Scand J Work Environ Health 1978;4(2):78-83
https://doi.org/10.5271/sjweh.2731
Issue date: 1978

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This article in PubMed: www.ncbi.nlm.nih.gov/pubmed/734421

Print ISSN: 0355-3140 Electronic ISSN: 1795-990X

Scand. j. work environ. & health 4 (1978): suppl. 2, 78—83

Accumulation of styrene monomer and neurochemical effects of long-term inhalation exposure in rats

by HEIKKI SAVOLAINEN, M.D., and PIRKKO PFÄFFLI, M.Sc.[1]

SAVOLAINEN, H. and PFÄFFLI, P. Accumulation of styrene monomer and neurochemical effects of long-term inhalation exposure in rats. *Scand. j. work environ. & health* 4 (1978): suppl. 2, 78—83. A simplified compartment model indicated that fat styrene content might be used as an index of the whole body styrene burden. Intermittent exposure of adult male rats to 300 ppm 5 d a week, 6 h daily for 1 to 11 weeks showed an initial steady increase in the fat styrene content up to the 4th week and an exponential decrease thereafter. This phenomenon might have resulted from the enhancement of styrene oxidation due to the prolonged exposure. The effect of the increased oxidation on the body styrene burden is theoretically doubled after 9.1 weeks of exposure. Neurochemical effects after that time might have resulted from an increased binding of styrene oxide in the cellular macromolecules, e.g., to their sulfhydryl groups. The activity of the metabolite towards the cellular constituents could very well explain the increased proteolysis possibly caused by lysosomal labilization. The present data also lend support to the theory of the importance of metabolic activation to styrene and other solvent toxicity.

Key words: inhalation exposure, metabolic activation, styrene, styrene oxide.

Styrene monomer is one of the principal precursors in the industrial production of artificial polymer materials. Its wide use poses a potential occupational hazard of significant magnitude because of the great number of workers exposed to its liquid form or its vapors. Styrene is highly lipophilic with a lipid/blood partition coefficient of 135 (9) and might be assumed to accumulate markedly in the body of an exposed person. Furthermore, styrene is metabolized rapidly by human and animal organisms through oxidation to form initially an epoxide (12). Styrene oxide is highly reactive, and it may be the means through which styrene exerts its toxic effects or promotes carcinogenesis (5).

Neurological symptoms among workers exposed to styrene vapor include distal hypoesthesia and impaired conduction velocity in peripheral nerves (8). The toxic effects on the nervous system might be mediated through the action of styrene oxide on the nerve cell constituents, as the binding of labeled styrene oxide in rat brain macromolecules has been demonstrated (20). Early neurotoxic effects include changes in the protein pattern of spinal cord axons in rats exposed for weeks to 300 ppm of styrene vapor (19).

The aims of the present study were to examine the accumulation of inhaled styrene monomer in a simplified compartment model during a long-term inhalation exposure and to compare the eventual neurochemical effects with changes in the body styrene burden. We also wished to characterize protein fractions with signifi-

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cant styrene oxide association more closely.

MATERIALS AND METHODS

Adult male Wistar rats with an average body weight of 300 ± 24 g were exposed to 7.9 μmol/l (300 ppm) of styrene vapor for 1 to 11 weeks, 5 d a week, 6 h daily in a dynamic exposure chamber of 1 m^3. The exposure level was monitored continuously as described earlier (19). Rats were decapitated after 1, 2, 4, 6, 7, 8, 9, 10 and 11 weeks of exposure, and the styrene concentrations in various organs were analyzed gas chromatographically (19). Other similar rats received an intraperitoneal injection of 460 μmol of labeled styrene oxide (20). The rats were sacrificed by decapitation 3 h after the injection, and the association of label with the protein molecules of (a) the spinal cord homogenate, (b) spinal cord axons, and (c) the water-soluble fraction of the cerebral hemispheres was analyzed with a liquid scintillation counter (18, 20). The results from the injected rats were compared with animals dosed intraperitonelly with 650 μmol of ^{35}S-labeled carbon disulfide (16).

The effects of the inhalation on the brain protein metabolism were studied by the analysis of the rate of proteolysis and the amount of ribonucleic acid (19).

