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**Uptake, distribution and elimination of styrene in man. Concentration in subcutaneous adipose tissue.**

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**Key terms:** adipose tissue; adipose tissue concentration; alveolar air; body fat; concentration; distribution; elimination; exercise; exposure; man; rest; styrene; subcutaneous adipose tissue; uptake

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# Uptake, distribution and elimination of styrene in man

## Concentration in subcutaneous adipose tissue

by JÖRGEN ENGSTRÖM, M.D., RASMUS BJURSTRÖM, B.Sc., IRMA ÅSTRAND M.D., and PER ÖVRUM, M.Sc.[1]

ENGSTRÖM, J., BJURSTRÖM, R., ÅSTRAND, I. and ÖVRUM, P. Uptake, distribution and elimination of styrene in man: Concentration in subcutaneous adipose tissue. *Scand. j. work environ. & health* 4 (1978) 315—323. Seven male subjects were exposed to 210 mg/m$^3$ of styrene in inspired air during 30 min at rest and three 30-min periods of work on a bicycle ergometer at intensities of 50, 100 and 150 W. The uptake in the organism was measured by the Douglas bag technique. The mean uptake was 490 mg, corresponding to 63 % of the amount inspired. During the last 30-min period, the uptake in the organism was 5—6 times higher than during the first period at rest. The elimination of styrene by the airways during 19 h after the exposure was estimated to be about 3 % of the amount retained in the body during exposure. Needle biopsy of subcutaneous adipose tissue was performed on all the subjects before exposure and 0.5, 2, 4 and 20—24 h after the exposure. In addition, four of the men were subjected to biopsies during the 1—2 weeks following exposure. The concentration of styrene in adipose tissue was determined by gas chromatography after evaporation into nitrogen at a high temperature. About 24 h after the exposure the mean concentration of styrene in adipose tissue was on about the same level as 2—4 h after exposure, i.e., about 3.5 mg/kg. Retention of styrene in adipose tissue was noticed as late as 13 d after the short exposure at a concentration in inspired air corresponding to the Swedish threshold limit value. The estimated half-life of the concentration of styrene in adipose tissue was 2—4 d. In spite of the rapid metabolism of styrene, industrial exposure is considered to be accompanied by the risk of accumulation in adipose tissue because of the slow elimination rate.

*Key words:* adipose tissue concentration, alveolar air, body fat, exercise, exposure, rest, styrene, uptake.

Styrene (vinyl benzene) is widely used in the plastic industry, for example, in the manufacture of recreational boats and tanks. The compound is simultaneously employed as a solvent and as the starting point for polymerization.

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Basal results of alveolar air and blood concentrations have been reported from experimental exposure to styrene during rest and exercise in this laboratory (2). The alveolar concentration rose only to about 25 % of the concentration in the inspired air when the rate of work was successively raised to 150 W during constant exposure. The arterial concentration increased considerably throughout the entire 2-h exposure. It was concluded that styrene was easily soluble in blood.

Wolff et al. found styrene in subcutaneous fat samples of 13 out of 17 employees who had been exposed to more than 5 ppm of styrene within the previous 3 d in a polymerization plant (22). The same authors pointed out that, although urine metabolites and breath levels of styrene are reported to be detectable for less than 24 h after exposure, styrene was found in subcutaneous fat for as long as 3 d after the most recent exposure. The concentrations in adipose tissue ranged from 0.1 to 1.2 mg/kg of fat.

The concentrations of methylene chloride in subcutaneous adipose tissue after experimental exposure were determined in a previous study from this laboratory (8, 9). Methylene chloride is a volatile solvent with a relatively poor affinity for adipose tissue. Xylene, which is easily soluble in blood and adipose tissue, has also been studied (10). The purpose of the present study was to measure the concentrations of styrene in adipose tissue after standardized experimental exposure.

Great solubility in blood and tissues and a rapid biotransformation present conditions resulting in great uptake in the organism. Although approximately 95 % of the amount of styrene taken up in the organism is biotransformed and excreted (4), there is a potential risk of accumulation during industrial exposure, because of the great solubility of styrene in fat (17). Since styrene and its metabolites have been shown to have neurotoxic effects on man (5, 11, 12, 14) and mutagenic (16, 21) and teratogenic effects (20) and since an increased incidence of chromosome aberations has been reported in people during long-term exposure (15), there is need for methods to measure the accumulation in the organism. Needle biopsy of subcutaneous adipose tissue and gas chromatographic analysis of styrene concentrations provide increased opportunities for estimating the degree of indirect exposure of sensitive organs, through release of the solvent from fat depots after exposure.

