6 hours, but remained approximately constant in the liver, lungs, kidneys, duodenum, brain, and spinal cord. Radioactivity (3H) from styrene oxide decreased in the liver and kidneys from 3 to 6 hours after injection, but remained constant in all other organs. After styrene injection, about 91% of the radioactivity in the brain was found in the water-soluble and chloroform-methanol-soluble fractions. After styrene oxide injection, about

83% of the radioactivity was found in the water-soluble fraction. The investigators [204] did not consider that the observed differences might have been due to the different isotopes used. Because tritium readily exchanges with hydrogen of other molecules, particularly proteins, nucleic acids, and water, it would be necessary to isolate and identify suspected metabolites of styrene and styrene oxide.

In 1978, Plotnick and Weigel [205] studied the tissue distribution and excretion of uniformly ring-labeled-14C-styrene in male and female Sprague-Dawley rats at various time intervals following a single oral dose of 20 mg/kg. Peak tissue levels of radioactivity were achieved at or before 4 hours after administration. The organ with the highest concentration of radioactivity at all time intervals studied (2, 4, 8, 12, and 24 hours), and in both sexes, was the kidney, followed in order of decreasing concentration by the liver and pancreas. Approximately 90% of the radioactivity administered was excreted in the urine within 24 hours of administration. Less than 2% of the dose was found in the feces. The investigators [205] speculated that the high levels found in the pancreas might be related to increased glucose tolerance, which has been reported in some styrene workers [115,116,120].

In 1976, Sauerhoff et al. investigated the metabolism and distribution of styrene in adult Sprague-Dawley rats after oral administration of a single dose of 50 or 500 mg styrene/kg [206] and after exposure to styrene vapor at 60 or 600 ppm for 6 hours [207]. For both investigations, 14C-styrene (uniformly ring-labeled) was used and the animals were sacrificed 72 hours after exposure. Radioactivity recovered in the urine, feces, expired air, and carcass was about 95, 4, 1.5, and 0%, respectively, after the oral dose of 50 mg/kg and about 90, 1.5, 9, and 0%, respectively, after the 500 mg/kg dose. In both cases, the percentage of radioactivity in expired air was lower in female than in male rats (0.9 vs. 1.8% and 5.3 vs. 12% at 50 and 500 mg/kg, respectively), indicating that female rats retained styrene longer than male rats [206].

After a dose of 50 mg/kg, six 14C-labeled metabolites were found in the urine. After a dose of 500 mg/kg or exposure at 60 or 600 ppm, seven radioactive metabolites were found. After all doses and exposures, four of these metabolites were identified as phenylglyoxylic, mandelic, benzoic, and hippuric acids. The recovered radioactivity associated with phenylglyoxylic, mandelic, benzoic, and hippuric acids was about 33, 30, 1.5, and 18%, respectively, in male rats; in females, it was about 25, 41, 2, and 20%, respectively [206,207].

In 1978, in a later investigation by Ramsey and Young [208] at the same laboratory as Sauerhoff et al. [206,207], adult rats were exposed to styrene vapor at 80, 200, 600, or 1,200 ppm for up to 24 hours. Styrene concentrations in the blood of rats were measured during and after a 6-hour exposure. The investigators [208] concluded that styrene at exposures up to 200 ppm did not accumulate in the body. Styrene was highly concentrated in fat relative to blood; the ratio of the concentration of styrene in fat to that in blood was approximately 40:1 at 80 ppm and 80:1 at 1,200 ppm.

In 1978, Savolainen and Pfaffli [209] exposed male rats to styrene vapor at 300 ppm for 6 hours per day, 5 days per week, for 1-11 weeks. The content of styrene in the fat increased linearly during the first 4 weeks and decreased in an exponential manner thereafter. Half of the peak styrene concentration in fat was detected after 9 weeks of exposure.

In 1958, El Masri et al. [210] investigated the metabolism of styrene in rabbits. Hippuric, mandelic, and mercapturic acids, and the glucaronide of phenylglycol were isolated from the urine of rabbits that had been administered styrene orally. The main metabolite (30-40% of the dose) was hippuric acid.

In 1965, Ruvinskaya [211] reported the conversion of styrene to mandelic acid in guinea pigs and rabbits. When guinea pigs were given subcutaneous injections of styrene in sunflower oil (1:1) at 50, 100, or 500 mg styrene/kg, the total mandelic acid found in the urine was 4.0, 7.9, and 36 mg, respectively. At 50 mg styrene/kg, excretion was complete within 1 day; 2 and 3 days were needed to complete excretion of mandelic acid after injection of 100 and 500 mg/kg, respectively. When guinea pigs were exposed to styrene at 1,410 ppm for 5 hours, they excreted mandelic acid for 4 days. The total excreted mandelic acid was 121-157 mg, 80% of which was excreted on the first day.

Ruvinskaya [211] also exposed guinea pigs and rabbits to styrene at 1.2, 12, 235, 705, or 1,175 ppm for 4 hours daily for 3 days. Urine was collected from each animal for 20 hours following each exposure. Mean levels of mandelic acid excreted daily by rabbits exposed at the four highest concentrations were 0.9, 11.2, 26.8, and 40.2 mg, respectively, and, for guinea pigs, they were 1.0, 12.0, 30.1, and 45.5 mg, respectively. There was no elevation of mandelic acid in the urine of rabbits or guinea pigs exposed at 1.2 ppm. When rabbits were exposed at 12 ppm for 4 hours daily for 30 days, mandelic acid excretion was relatively constant at about 1 mg/d. Having assumed a respiratory volume of 500 ml/min and a retention of 50%, Ruvinskaya [211] calculated that about 30% of the absorbed styrene was excreted in the urine as mandelic acid.

In 1971, Bardodej et al. [212] reported on the excretion of styrene metabolites by male Wistar rats exposed to styrene at 446, 728, or 1,856 ppm of styrene vapor for 2-104 hours. Mandelic and phenylglyoxylic acids in the urine were determined by polarography and spectrophotometry. The ratio of mandelic acid to phenylglyoxylic acid averaged 0.61 and ranged from 0.47 to 1.15. There was some indication that the ratios became larger with increasing duration of exposure. Only traces of phenylglycol were found by paper chromatography.

