

CHAPTER III

STYRENE

Styrene, $C_6H_5CH=CH_2$, is a clear, colorless or yellowish liquid with a strong and disagreeable odor. The addition of an ethylene group to the aromatic ring makes styrene considerably less volatile and more fat soluble than benzene (Appendices 1 and 2). Styrene is used extensively in the production of synthetic rubbers and plastics (e.g. polystyrene, styrene-acrylonitrile and styrene-acrylonitrile-butadiene copolymers), as a modifier or solvent of polyester resins and as a starting material and intermediate in chemical syntheses (51, 146). The U.S. production of styrene in 1977 was about 3.1 million tons (432).

TOXICITY OF STYRENE

Styrene is primarily a skin and mucous membrane irritant and a narcotic. Spencer *et al.* (413), performing single exposures of rats and guinea pigs to 1300-10000 ppm styrene, noted increasing eye and nose irritation with increasing atmospheric concentrations. General weakness and unsteadiness was observed after 12-30 hours exposure to 1300 ppm. Exposure to 2500 ppm resulted in weakness, stupor, incoordination, loss of equilibrium, tremors and unconsciousness after 10-12 hours.

All rats and guinea pigs died within 21 and 14 hours respectively. At 5000 ppm the animals showed signs of CNS effects immediately and became unconscious within 1 hour. 10000 ppm styrene was lethal for all animals within 3 hours. 2-4 weeks after exposure the surviving animals were autopsied. The most prominent organ lesions were found in the lungs, characterized by congestion, hemorrhage and edema. There were also some parenchymatous liver and kidney changes. Wolf *et al.* (475) determined the oral LD₅₀ of styrene in rats to be 5.0 g/kg. Compared to benzene, toluene and xylene, styrene was more acutely toxic than benzene (LD₅₀ 5.6 g/kg) and toluene (LD₅₀ 7.0 g/kg) but less toxic than xylene (LD₅₀ 4.3 g/kg). When the rats were autopsied, slight liver and kidney changes were noted. Stewart *et al.* (423) exposed human volunteers to 52-376 ppm styrene for 1-7 hours. Exposure to 52 ppm for 1 hour or 117 ppm for 2 hours produced no objective or subjective signs of illness. Exposure to 216 ppm produced nasal irritation after 20 minutes. When exposed to 376 ppm all subjects noted some eye and nose irritation within 15 minutes. After 45-60 minutes exposure to 376 ppm nausea and feelings of inebriation appeared and some subjects showed abnormalities upon neurological examination. Clinical laboratory studies of blood and urine were normal after all exposures.

Little is known about the consequences of chronic styrene exposure. Exposure of rats, guinea pigs, rabbits and monkeys to 1300 ppm styrene 7-8 h/day, 5 days/week for a period of at least 6 months produced no gross or microscopic pathological lesions or hematological changes. Some rats showed a very slight increase of liver and kidney weight as compared to

controls. All animals survived the exposures except 10% of the guinea pigs which died from acute lung irritation. Twenty oral doses of 0.1 or 0.5 g/kg to rats were harmless except some local irritation of the esophagus and stomach at the higher dose level (413). Rats, rabbits, guinea pigs and monkeys exposed 7-8 h/day, 5 days/week for up to 6 months to 650-2000 ppm showed no histopathological or hematological abnormalities. Eye and nose irritation was noted in the rats and guinea pigs as well as slight to moderate growth depression (475). Human chronic exposure to styrene may give rise to the so called "styrene sickness", characterized by nausea, vomiting, loss of appetite and general weakness and workers with prolonged styrene skin contact may develop dermatitis (51, 146). Browning (51) states that no hematological disturbances have been reported from chronic human styrene exposure. Aldyreva (14), however, claims that chronic exposure to styrene may cause leukopenia and lymphocytosis as well as toxic hepatitis and disturbances of liver function. Some relatively recent studies deal with occupational styrene neurotoxicity. Lilis *et al.* (252) examined 494 workers exposed to styrene in a monomer manufacturing and polymerization plant. The findings indicate a possible styrene peripheral neuropathy since 8.5% of the workers showed hypoesthesia of the lower extremities and 16.4% of the workers decreased peroneal nerve conduction velocities, both phenomena positively correlated with duration of styrene exposure. Effects on the CNS have also been described. Seppäläinen and Härkönen (389) found abnormal EEGs in 23 out of 96 workers exposed to styrene in plants producing reinforced polyester products. The number of abnormal EEGs was larger in workers

with high exposure to styrene evidenced by the amount of styrene metabolites excreted in the urine. There were no symptoms of peripheral nerve dysfunction. Both abnormal EEGs and changes in psychological functions were found to be dependent on styrene exposure estimated from urinary excretion of styrene metabolites in another study of the same workers (171).