RESULTS AND DISCUSSION

In the major aqueous organ compartments, previously unexposed rats are near equilibrium after inhaling styrene vapor

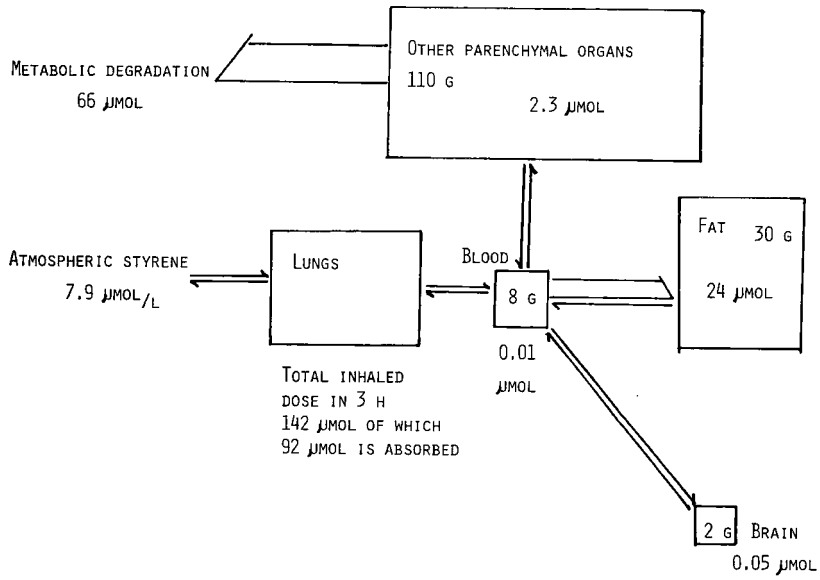

Fig. 1. Simplified model of styrene distribution in previously unexposed rats after they inhaled 7.9 μmol/l (300 ppm) of styrene vapor for 3 h. The atmospheric styrene content and organ styrene concentrations are measured values, whereas the metabolized amount is estimated from enzyme activities. Compartment weights are given together with the total amount of styrene 3 h after the beginning of exposure. A very significant fraction of styrene present in the organism can be found in the fat, and the fat styrene concentration can be used as an index of the body styrene burden if the body weight does not change significantly.

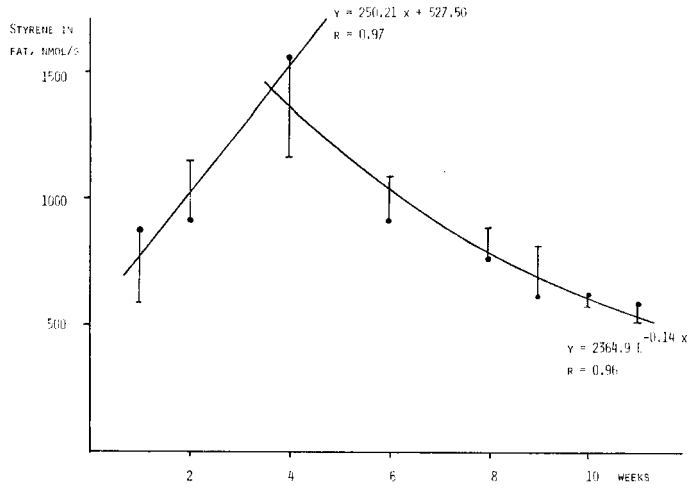

Fig. 2. Changes in the fat styrene concentration during extended inhalation exposure. The fat styrene content increases rather linearly during the first four weeks of exposure, and it tends to decrease in an exponential manner thereafter. Half of the peak styrene concentration in fat is detected after nine weeks of exposure.