In 1971, Ohtsuji and Ikeda [213] reported studies on the metabolism of styrene (455 mg/kg), styrene oxide (527 mg/kg), phenylglycol (603 mg/kg), phenylglyoxylic acid (500 mg/kg), and mandelic acid (500 mg/kg) in female Wistar rats. Each compound was dissolved in soybean oil, and 2 ml/kg of the mixture was injected ip into rats that weighed about 50 g. Groups of six

rats were studied with each chemical administered. Urine collected from each rat during consecutive 2-hour periods for 10 hours after injection was assayed for glucuronides and hippuric, phenylglyoxylic, and mandelic acids. Some rats were pretreated with phenobarbital twice daily for 4 days before injection to induce hepatic microsomal enzymes; others were treated with a microsomal enzyme inhibitor, 2-diethylaminoethyl-2,2-diphenyl valerate hydrochloride (SKF-525A), before administration of styrene [213].

Injection of styrene and phenylglycol into the rats resulted in increased urinary excretion of a glucuronide (unidentified, but thought by the investigators [213] to be phenylglycol glucuronide) and phenylglyoxylic, mandelic, and hippuric acids. Styrene oxide may have increased glucuronide excretion in the 2 hours after injection; however, the total glucuronide excreted in 10 hours was the same as in controls. Excretion of the acid metabolites was increased after administration of styrene oxide. When phenylglyoxylic acid was injected, it alone was found in large quantities in the urine, indicating that it is an end product of styrene metabolism in the rat. When mandelic acid was injected, phenylglyoxylic, hippuric, and mandelic acids were excreted. When rats were injected with phenobarbital before injection of styrene, the level of styrene metabolites in the urine was increased, but this same effect of phenobarbital was not observed after injection of styrene oxide, phenylglycol, or phenylglyoxylic and mandelic acids [213].

Administration of styrene oxide and phenylglycol resulted in excretion of greater amounts of all the styrene metabolites during the first 2 hours than was observed when styrene was administered [213]. Inhibition of microsomal enzyme activity caused a decrease in the excretion of glucuronide from styrene, but did not affect excretion of the other metabolites. Administration of the microsomal enzyme inhibitor SKF-525A also resulted in a decreased excretion of mandelic acid in the first 2 hours after styrene administration, but no difference in mandelic acid excretion was noted after 10 hours in either the SKF-525A-treated rats or the untreated control rats. Mandelic acid was an apparent precursor of both phenylglyoxylic and hippuric acids. The data are presented in Table IV-24.

TABLE IV-24

EXCRETION OF URINARY METABOLITES 2 AND 10 HOURS AFTER INJECTION OF STYRENE, STYRENE OXIDE, PHENYLGLYCOL, PHENYLGLYOXYLIC ACID (PGA), OR MANDELIC ACID

Substance Injected	Glucuronides mg/kg		PGA mg/kg		Mandelic Acid mg/kg		Hippuric Acid mg/kg	
	2 h	10 h	2 h	10 h	2 h	10 h	2 h	10 h
Saline only	9.2	52.1	0.4	1.8	1.4	7.1	3.2	19.4
Styrene	15.6	70.4	9.1	70.7	11.0	58.8	14.7	78.0
Styrene/PB*	48.1	130.0	39.3	122.6	51.9	131.2	75.7	166.4
Styrene/SKF-525A	8.6	51.5	3.9	54.8	5.1	56.9	5.1	47.0
Styrene oxide	21.8	53.7	14.7	24.1	27.2	45.5	24.9	43.7
Styrene oxide/PB	24.1	59.5	18.7	27.0	31.7	47.6	31.3	52.4
Phenylglycol	41.1	77.4	35.6	89.0	45.4	60.6	75.0	110.0
Phenylglycol/PB	52.5	84.5	41.7	85.5	69.6	85.1	69.3	93.4
PGA	6.7	16.3	38.8	110.4	1.5	8.5	4.0	18.4
PGA/PB	6.9	19.7	47.5	131.7	1.6	9.1	4.2	19.9
Mandelic acid	2.8	16.0	67.9	129.8	110.2	122.1	11.9	35.9
Mandelic acid/PB	3.2	17.6	64.7	136.4	89.3	99.6	11.6	36.9

*PB = Phenobarbital
Use of a slash (/) indicates pretreatment with the substance that follows it.
Taken from Ohtsuji and Ikeda [213]

In 1974, Ikeda et al. [159] found that the 24-hour urinary excretion of mandelic and phenylglyoxylic acids of rats exposed to styrene vapor for 8 hours was linear with styrene exposures up to about 100 ppm, whereas hippuric acid excretion was linear with respect to styrene exposures up to 200 ppm.

In 1978, Seutter-Berlage et al. [214] identified, and determined the molar ratios of, three mercapturic acids that appeared in the urine of adult female Wistar rats after the ip injection of 250 mg styrene/kg once a day, 5 days per week, for 3 weeks. Compounds identified as metabolites of styrene were: N-acetyl-S-(1-phenyl-2-hydroxyethyl) cysteine, N-acetyl-S-(2-phenyl-2-hydroxyethyl) cysteine, and N-acetyl-S-(phenacyl) cysteine. These metabolites were present in a molar ratio of 65:34:1. In a separate

experiment, five rats each received a single ip injection of styrene (250 mg/kg); total mercapturic acids as a percentage of the initial dose were determined to be 10.4% on the first day, and 0.26% on the second day. No mercapturic acids were found on the third day.

Later, in 1980 these same investigators, Delbressine et al. [215] studied whether phenaceturic acid was a urinary metabolite in adult female rats after ip injections of styrene (150 mg/kg) or styrene oxide (150 mg/kg) in sesame oil 5 days per week for 3 weeks. To exclude interference from naturally occurring phenaceturic acid, the experiments were repeated with d8-styrene (styrene with deuterium in the 8 position). After administration of styrene to rats, significantly greater amounts ($p<0.0025$) of phenaceturic acid were isolated from urine during the first 24 hours (as compared with controls) and identified as its methyl ester by thin layer chromatography with the structure confirmed by nuclear magnetic resonance spectroscopy. Quantitative results obtained with gas-liquid chromatography showed 1.4% of a single administered dose to be phenaceturic acid. Neither a single dose nor continuous administration of styrene oxide resulted in any significant increase in urinary phenaceturic acid excretion compared to the control group; the reason for this finding was not clear to the investigators [215]. The studies with d8-labeled styrene and styrene oxide confirmed the findings with the unlabeled substances.