## SUBJECTS, EXPERIMENTAL DESIGN AND METHODS

The subjects were seven healthy men, 22 to 30 years of age. Tables 1 and 2 list anthropometric data and results from pulmonary tests and exercise tests on a bicycle ergometer. [See Åstrand et al. (3) for details of the methods.] The amount of body fat was calculated according to von Döbeln by means of underwater weighing and skeletal measurement (6, 7).

Exposure to styrene was performed with the aid of a breathing valve and mouthpiece. The concentration in inspiratory air amounted to about 210 mg/m$^3$ (50 ppm) throughout the entire exposure. Subjects were exposed for 30 min at rest and for three 30-min periods during exercise on a bicycle ergometer at intensities of 50, 100 and 150 W. Exposure was interrupted for 20 min before the start of the physical exercise. The methods were basically the same as in previous solvent experiments with respect to the administration of the solvent, measurement of uptake in the organism, and determination of the concentrations in inspiratory, expiratory and alveolar air, and in arterial and venous blood (3).

*Table 1.* Body height, body weight, estimated amount of body fat and the results of pulmonary function tests of seven male subjects aged 22 to 30 years. (Mean values and standard errors)

| Body height (cm) | Body weight (kg) | Estimated weight of body fat (kg) | Vital capacity (l) | Residual volume (l) | FEV% [a] | $MVV_f$ [b] (l/min) |
|---|---|---|---|---|---|---|
| 182±2.5 | 78.0±2.3 | 10.8±2.1 | 6.2±0.2 | 1.5±0.1 | 83±2 | 207±8 |

[a] FEV% = forced expiratory volume for 1 s as the percentage of vital capacity.
[b] $MVV_f$ = maximal voluntary ventilation at a free rate.

*Table 2.* Results of seven male subjects from exercise tests on a bicycle ergometer. (Mean values and standard errors)

| Work load (W) | Heart rate (beats/min) | $\dot{V}_E$ BTPS [a] (l/min) | $\dot{V}_{O_2}$ STPD [b] (l/min) | Blood lactate concentration (mmol/l) |
|---|---|---|---|---|
| 50 | 93 ± 4 | 23.3 ± 1.7 | 0.98 ± 0.03 | 1.7 ± 0.2 |
| 100 | 112 ± 4 | 35.7 ± 2.3 | 1.49 ± 0.03 | 2.3 ± 0.4 |
| 150 | 136 ± 5 | 49.2 ± 3.8 | 2.03 ± 0.06 | 3.6 ± 0.6 |
| maximal work | 190 ± 4 | 151.8 ± 7.4 | 3.71 ± 0.22 | 14.7 ± 1.1 |

[a] $\dot{V}_E$ BTPS = pulmonary ventilation at body temperature and pressure, saturated with water vapor.

[b] $\dot{V}_{O_2}$ STPD = oxygen uptake at 0°C, 760 mm Hg, dry.

Food intake and physical activity were standardized after exposure. Subjects had lunch after 1 h and performed physical exercise for 5 min of every 15-min period during the first 4 h following exposure. The exercise was performed on a treadmill at a work rate resulting in an oxygen uptake of about 1 l/min.

The styrene concentrations in alveolar air were followed for about 19 h after exposure, and the corresponding expired amounts were estimated. The mean amount of styrene expired in the first 4 h after exposure was measured. In the first 20 min the amount was continuously measured with the Douglas bag technique. Pulmonary ventilation was then measured for 5-min periods with the Wright respirometer (Medishield Harlow Essex CM 195 AB, England), and the respiratory rate was estimated. A total of 10 such measurements, evenly spread out in time up to 4 h after exposure, were made. The measurements were made alternately during rest and during exercise. The amount of styrene expired was calculated as the product of alveolar ventilation and alveolar concentration. Alveolar ventilation ($\dot{V}_A$) was calculated according to the following formula:

$$\dot{V}_A = \dot{V}_E - \text{dead space} \times \text{respiratory rate},$$

where $\dot{V}_E$ = total ventilation, dead space = 150 $cm^3$. The mean values for ventilation ($\dot{V}_E$) and respiratory rate during rest and exercise, respectively, were calculated for each individual and then for the seven subjects as a group. The mean value for the concentration in the alveolar air of the seven subjects at different times was plotted in a diagram, and a curve was drawn by hand. The mean amount of styrene expired by the subjects was calculated for each 5-min period. The calculation was based on the mean value for $\dot{V}_A$ and for the mean alveolar concentration estimated for each 5-min period on the basis of the drawn curve (fig. 1).