Despite the essentially negative results from both animal and human chronic styrene exposures, styrene has attracted considerable attention lately as a potential mutagen and carcinogen. This is due to the probable initial biotransformation of styrene to styrene oxide, an electrophilic compound with the ability to react with nucleophilic sites in tissue macromolecules. Milvy and Garro (276) found styrene oxide but not styrene to be mutagenic for histidine requiring *Salmonella* strains revertable to prototrophy by base substitution mutagens. Vainio *et al.* (457), de Meester *et al.* (93) and Stoltz and Withey (431) using the same *Salmonella* strains as Milvy and Garro added liver microsomal preparations to the incubation media. All authors found styrene oxide to cause mutations in *Salmonella* strains with or without the presence of liver microsomes, i.e. with or without metabolic activation. Styrene was found to be mutagenic in two of the investigations (93, 457) but only after metabolic activation. It was suggested that styrene is metabolically transformed to styrene oxide, which is a direct mutagen causing base pair substitutions. Stoltz and Withey (431) did not find styrene to be mutagenic even in the presence of liver metabolizing systems. Loprieno *et al.* (257) tested styrene and styrene oxide for

mutagenic effects on yeast fortified with mouse liver microsomes, on Chinese hamster cells *in vitro* and on yeast *in vivo* in a host-mediated assay in mice. Styrene oxide was mutagenic in all test systems whereas styrene was mutagenic for yeast cells only in the host-mediated assay. The connection between styrene exposure and mutagenic events has also been strengthened by the observation that workers exposed to styrene show an increase in chromosomal aberrations, mostly chromosome breaks, in peripheral lymphocytes, when compared to controls (270). Similar aberrations could be induced in rat bone marrow cells after 9 weeks exposure of rats to 300 ppm styrene, 6 h/day, 5 days/week (269). So far, there are no reports of styrene carcinogenesis in humans. Weil *et al.* (469) could not observe skin tumors in C₃H mice painted on the dorsal skin with a 5% solution of styrene oxide in acetone three times weekly for life. Van Duuren *et al.* (460) painted 30 Swiss ICR/Ha mice with a 10% solution of styrene oxide in benzene three times weekly for life and observed 3 skin tumors. However, out of 150 controls, painted with pure benzene, 11 developed skin tumors. Styrene oxide has also been shown to bind covalently to whole homogenate, microsomes, protein and nucleic fractions of rat liver after intraperitoneal injection (264). The binding was highest 1 hour after administration, accounting for 2-3% of a dose of 50 mg styrene oxide/kg and 30% of a dose of 200 mg styrene oxide/kg. The protein fraction contained the largest proportion of the covalently bound material. Covalent binding also occurred *in vitro* upon incubation of liver fractions with styrene oxide.

ABSORPTION, DISTRIBUTION, BIOTRANSFORMATION AND ELIMINATION OF STYRENE

Styrene is readily absorbed by inhalation of vapors or mists, from the gastro-intestinal tract after oral administration and also by application to the skin (51, 146). Dutkiewicz and Tyras (110) found a skin absorption rate of 9-15 mg/cm²/h for liquid styrene and calculated that a few minutes exposure of the hands would result in the absorption of as much styrene as an 8 hour exposure to 12 ppm. Humans inhaling 22 ppm styrene for 8 hours retained 61% of the vapor but no styrene was found in the exhaled air after inhalation was terminated (31). Human volunteers inhaling 100 µg/l styrene retained 66% at nasal inspiration and 59% at oral inspiration (130). No styrene was detected in the exhaled air. However, other animal and human investigations indicate that a small part of the retained or absorbed amount is expired unchanged. Danishefsky and Willhite (89) administered c:a 0.2 ml styrene-β-¹⁴C to rats subcutaneously and recovered 2-3% of the radioactivity as unchanged styrene and 12% of the radioactivity as ¹⁴CO₂ in the exhaled air within 24 hours. It was suggested that oxidative cleavage of the double bond in the side chain resulted in the ultimate formation of ¹⁴CO₂. Rabbits dosed with 1.8 g styrene orally excreted about 2% of the dose in the expiratory air (117). Human volunteers exposed to 52-376 ppm styrene for 1-7 hours excreted a small amount rapidly and exponentially via the lungs (413). It was noted that when individuals were exposed to different styrene concentrations, the individual exposed to the higher concentration also excreted a higher concentration of styrene in his breath in the immediate postexposure period. Also, when two individuals