In 1970, Bakke and Scheline [216] studied the metabolism of styrene to phenolic compounds in five male rats. The rats were fed neomycin for 6 days before administration of styrene to inhibit formation of natural phenolic compounds. Styrene, dissolved in 1 ml of propylene glycol, was administered by stomach tube at 100 mg/kg and urine was collected for 48 hours. To convert any metabolites from their conjugated form, the urine samples were treated with an enzyme preparation that contained beta-glucuronidase and sulfatase. During the 48 hours, 0.1% of the initial dose of styrene was recovered as 4-vinylphenol. Other alcoholic metabolites identified were 1-phenylethanol and a trace of 2-phenylethanol. Bakke and Scheline [216] also looked for, but did not find, phenylglycol. Excretion of p-ethylphenol was not greater than control values.

In 1978, Pantarotto et al. [217] administered styrene ip to Sprague-Dawley rats. In addition to phenylethylene glycol and mandelic, benzoic, and hippuric acids, phenolic metabolites, namely, 4-vinylphenol, p-hydroxymandelic acid, p-hydroxybenzoic acid, and p-hydroxyhippuric acid were identified in the urine of the treated animals. These metabolites were characterized by mass spectrometry and by comparative thin layer chromatography with standard compounds. The investigators [217] theorized that the phenolic metabolites could have been formed as a result of chemical rearrangements of unstable arene oxides.

In 1972, Ikeda et al. [218] studied the effects of coadministration of toluene or pretreatment with phenobarbital on the metabolism of styrene in female Wistar rats. Both styrene (228 mg/kg) and toluene (217 mg/kg) were administered ip in soybean oil. Simultaneous administration of toluene

suppressed and delayed the excretion of mandelic and phenylglyoxylic acids, which suggested a competitive inhibition of styrene metabolism by toluene. The observed effect of toluene was attributed to competitive inhibition of the oxidative processes involved in the metabolism of styrene, since phenobarbital pretreatment counteracted this effect [218]. The suppression of oxidation of styrene was a transient effect, since the levels of styrene metabolites in the urine returned to control values 6-8 hours after injection of toluene.

In 1974, Ikeda et al. [159] further studied the metabolism of styrene in rats when styrene was injected ip at doses up to 910 mg/kg into rats and the time course of the urinary excretion of mandelic, phenylglyoxylic, and hippuric acids was monitored. There was a delay in excretion of mandelic and phenylglyoxylic acids; the amounts of these acids excreted were linear with respect to dose only up to styrene doses of 200-250 mg/kg. There were no significant differences between control and styrene-treated animals with respect to urinary hippuric acid excretion at styrene doses below 100 mg/kg. Excretion of hippuric acid became linear with respect to styrene doses above 100 mg/kg [159].

In 1967, James and White [219] studied the formation of mercapturic acids in adult female rabbits and rats treated with styrene and styrene oxide. In one experiment, rats that had been fed yeast containing radioactive sulfur (35S) were given styrene and styrene oxide by stomach tube at doses of about 200 or 250 mg/kg, respectively. Urine was collected over the next 24 hours and assayed for radioactivity. Administration of styrene and styrene oxide resulted in excretion of relatively large amounts of radioactively-labeled hydroxyphenylmercapturic acid, indicating that hydroxylation of the benzene ring of the parent compound had occurred. Traces of labeled phenethylmercapturic acid and an unidentified labeled metabolite were also found.

In another experiment by James and White [219], hydroxyphenylmercapturic acid but no phenethylmercapturic acid was found when unlabeled styrene (145 mg/kg) and styrene oxide (185 mg/kg) were administered by stomach tube to rabbits. No mercapturic acids were found when phenylglycol was given to either rabbits (185 mg/kg) or rats (170 mg/kg). After administration of phenethylmercapturic acid, the rabbits excreted only small amounts of the hydroxy derivative, indicating that another source of this compound was probable. Administration of 8-14C-labeled styrene oxide to rabbits resulted in large amounts of labeled mandelic acid and no naphthoresorcinol-reactive substances. In rabbits, 32% of the administered styrene and 30% of the styrene oxide were converted to mandelic acid. James and White [219] also found that 15-32% of the styrene oxide administered was excreted in rabbits as hippuric acid. With phenylglycol, 17-29% was recovered as mandelic acid, and 4-13% as hippuric acid [219]. No phenolic compounds other than those normally in urine were observed in rabbits given styrene oxide.

In 1977, Vainio and Makinen [220] found that styrene in olive oil, administered ip at 150-1,000 mg/kg, caused a reduction in the hepatic nonprotein sulfhydryl content of female mice, male Wistar rats, female hamsters, and male guinea pigs in about an hour after the injection. Vainio and Makinen [220] suggested that this might reflect the conjugation of reactive alkylating metabolites with hepatic glutathione; they pointed out that the mouse was the most sensitive to this action of styrene, and the rat the least sensitive, possibly because mouse liver has a high epoxide-forming capacity and a low epoxide-inactivating capacity as compared to rat liver.

In 1976, Parkki et al. [221] reported that the activity of epoxide hydratase in Wistar rats increased with increasing styrene doses. Styrene was injected ip in corn oil in daily doses of 100 mg/kg for 3 or 6 days, or 500 mg/kg for 1, 3, or 6 days, or 2,000 mg/kg in one dose. No increases in epoxide hydratase were observed after 3 or 6 doses of 100 mg/kg; statistically significant increases occurred after a single dose of 2,000 mg/kg and 3 or 6 doses of 500 mg/kg. No increase in the activity of uridine diphosphate-glucuronosyltransferase was observed with styrene at any dose, nor was any increase observed in activities of the hydratase or the transferase after one or three doses of styrene oxide (375 mg/kg) or phenylethylene glycol (750 mg/kg).

In 1976, Delag et al. [222] investigated the effect of styrene on carbohydrate metabolism in rats. Glucose, styrene, or glucose and styrene together were administered by stomach tube to two groups of Wistar rats that were either on standard diet or had been food-deprived for 17 hours. Two hours after administration of the compounds, the animals were sacrificed and the left caudate lobe of the liver, the left ventricle of the heart, and the left thigh muscle were removed, homogenized, and assayed for glycogen using anthrone. The results are presented in Table IV-25.