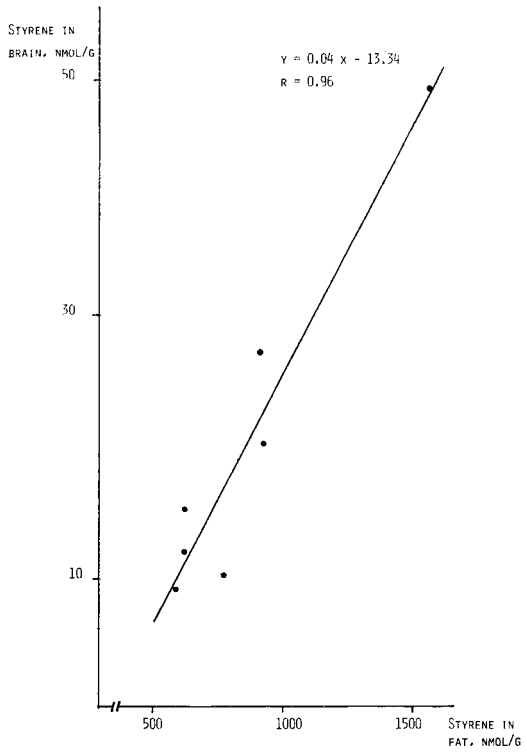

Fig. 3. Brain and fat styrene concentrations in inhalation exposure. Brain and fat styrene concentrations are linearly correlated immediately after daily exposure. This situation might not prevail after cessation of exposure for longer periods to allow the mobilization of styrene from the aqueous compartments.

(7.9 μmol/l) for 3 h, whereas a significant increase in the styrene concentration of the fatty tissues is probable even thereafter. A very significant fraction of styrene in rat body is to be found in the fat (fig. 1), and the fat styrene concentration might therefore be used as an indicator of body styrene burden. The fat styrene content increases rather linearly during the first four weeks of exposure (fig. 2). This phenomenon is not surprising in view of the high lipophilicity of the monomer. The fact that increased styrene accumulation takes place despite daily intermissions in exposure might justify the assumption that the biological half-life of styrene molecules in fat is sufficiently long to allow accumulation.

The apparent decrease in fat styrene content after the fourth week of exposure (fig. 2) might reflect the enhanced oxidation of styrene molecules, e.g., in liver (12). This phenomenon must have very important biological implications, and it might partially explain differences in the half-lives of styrene accumulation according to intensity and duration of exposure (3).

Changes in body styrene burden are reflected in the brain styrene concentrations. The latter are linearly proportional to fat styrene content immediately after the end of the exposure period (fig. 3). However, the higher brain styrene content during the early phase of exposure is not associated with appreciable neurochemical effects (19). This situation may pre-

vail at low levels of styrene exposure until significant metabolic reactions take place. Higher styrene concentrations cause coma and immediate death, and the mechanism might be similar to the effect on the nerve cell membrane as in cases of trichloroethylene (22) or of *n*-hexane (4). However, a secondary styrene metabolite, phenylethylglycol, also possesses a marked depressive action on mammalian brain (1, 12). The acute effects of styrene toxicity might therefore be caused by different mechanisms than those of chronic toxicity, which might result from organic rather than functional damage.

The enhanced oxidation of the absorbed styrene is associated with increased proteolysis. The onset of increased protein destruction coincides with the apparently doubled effect of the metabolic styrene conversion capacity at the ninth week of exposure (19). Reactive epoxide metabolites are able to labilize lysosomal membranes (15, 23), which would allow disinhibition of the proteolytic activity.

Rodent brain cells seem to be able to catalyze the oxidation of organic compounds, and, specifically, microsomes prepared from rat brain contain cytochrome P-450 together with monooxygenase activity (13). However, the brain cytochrome P-450 content might not be increased, e.g., by phenobarbitone (21). This is not a limiting factor in the case of styrene toxicity because styrene oxide is rather long-living and sufficiently apolar