were exposed to identical vapor concentrations but for different periods of time, the rates of styrene excretion from the lungs were different. It was concluded that an individual exposed for a longer period, who will have absorbed more styrene, excretes styrene over a longer period of time. Of the total amounts absorbed, 0.7-1.2% were found as unchanged exhaled styrene. Dose-dependent two-compartment pharmacokinetics for styrene in rat blood were observed by Withey and Collins (474) after intravenous injection of styrene with rate coefficients decreasing with increasing dose. They also studied the distribution of intravenously injected styrene at two dose levels, 13.37 or 4.01 mg/kg, in the rat. Styrene concentrations were determined in the heart, lungs, liver, spleen, kidney and brain at six sampling times up to 90 minutes after administration. Styrene was rapidly and evenly distributed among the organs at the higher dose level. Eight minutes after injection the liver, kidney and brain styrene levels were higher than the blood level whereas the heart and spleen levels were lower. All levels decreased rapidly and linearly with time, most rapidly in the liver followed by the heart, brain, spleen, blood and kidney. At the lower dose level the organ concentrations did not exceed that of the blood 8 minutes after the injection. Plots of organ concentrations against time gave biphasic curves with an initial rapid phase followed by a slower phase. The lung levels were irreproducible at both dose levels which was considered to be due to pathological changes from the irritating action of styrene. Danishefsky and Willhite (89) measured the radioactivity levels in several organs of the rat after subcutaneous injection of styrene- β -

^{14}C . One hour after administration more than half of the dose was still present at the injection site but the liver contained 4.62% of the dose followed by the kidney (1.82%) and the blood (1.02%). The pancreas, lungs, stomach, intestine, adrenals, heart, spleen, bone, brain and thymus all contained less than 1% of the dose. Six hours after the injection all organ levels had decreased and after 24 hours only minute amounts of radioactivity could be registered. The long-time retention of styrene in body fat was reported in a recent investigation of occupationally exposed persons (403). Styrene could be detected in fat samples from heavily exposed workers (>5 ppm) 2-3 days earlier. Savolainen and Vainio (369) compared the distribution of radiolabeled styrene oxide and styrene in rats after intraperitoneal injection with special interest focused on the binding of the two compounds in the nervous system. The blood, liver, lung, kidney, duodenum, brain and spinal chord radioactivity levels were measured at 3, 6 and 24 hours after injection of $577\text{ }\mu\text{mol }^{14}\text{C}$ -labeled styrene and 3 and 6 hours after injection of $460\text{ }\mu\text{mol }^3\text{H}$ -labeled styrene oxide. Three hours after injection the highest concentration of styrene was found in the kidneys followed by the brain, duodenum, liver, spinal cord, lungs and blood. The kidney styrene concentration was 19 times higher than the blood concentration. The styrene oxide tissue concentrations were all higher than the styrene concentrations and followed a different order. Three hours after injection the highest concentration of styrene oxide was observed in the duodenum followed by the liver, kidneys, brain, spinal cord, lungs and blood. The blood styrene oxide concentration was 8 times higher than the blood

styrene concentration. No significant decreases of either styrene or styrene oxide concentrations took place in the lungs and spinal chord between 3 and 6 hours after injection. In the styrene experiment, significant decreases were found at the 6 hour sampling time in the duodenum and brain whereas a significant increase was found in the blood. Styrene oxide concentrations decreased significantly in the liver and kidneys. Minor amounts of radioactivity were still present in all the examined organs 24 hours after injection of styrene. According to the authors, the high radioactivity levels found in the duodenum and kidneys point at biliary and urinary excretion, probably of styrene and styrene oxide metabolites. The major part of the radioactivity in brain tissue was soluble in water or chloroform-methanol in both experiments at all times of measurement. Styrene was not found to be associated with soluble brain proteins in contrast to styrene oxide. Small and slightly higher amounts of styrene oxide than styrene were found associated with non-soluble brain proteins. The binding of styrene and styrene oxide was less in the spinal cord than in the brain. Styrene was not associated with axonal proteins in contrast to styrene oxide which was found in axonal proteins in a very low concentration. The results indicate that styrene and its presumed metabolite styrene oxide may actually bind to brain proteins. Styrene oxide may also bind to axonal proteins in the spinal cord as well as to soluble brain proteins.

Savolainen (366) has suggested that compounds forming long lived intermediates reacting with axonal constituents, such as styrene forming styrene oxide, may cause encephalopathy and neuropathy.