TABLE IV-25

GLUCOSE AND GLYCOGEN IN LIVER AFTER ADMINISTRATION OF GLUCOSE, STYRENE, OR GLUCOSE AND STYRENE TO RATS

Substance(s) Administered	Glucose (mg/g tissue)*		Glycogen (mg/g tissue)*	
	Fed	Fasted	Fed	Fasted
None (control)	5.0	6.3	49.1	13.4
Glucose**	5.6	5.6	39.3	16.6
Styrene**	4.0	5.9	21.2	10.7
Glucose and styrene**	5.9	6.1	33.0	18.2

*Mean of 7 determinations
**Each administered at 2.5g/kg body weight
Taken from Delag et al. [222]

Although styrene caused depletion of hepatic glycogen in both fed and fasted animals, the data [222] indicate that its effect could be reversed by the simultaneous administration of glucose.

In a 1968 abstract, Leibman and Ortiz [223] presented evidence that in the conversion of styrene to phenylethylene glycol in mammalian liver microsomes requiring NADPH and oxygen, styrene oxide was the primary product of the NADPH-dependent oxidation. Mixtures containing styrene, nicotinamide adenine dinucleotide phosphate (NADP), glucose-6-phosphate dehydrogenase, and 9000 x g supernatant fraction of rabbit liver were incubated in air for 5 minutes, and extracted. Styrene oxide was identified either by direct gas chromatography of the extracts or by thin-layer chromatography of the picric derivative. No styrene oxide was found when NADP and glucose-6-phosphate dehydrogenase were omitted. When 8-14C styrene was incubated in the above system with a pool of unlabeled styrene oxide, about equal radioactivity was found in the phenylethylene glycol and in the picric derivative of styrene epoxide.

Later, in 1969, Leibman and Ortiz [224] demonstrated the in vitro formation of phenylglycol from styrene by hepatic microsomal preparations in rats and rabbits pretreated with phenobarbital. Ethyl acetate extracts of a reaction mixture that contained 50 µmol of styrene in 0.5 ml of dimethylformamide were qualitatively analyzed by gas chromatography. Peaks having the same retention times as a reference standard of phenylglycol appeared following incubation. In addition, thin layer chromatography of the ethyl acetate extracts after both acetylation and trimethylsilylation yielded areas having the same reference values in three different solvent systems as the acetylated and silylated derivatives of standard phenylglycol [224].

In 1970, this work was further extended by Leibman and Ortiz [225] to demonstrate that styrene oxide may be an intermediate in the formation of phenylglycol. Styrene oxide was identified in ethyl acetate extracts of rabbit liver microsomal preparations that had been incubated with unlabeled styrene and styrene labeled with carbon-14 at the 8 position. The oxide intermediate was demonstrated by three methods: gas-liquid chromatography, thin layer chromatography, and by accumulation of radioactivity in a pool of styrene oxide added to reduce the effect of epoxide hydratase.

In 1976, Salmona et al. [226] studied the enzymatic capacity for epoxidation in the liver, lungs, kidneys, heart, spleen, and brain of male and female rats. Microsomes from the organs were incubated with styrene or styrene oxide, and the activities of monooxygenase and of epoxide hydratase were assayed. The investigators [226] found that styrene oxide formation, i.e., monooxygenase activity, is dependent on the availability of NADPH. Inhibitors of cytochrome P-450 (metyrapone and SKF-525A) and inhibitors of epoxide hydratase (cyclohexene oxide and epoxytrichloropropene) were studied for their effect on enzyme activity of hepatic microsomes. The cytochrome P450 inhibitors significantly inhibited ($p<0.01$) both styrene monooxygenase

and epoxide hydratase activities. The epoxide hydratase inhibitors markedly inhibited epoxide hydratase but not monooxygenase activity.

In 1978, Cantoni et al. [227] studied the activities of styrene monooxygenase and epoxide hydratase in rats, mice, guinea pigs, and rabbits of each sex and found the highest activity of each enzyme in the liver, with the hydratase activity being higher than that of monooxygenase. Each enzyme was also found in microsomal fractions from the heart, lungs, spleen, and kidneys. Styrene or styrene oxide was the substrate used in incubating the microsomal preparations prior to enzyme assay. Because of the high ratio of hydratase to oxygenase activity in the rat, Cantoni et al. [227] suggested that the rat would be relatively resistant to styrene toxicity, assuming that oxygenase caused styrene oxide to form and that hydratase (as well as glutathione-S-transferase) inactivated the styrene oxide. Because of the low activity of epoxide hydratase in mouse and rabbit lungs in comparison to activating capacity, the investigators [227] speculated that the lungs of these two species would be the most sensitive site for styrene toxicity.

Because of the work of Bakke and Scheline [216], who found 4-vinylphenol in the urine of rats given large amounts of styrene, Watabe et al. [180] suggested in 1978 that 1-vinylbenzene-3,4-oxide (styrene-3,4-oxide) might be the precursor of both 4-vinylphenol and 1,2-dihydroxy-1,2-dihydro-4-vinylbenzene, and that an oxygenated metabolite of the latter compound is a proximate mutagen. In another 1978 investigation, Watabe et al. [228] incubated rat liver microsomes with styrene, and identified styrene oxide (or at least a chromatographic peak with the same retention time as styrene oxide). Maximal amounts of the oxide were found after an incubation period of 10 minutes, thereafter decreasing so that at 40 minutes the oxide was not detectable. Styrene glycol was formed in increasing amounts until a maximum was reached at 60 minutes.

In 1981, Belvedere and Tursi [229] studied the oxidation of styrene to styrene oxide in human blood erythrocyte and lymphocyte suspensions. Styrene oxide formed enzymatically was quantitatively hydrated by acidification to styrene glycol, a compound more suitable for gas chromatographic analysis. Styrene oxidation in human erythrocytes was inhibited by carbon monoxide, occurred in the absence of NADPH and NADP, and was undetectable in the absence of oxygen. The finding that oxygen was required to catalyze the formation of styrene glycol is indicative that the glycol was in fact formed from styrene oxide and not directly by the hydration of styrene. Lymphocytes (with the addition of NADPH and NADP) were more active than red blood cells in styrene oxidation.