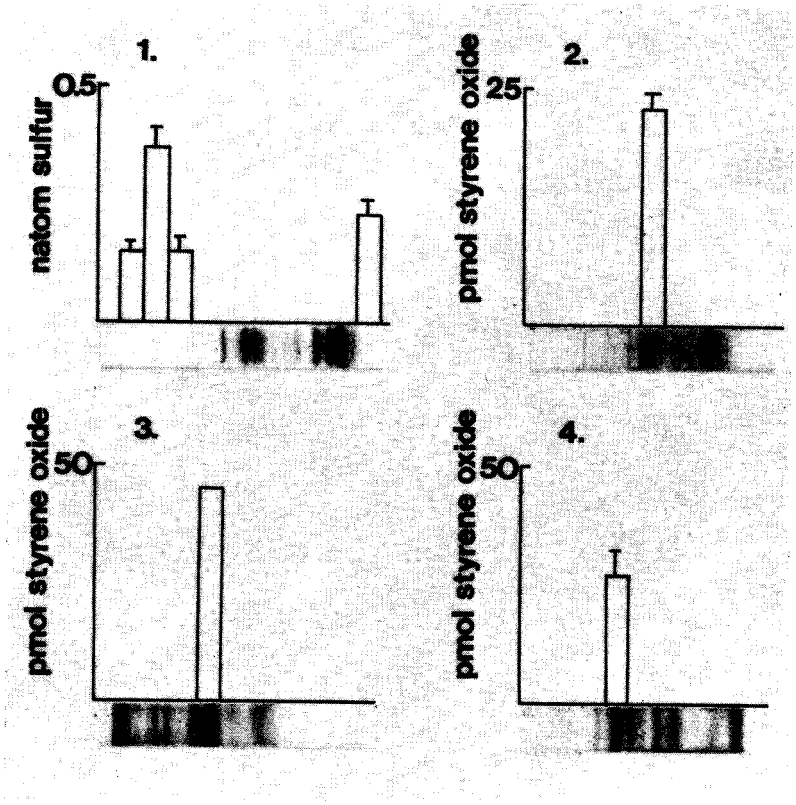

Fig. 4. Radioactivity in spinal cord (1, 2), spinal axon (3) and water-soluble cerebral protein fractions (4) after an intraperitoneal injection of 650 μmol of ^{35}S-labeled carbon disulfide (1) or 460 μmol of ^{3}H-labeled styrene oxide. The cathode and origin of the electrophoretic separation are on the left. Bars indicate 1 SD (N = 5). Styrene oxide is associated with the protein molecules present in significant concentrations in spinal cord homogenate and in cerebral water-soluble fraction. In axons, styrene oxide binds to neurofilament filarin. The carbon disulfide sulfur-binding fractions near the origin of the electrophoresis visualize only faintly (note that a significant fraction of the labeled sulfur does not co-migrate with protein).

to be transferred, e.g., from the liver across "the blood-brain barrier" to the central nervous system. The styrene oxide half-life in buffered saline in vitro is 23.4 h.

As to the protein fractions which bind styrene oxide, no particular group of molecules is specifically affected. The protein fractions with a binding capacity in spinal cord homogenate or in the water-soluble fraction of the cerebral hemispheres are present in large quantities (fig. 4). The major binding protein in the spinal cord axons is the neurofilament filarin, which contains three sulfhydryl groups per protein molecule of 50,000 Daltons (10). The neurofilaments are implicated in the rapid transport of protein in the axoplasm (25), and they have an important role in the pathogenesis of axonopathy in chronic carbon disulfide intoxication (6), or in dying-back neuropathies in general (24). However, they may not be the first protein component affected in the beginning of styrene axonopathy, but a protein class that is not associated with purified neurofilaments is lost as the earliest sign of styrene toxicity after nine weeks of exposure (19).

Sulfhydryl groups may, indeed, serve as binding sites for reactive styrene oxide. For instance, styrene oxide is reported to bind to cysteine moieties at the active site of alcohol dehydrogenase with the resultant loss of enzyme activity, e.g., in rat liver (7). Sulfhydryl groups also bind the oxidatively liberated carbon disulfide sulfur (2) although the toxin is bound very rapidly after its formation because of the very high reactivity of the atomic sulfur. Its principal binding protein in rat liver microsomes is the monooxygenase itself (11, 17). One might speculate that the carbon disulfide sulfur-binding protein in spinal cord homogenate is near the place of oxidation. If so, the very high binding activity despite a very low quantity of protein (fig. 4) would be explained.

In conclusion, exposure of adult rats to styrene vapor causes a marked intial accumulation of the monomer in the body with decreased body burden after several weeks of exposure. The increased formation of styrene oxide after nine weeks of exposure is associated with neurochemical effects. Styrene oxide may bind to a variety of protein molecules, and sulfhydryl groups may serve as the binding sites. The present findings support the hypothetical relationship of the formation of reactive metabolites and neurotoxic effects in various types of solvent exposure (14).

ACKNOWLEDGMENTS

We wish to thank Ms. E. Elovaara, Ms. T. Korhonen, Ms. E. Leskinen, and Ms. P. Toropainen for their technical assistance and the Finnish Academy for its financial aid.

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QUESTIONS AND ANSWERS

Question to Dr. SAVOLAINEN

Prof. IKEDA: Have you isolated styrene oxide from the nervous tissue of rats repeatedly exposed to styrene until peripheral neurotoxicity is observed?

Dr. SAVOLAINEN: Unfortunately, we do not yet have the methods to do this although it would be very important to know the styrene oxide concentrations.