The mean amount of styrene expired by the seven subjects during the following 15 h (the rest of the afternoon and the night) was estimated. In the calculation an alveolar ventilation of 8 l/min was used for the 5 afternoon hours and of 5 l/min for the 10 night hours. The mean of the alveolar concentration was plotted against time, and a rectilinear decay over time was assumed to occur. The amount of expired styrene was calculated in the same manner as for the first 4 h as the product of alveolar ventilation and alveolar concentration.

The adipose tissue biopsies were performed in the upper lateral gluteal quadrant after intracutaneous anesthesia with a 1 % lidocain solution (Xylocain, Astra). The needle was lance-shaped and the dimensions were 2.0 × 80 mm (KN 1480 W, Mediplast AB). The needle was connected via a Luer-Lok to a 50-cc syringe with a glass barrel and plunger (B-D Yale, Becton, Dickinson & Co.) [See Engström and Bjurström (10) for details of the method.]

Adipose tissue specimens were taken prior to exposure in order to reveal substances interfering with the solvent analyses. Biopsies were also performed 0.5,

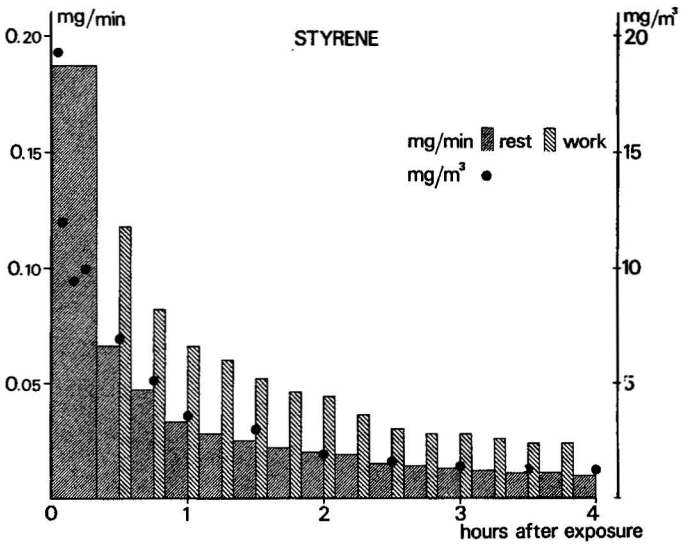


*Fig. 1.* Concentration of styrene in alveolar air and the calculated amount expired per minute by the subjects for 5-min periods during exercise (with an oxygen uptake of about 1 l/min) and at rest. Mean values for seven subjects during 4 h after 2 h of exposure to about 210 mg/m³ in inspiratory air (at rest and during exercise at intensities up to 150 W).

2, 4, and 20—24 h after exposure. Six of the subjects were subjected to double biopsies on one occasion after 2 or 4 h. When the second sample was taken, the needle was inserted into the same skin puncture, but its direction was changed 180°.

The study was extended in time for four subjects. Thus, adipose tissue specimens were taken from two subjects 7 d after exposure, from one subject after 7, 9 and 13 d and from one subject 8 and 11 d after exposure. The material comprised a total of 48 biopsy specimens.

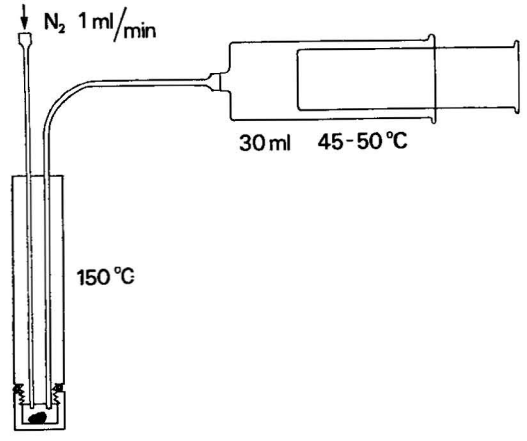


*Fig. 2.* Diagram of the evaporation unit. Nitrogen from a bag at atmospheric pressure is drawn over the specimen in an evaporation chamber into a motor-operated gas-tight glass syringe.

In addition to the aforementioned double determinations, white adipose tissue from rat was assayed so that the error of the method in solvent assays could be estimated. In the study, to be published by Carlsson, one rat was exposed to 50 ppm of $^{14}C$-labeled styrene in inspiratory air for 4 h and sacrificed immediately following exposure. An adipose tissue specimen was divided into eight subspecimens, two of which were analyzed by liquid scintillation counting and six by the gas chromatographic method of the present study.