However, in 1982, Beije and Jenssen [230] conducted a mutagenicity study with an isolated perfused rat liver as a metabolizing system and Chinese hamster V79 cells as genetic target cells. Styrene oxide was rapidly metabolized by the perfused liver, and thus, no mutagenic effect was detected. However, when styrene was added to the perfusion system, an increase in mutations in the V79 cells were observed regardless of where in

the circulation perfusion medium the V79 cells were placed. The mutagenicity of styrene was five times higher as compared to using an S9 mix from the same rat strain as the metabolizing system. The simultaneous analysis of styrene oxide in the perfusion medium indicated concentrations only 2-4% of the original amount of styrene. Beije and Jenssen [230] suggested that their results using the liver perfusion/cell culture system mimics the metabolism expected to be found in the intact animal, thus indicating that styrene oxide is not the principal mutagenic metabolite of styrene *in vivo*.

In 1977, Ryan and Bend [231] demonstrated that biliary excretion of the glutathione conjugate was dose-dependent with respect to styrene oxide in perfused rat liver preparations. In 1977, additional work with styrene oxide was reported by Bend et al. [232] who perfused rat livers *in vivo* by cannulation of the portal vein, the thoracic vena cava, and the bile duct. Using styrene oxide at 0-500 µmol per liver, they found labeled 8-14C styrene oxide bound to DNA, RNA, and protein, even when the organ was challenged with only 1 µmol of styrene oxide. The binding increased with increasing styrene oxide concentrations, and at 500 µmol a marked increase in its binding to protein was observed simultaneously with microscopic changes of the liver and depletion of hepatic glutathione [232].

SUMMARY

A summary of information on possible effects of styrene on health follows.

Effects on the Nervous System

Experimental exposures in humans to styrene have demonstrated that styrene causes CNS depression [68,69,70,71,72]. Within minutes of an exposure at 800 ppm, 2 men experienced listlessness, drowsiness, and impaired balance [68]. During a 1-hour exposure to 376 ppm, decrements in balance, coordination, and manual dexterity tests were measured, and subjective complaints of headache, nausea, and a feeling of slight inebriation were reported [69]. A significant difference in reaction time was noted in 12 subjects after a 2-hour exposure that included consecutive 30-minute styrene exposures of 50, 150, 250, and 350 ppm [71]. Slower reaction times were found in 2 or 3 subjects during 90-minute exposures of 50, 100, and 200 ppm; loss of balance was found for 3 subjects during the 90-minute exposure at 200 ppm [72]. Subjective complaints reported by 6 subjects during 90-minute styrene exposures at 50, 100, or 200 ppm included headache, fatigue, sleepiness, malaise, difficulty in concentrating, and a feeling of intoxication [72]. During the course of a study lasting a couple of weeks, which included a series of styrene exposures at 20, 100, and 125 ppm for 7.5 hours, there were some changes in 3 of 6 subjects in both visual

evoked response and EEG amplitude, which the investigators [70] deemed to be consistent with CNS depression.

However, there have also been experimental studies showing styrene to have little effect on the central nervous system. After experimental styrene exposures of 50 ppm for 1 hour, 99 ppm for 7 hours, 117 ppm for 2 hours, or 216 ppm for 1 hour, no significant CNS effects (e.g., decrements in balance, coordination, and manual dexterity) were noted [69]. No changes in manual dexterity or perceptual tests were noted after consecutive 30-minute exposures to 50, 150, 250, and 350 ppm [71]. Loss of balance was not found during 90-minute exposures to 50 or 100 ppm [72]. In exposures for 1, 3, or 7.5 hours to 20, 100, or 125 ppm of styrene, no deleterious effects on equilibrium, cognitive testing scores, or EMGs were found [70]. No significant optokinetic changes were noted in 5 subjects exposed to 300 ppm of styrene for 1 hour [73].

Workers exposed to styrene have experienced weakness [105], increased reaction times [111,392], abnormal EEGs [63,106,108,118,124,125], and headache, fatigue, malaise, tension, or dizziness [59,63,67,82,84,94,101,102, 105,106,108,109,110,112,113,123,138]. Styrene exposures in many of these studies were either not determined [63,67,101,108], or were at times greater than 100 ppm for some of the workers [59,102,105,106,111,112,113,123,138]. However, at one RP/C facility where the average styrene exposure was estimated from urinary mandelic acid measurements to be about 30 ppm, 24% of the workers had EEGs judged to be abnormal [124].

There is some evidence of styrene-induced peripheral neuropathy. In an investigation of workers at a U.S. styrene and polystyrene production plant (with potential exposures to styrene, benzene, ethylbenzene, and toluene), radial and peroneal nerve conduction velocities were said to be reduced, but comparative data on normal velocities were not given [81]. It was also reported in the same study [81] that radial and peroneal nerve conduction velocities decreased with increasing duration of exposure, but because appropriate corrections for age were not made, it is not evident whether the decrement with longer exposure was due to the longer exposure, increasing age, or a combination of both. In a Swedish study [123], 10 of 33 workers exposed to about 5-125 ppm of styrene had evidence of a mild sensory neuropathy with polyphasic sensory responses; these 10 workers were older than those not having signs of neuropathy, and they were more heavily exposed. The investigators [123] speculated that age alone did not cause these effects, but that the effects of age and styrene exposure might have been synergistic. It seems evident that more investigation of nervous system effects is needed.

Irritant Effects

Irritation of the eyes has occurred in human subjects undergoing single exposures [68,69,70,72] and in workers during repeated exposures

[35,53,58,61,104,110,113,126] to styrene. Styrene has also been reported to cause superficial transient burns of the human cornea [55]. Irritation of the respiratory tract has occurred in human subjects [68,69,70,72] and in workers [35,53,59,56,93,104,113,123,126] exposed to styrene. As would be expected since styrene is a defatting agent, styrene is a primary skin irritant [234], and has caused dermatitis, including a rash and chapped skin in workers [54,56,61,91,104,113,122,126].

Volunteer subjects exposed to styrene vapor at 800 ppm had immediate eye, nose, and throat irritation [68]. At 376, 216, and 99 ppm, human subjects developed eye or respiratory tract irritation within 20 minutes of the start of exposure [69]. In another experimental study [70], the incidence of eye, nose, and throat irritation for male subjects was 13% at 0 ppm, 17% at 20 ppm, 20% at 100 ppm, 33% during exposures fluctuating between 75 and 125 ppm, and 45% at 125 ppm styrene; the incidence of eye, nose, and throat irritation for female subjects was 8% at 0 ppm and 32% at 100 ppm. Irritation of the eyes was noted by volunteer subjects in another study [72] during styrene exposures of 50, 100, 200, and 300 ppm.

Workers exposed to styrene in reinforced plastics applications complained of eye and respiratory tract irritation, but were able to tolerate styrene exposures in excess of 500 ppm for several hours at a time [35]. Among 35 reinforced plastics workers exposed to styrene at 44-550 ppm, 34 complained of some sort of eye, nose, or throat irritation; and about half of them complained of wheezing, shortness of breath, or chest tightness [104]; exposure to an isocyanate (MDI) might have contributed to some of these effects. Complaints of wheezing and chest tightness have also been noted in workers during an investigation of a styrene and polystyrene production plant [82]; however, spirometric studies of airway effects did not suggest significant changes, nor was there any radiologic evidence of significant lung change observed. In a factory where reinforced plastics were made, workers exposed to TWA styrene concentrations of 9-111 ppm (average 69 ppm) complained of chest tightness (23% of the workers), wheezing (18%), and shortness of breath (54%), but ventilatory function was significantly changed during the shift only in those workers that smoked [113]. Among 21 workers exposed to styrene at about 75 ppm for about 10 years at another RP/C plant [114], there were four cases of reduced FEV_1, but whether the cause was age, styrene exposure, or other factors was not clear. As compared to controls, a significantly greater number of RP/C workers in another clinical study had abnormal pulmonary function [91].

Irritation of the eyes and upper respiratory tract has occurred in some workers and experimental subjects at styrene concentrations well below 200 ppm. More research into long-term respiratory effects of styrene is needed.

Effects Involving the Liver

Various clinical studies [82,96,103,113,115,116,120] have suggested that styrene exposure has affected liver function, based on several tests. Among the findings, which may indicate liver function changes, were elevated serum enzyme activities [82,96,103], elevated serum uric acid [113], increased glucose tolerance [115,116,120], and a low glucose assimilation coefficient [116]. In one study designed to investigate liver function among polystyrene production workers, the authors [96] concluded that styrene exposure caused hepatic dysfunction reflecting metabolic disturbances, but not of a pathological degree.

A clear-cut trend toward altered liver function has not been demonstrated. In addition, the more commonly used tests such as changes in serum enzyme levels of hepatic origin were not always used. Animal studies [53] have suggested that styrene is hepatotoxic only at concentrations much higher than those found in the workplace. Damage to the hepatic parenchyma of rats after a single exposure to styrene vapor at 2,500 ppm for up to 21 hours was reported by one group of investigators [53], who also reported increases in the liver weights of rats repeatedly exposed to styrene vapor at 1,300 ppm for 130-139 days. However, they found no liver damage in guinea pigs exposed at 650 ppm or rabbits and monkeys exposed at 1,300 ppm for similar periods. Another group [169] noted parenchymal alterations and congestion in the liver of rats intermittently exposed for 2 weeks to 300 ppm of styrene 6 hours per day, 5 days per week. Research to clarify the role of styrene on liver status is needed. Meanwhile, for worker protection, liver function should be periodically assessed due to the importance of this organ in the biotransformation and detoxification of toxic substances.

Mutagenicity

Many *in vitro* tests of mutagenicity have indicated that styrene has no mutagenic activity. This has been found with *S. typhimurium* strains TA 98, TA 100, TA 1535, TA 1537, and TA 1538 [172,173,174,176] as well as with *E. coli* K12 [174], the yeasts *S. pombe* and *S. cerevisiae* [175], and cultured Chinese hamster V79 cells [175,176]. However, a few studies [171,177,178] have found weak evidence of the mutagenicity of styrene in *S. typhimurium* strains TA 98 and TA 1538. Styrene oxide has been shown to be mutagenic toward *S. typhimurium* TA 100 and TA 1535 [171,172,173,174,176,178], *E. coli* K12 [174], the yeasts *S. pombe* and *S. cerevisiae* [175], and Chinese hamster V79 cells [175].

There was increased uptake of tritiated thymidine into the DNA of heteroploid human cells, reflecting unscheduled DNA synthesis, following treatment with styrene oxide but not with styrene [176]. Chromosomes from human lymphocytes incubated with styrene or styrene oxide had more abnormalities than controls, though the abnormalities in chromosomes in styrene-treated cells were different from those in the styrene oxide-treated

cells [179]. Doubts about the applicability of this *in vitro* test are amplified by the suggestion of the investigators [179] that impurities in the styrene preparation might have been responsible for the effects. However, a later study by the same group [181] showed a clear dose-response relationship in the induction of sister chromatid exchanges (SCE) in human whole blood lymphocyte cultures treated with styrene.

In vivo tests have also given conflicting results. In a host-mediated assay [175], a high dose of styrene (i.e., 1 g/kg) produced gene conversions in the yeast *S. cerevisiae* which had been injected ip into mice. Mice intubated with styrene oxide, but not those intubated with styrene, had a significantly elevated incidence of chromosomal aberrations in bone marrow cells removed 24 hours after administration of the dose [176]. An increase in the frequency of SCE in alveolar macrophages, in bone marrow cells, and in regenerating liver cells was observed in mice exposed to styrene at 565 ppm for 6 hours per day for 4 days [183]. Chromosomal breaks were also found in the bone marrow of rats exposed to styrene vapor [184], but not in mice [176] or hamsters [185]. Styrene has also caused recessive lethal mutations in fruit flies [187].

Chromosomal changes were found to be more frequent in the lymphocytes of styrene-exposed workers in several European RP/C factories than among controls [76,129,130,131,132]. An increased frequency of SCE in the lymphocytes of styrene-exposed workers has also been reported [132]. However, other studies of workers exposed to styrene have shown no significant increases in either chromosomal aberrations [134,136] or SCE [136]. In a study of unscheduled DNA synthesis in lymphocytes of styrene-exposed workers, styrene did not alter the efficiency of DNA repair, but rather predisposed the lymphocytes to an increased risk for DNA damage from subsequent exposures to genotoxic agents [137].

The reasons for the different results in the many tests of mutagenicity of styrene are not known, so a conclusion on whether styrene is a mutagen seems premature. It may be that these different results reflect the different capabilities or efficiencies of metabolism by the various species or mutagen assay systems; for example, if one species hydrates styrene epoxide as soon as it is formed, styrene should be less potent, or even inactive, as a mutagen than in another species lacking the ability to inactivate the epoxide so rapidly. (This argument is based on the reasonable although unproved premise that styrene epoxide formation is the basis for any mutations.)

Reproductive Effects

Congenital defects in children whose mothers had been occupationally exposed to styrene were reported by Holmberg [66]; a more thorough investigation is needed to verify the implications of these findings. The finding of styrene, at unstated concentrations, in umbilical cord blood [65] suggests that styrene can cross the placenta; in these cases, the

source of the styrene exposure of the mothers was not found. Placental transfer of styrene in rats has also been noted [189]. An increased rate of spontaneous abortions observed among Finnish styrene workers [139] might be compatible with findings of cytogenetic effects or of terata; thus, despite lack of confirmation of this study, the finding warrants concern. A later study, however, found no difference in the number of spontaneous abortions between styrene workers and a group of age- and social class-matched controls with no solvent exposures [140].

Ragul'ye [190] found an increased incidence of pre-implantation loss and total embryo mortality in female rats that had been exposed to styrene at 12 ppm for 4 hours per day throughout gestation. The study by Vergiyeva et al. [191], conducted like that of Ragul'ye [190] but involving a higher styrene exposure concentration of 47 ppm, found no significant evidence of embryotoxicity, thereby refuting the Ragul'ye study. Murray et al. [192] investigated teratogenicity in rats and rabbits exposed to styrene by inhalation of vapor or by intubation of liquid and found no significant evidence that styrene is teratogenic; however, the dams were not exposed during the first or last trimester of gestation. None of these three investigations [190,191,192] found evidence of teratogenicity. Similarly, Kankaanpaa et al. [193] did not find a significant excess of malformed fetuses from mice and hamsters exposed to styrene vapor; however, fetotoxicity (an excess of dead or resorbed fetuses) was found from hamsters exposed on days 6-18 of pregnancy at 1,000 ppm styrene, but not at lower concentrations. Ponomarkov and Tomatis [196] found a significantly greater pre-weaning mortality of progeny of female 020 mice administered a single 1,350 mg styrene/kg dose by stomach tube on day 17 of gestation; significant results were not found in similar experiments with C57 Bl mice or BD IV rats. A study [170] sponsored by the Chemical Manufacturers Association concluded that styrene administered in the drinking water had no deleterious effects on the reproductive capacity of rats through three generations.

In summary, styrene does not appear to be teratogenic, embryotoxic, or fetotoxic but further investigations of the reproductive outcomes of workers exposed to styrene are needed.

Carcinogenicity

The possible carcinogenicity of styrene has been investigated by long-term administration to rodents [195,196,197,198]. One of these investigations [195] involved vapor exposure. That study, which used rats, resulted in an increase in the combined incidence of leukemia and lymphosarcoma in female rats only that was not statistically significant. There was a high incidence of intercurrent disease (i.e., chronic murine pneumonia) in the male rats such that conclusions from the investigation are inappropriate. In another study [196], styrene was administered in olive oil by stomach tube to one strain of rats and two strains of mice. There was a statistically significant increase in lung adenomas and adenocarcinomas only in female mice of one strain; the authors [196]

suggested that their data provided weak evidence of the carcinogenicity of styrene. An NCI investigation [197] also developed equivocal evidence. There was a significant increase in lung adenomas and adenocarcinomas in male mice (when compared with matched controls but not when compared with historical controls), but not in female mice or rats. NCI [197] concluded that the data provided "suggestive" but not "convincing" evidence of the carcinogenicity of styrene, and recommended another experiment to help resolve the point. A companion experiment [198], involving the administration of a mixture of styrene and beta-nitrostyrene, also gave equivocal results.

Mortality studies of workers exposed to styrene have not shown an excess cancer mortality [31,78,83,84,142]. There have, however, been suggestions of an excess of leukemia. Nicholson et al. [83] studied the mortality experience in one U.S. styrene and polystyrene production plant, and found 83 total deaths vs. 106 expected among those exposed 5 years or more. There was no excess of deaths from cancer or from any of the nonneoplastic diseases examined such as cardiovascular or respiratory disease. The investigators [83] anticipated an excess of leukemia because of previous exposure of many of these workers to benzene; they found two cases of leukemia and one of lymphoma in the study group. They examined 361 death certificates of other workers employed at least 6 months at the plant and five additional cases of leukemia and four of lymphoma were found. The investigators [83] concluded that the information found regarding leukemia was not definitive.

In a U.S. styrene production plant, Ott et al. [31] found fewer total deaths (303 vs. 425) and fewer cancer deaths (58 vs. 76.5) among 2,904 workers at four plants than would be expected from the U.S. white male population. Most of these workers had been exposed to low levels of styrene during their employment (TWA concentrations were 10 ppm or less). The mortality data on the styrene workers (i.e., production and non-professional research) was also compared with previous mortality experiences from that company, and the styrene workers had a lower total mortality and a lower cancer mortality. However, there was a significant excess ($p<0.05$) of leukemia deaths compared with other company workers (6 observed vs. 1.6 expected), although the excess was not significant when compared with the U.S. population (6 vs. 2.9). Most of the excess was due to lymphatic leukemia. Some workers had been exposed many years earlier to high levels of benzene, but whether those men with leukemia had been exposed to benzene was not evident.

No excess of cancer was seen in a German styrene and polystyrene plant [78]. In that study, 74 death certificates were examined, and there were 12 deaths from cancer, 3 of them from lung cancer, neither significantly different from the comparison groups.

In a study of 1,205 reinforced plastics workers in Sweden [142], where exposure levels were thought to be 150-300 ppm styrene in recent years and higher in the past, a slight but nonsignificant decrement in cancer

incidence from that expected in the Swedish population was found. The latency period was not high for many of the workers, so further followup might reveal more cases of cancer.

From the experimental animal investigations and from the epidemiological studies, there seems little basis to conclude that styrene is carcinogenic. If styrene oxide is an intermediate metabolite, covalent binding to nucleic acids leading to cancer development might be predicted; however, there is little evidence that this epoxide is formed *in vivo*. Nonetheless, the enzyme catalyzing the formation of this epoxide from carbon-carbon double bonds exists in many tissues, as do the enzymes catalyzing the hydration or other inactivation of the epoxide. If these enzymes are in the same cells, it may be that the epoxide is formed and then rapidly deactivated; if it exists long enough, it might be carcinogenic. This speculation, if valid, could suggest that styrene is a weak carcinogen. Examination of the ratios of these enzymes in various tissues of several animal species [220,227] suggests that the mouse should be one of the more sensitive species. Two studies of experimental carcinogenesis [196,197] found positive results only in mice, a point compatible with the speculation, even if not proving it. Suspicions that the epoxide may be stable long enough to react with other molecules before being inactivated are enhanced by evidence [235] that the cytochrome P-450 monooxygenase system is not close, in a spatial sense, to epoxide hydratase and to transferases in the endoplasmic reticulum.

Thus, while it does not seem appropriate from presently available evidence to conclude that styrene can cause cancer among exposed workers, there is enough reason to suggest it might be at least a weak carcinogen, and priority should be given to further studies of this problem.

While several experimental tests of the carcinogenicity of styrene oxide have been inconclusive [199,200,201], an investigation in Italy [202] developed persuasive evidence that styrene oxide can cause stomach tumors in rats administered the compound by intragastric catheter. It seems reasonable to predict that administration of this compound by inhalation could cause tumors of the respiratory tract, and that styrene oxide could cause cancer in humans.

Uptake, Metabolism, and Elimination

Liquid styrene is readily absorbed through intact skin of man [151] and of animals [165]. Moreover, styrene vapor can penetrate the skin of man [154], although less efficiently than the liquid. Styrene is also retained after inhalation of the vapor, with retention rates reported to vary from 60 to 75% [69,88,143,146].

Absorbed styrene is eliminated from humans mostly in the urine as metabolites, but some excretion of unchanged styrene from the lungs occurs [91]. Metabolites reported in human urine include mandelic acid [35,72,88, 109,125,144,151,159], phenylglyoxylic acid [35,144,159], and, at higher

doses, hippuric acid [68,159]. These are also the most commonly found metabolites in the urine of styrene-treated experimental animals [53,206,207, 210,211,212,213,217,219], but other metabolites have also been found, namely, mercapturic acids [53,210,214,219], benzoic acid [206,207,217], 4-vinylphenol [216,217], 2-phenylethanol [216], phenaceturic acid [215], and phenylglycol [210,212], some of which may be excreted as conjugates of glucuronic acid or glutathione [68,203,210,213].

Styrene oxide, also known as styrene epoxide, has been proposed [224,225] as an intermediate in the metabolism of styrene, but it has not been found *in vivo*. However, phenobarbital-activated rat microsomes converted styrene to the epoxide [224] as determined by an indirect method.

An epoxide-forming enzyme (monooxygenase) has been found in a number of tissues of mice, rats, guinea pigs, and rabbits [226,227], but an epoxide inactivating enzyme (glutathione-S-transferase or epoxide hydratase) was also present in these same tissues. Thus, it seems possible that styrene is converted to styrene epoxide and is then further converted to such final products as mandelic and phenylglyoxylic acids. Whether styrene oxide (if formed) is present long enough to bind covalently to genetic material or other macro molecules is not known; from indirect evidence, styrene's lack of potency in causing mutations or tumors suggests that styrene oxide, if formed, is present only briefly.

This ability of epoxides to alkylate or arylate nucleic acid, thereby presumably leading to germinal or somatic mutations, is the major concern in the question of intermediary metabolism of styrene.

Other Effects

Other effects reported among styrene-exposed persons include gastralgia and nausea [72,101,102,104,109,110], nose bleed [93,104,105], blood dyscrasias [102], lowered arterial blood pressure [94], elevated blood pressure [105], enlarged thyroid [113], retrobulbar neuritis [57], thrombosis of the central retinal vein [62], gall bladder inflammation [74,105], decreased vision [59], decreased alcohol tolerance [128], abnormal thyroid size [113], and psychasthenia [64]. It does not seem appropriate to attribute these to styrene absorption (except perhaps for the gastrointestinal effects) on the basis of these few, unconfirmed observations.

Conclusion

Styrene is readily absorbed by the respiratory and gastrointestinal systems, and the skin. Exposures to styrene have caused CNS depression; subjective complaints included headache, fatigue, sleepiness, nausea, malaise, difficulty in concentrating, and a feeling of intoxication.

Decrements in balance, coordination, and manual dexterity tests have also been reported, as have slower reaction times and abnormal EEGs. Styrene vapor is also an irritant to the eyes and upper respiratory system, and liquid styrene is a skin irritant. Various clinical studies have suggested that styrene exposure has affected liver function.

Limited human data suggest that styrene might be teratogenic, but several studies with experimental animals indicate that it is not. Most, but not all, *in vitro* studies suggest that styrene is not mutagenic, but some mammalian studies, including observations of several groups of styrene workers, suggest cytogenetic changes may result from working with styrene. An increased rate of spontaneous abortions was observed in one group of RP/C workers, but not in another group. Styrene has been associated with an increase in lung tumors (although not consistently among species) in two experimental animal studies, while another study showed an elevation, though not statistically significant, in the combined incidence of leukemia and lymphosarcoma in female rats. Mortality studies of styrene workers have shown no excesses in overall cancer incidence. However, excesses of deaths, though not statistically significant, have been reported in the specific cancer categories "Lymphatic and Hematopoietic, except Leukemia" and "Leukemia."

Most of the styrene absorbed by humans is excreted in the urine as mandelic and phenylglyoxylic acids, and the urinary concentrations of the two or of just mandelic acid reflect amounts of styrene absorbed through the respiratory tract and through the skin (as well as through the gastrointestinal tract, if poor hygiene and work practices allow ingestion).