The styrene concentrations in the adipose tissue of the subjects were determined after evaporation of the solvent at 150°C into nitrogen which was continuously exchanged. The gas was collected in 30-ml glass syringes and assayed with gas chromatography. The evaporation lasted 60 min. The operation of the evaporation unit is illustrated in fig. 2. Details of the method have already been presented in another report (10). The analytical conditions were as follows: column: SCOT stainless steel column (Perkin Elmer), 15 m long with an internal diameter of 0.5 mm; stationary phase: carbowax "400"; carrier gas: nitrogen with a flow rate of 5 ml/min; temperature: column 90°C, injection block and detector 150°C. The styrene contents

were determined from the chromatogram with the aid of air samples containing known solvent concentrations.

Liquid scintillation counting of $^{14}C$-labeled styrene in adipose tissue of rat was performed according to the as yet unpublished method of Carlsson.

## RESULTS

The mean amounts of styrene supplied and taken up during the various periods of exposure are listed in table 3. The amount of styrene taken up in the organism rose from an average of about 40 mg during the first 30-min period at rest to 210 mg during the final period, i.e., during the 150-W exercise period. Thus uptake was 5—6 times greater during heavy work than at rest. Average uptake for the entire 2-h period of exposure amounted to 490 mg. The relative uptake declined from about 70 % of the amount supplied at rest to 60 % at the end of the last 30-min period; it averaged 63 % for the entire exposure. The uptake per kilogram of body weight amounted to 6.3 ± 0.5 mg (mean ± SD). Thus the differences between the seven subjects were relatively slight in this respect.

The mean alveolar concentration of styrene at the end of the first 30-min period was 16 % of the concentration in inspiratory air and 23 % at the end of the final period. These relative concentrations (x) displayed a relationship to the corresponding percentage of uptake (y) according to the equation $y = -0.86x + 79.3$ (1). Gaseous styrene was found to obey the same rules for uptake in the lungs as the other solvents studied in this laboratory, with the exception of butanol.

The arterial concentration rose successively as the work load increased for the three subjects in whom the concentrations were determined. The mean values were 1.2 mg/kg (range 0.9—1.5) at the end of the first period and 3.2 mg/kg (range 2.0—4.9) at the end of the final period of exercise. The ratio between the concentrations in arterial blood and alveolar air amounted to 47, 70, and 80, respectively. The concentration in the peripheral venous blood of these three subjects rose from an average of 0.2 mg/kg to 2.9 mg/kg (range 1.8—4.5) at the corresponding times.

The mean values for the concentrations in alveolar air and for the amount of styrene expired during the first 4 h after exposure are shown in fig. 1. The amount of styrene eliminated via expiratory air was about 2 % of the amount taken up. The amounts were about twice as great per unit of time during the periods on the treadmill as during the rest periods. An additional calculated mean amount of only 2 mg was expired during the following 15 h, i.e., during the rest of the afternoon and during the night. The total mean amount expired during 19 h after exposure was 2.6 % of the amount retained during exposure. The concentration in alveolar air was near the limit of detection 19 h after exposure.

*Table 3.* The amounts of styrene supplied and taken up by seven subjects at rest and during exercise. (Mean values and standard errors)

| Exposure [a] | Amount supplied (mg) | Amount taken up (mg) | Uptake in % of amount supplied |
|---|---|---|---|
| 1 | 57 ± 5 | 39 ± 2 | 70 ± 2 |
| 2 | 146 ± 6 | 95 ± 2 | 66 ± 2 |
| 3 | 229 ± 7 | 145 ± 4 | 64 ± 2 |
| 4 | 355 ± 17 | 210 ± 8 | 59 ± 2 |
| 1—4 | 777 ± 24 | 490 ± 12 | 63 ± 1 |

[a] 1 = Exposure at rest for 30 min, 210 mg/m³; 2 = exposure at 50 W, 30 min, 210 mg m³; 3 = exposure at 100 W, 30 min, 210 mg/m³; 4 = exposure at 150 W, 30 min, 210 mg/m³.

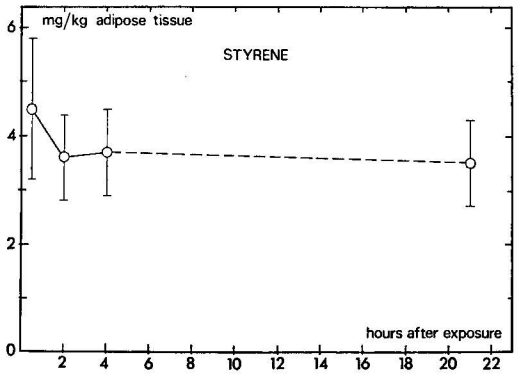


*Fig. 3.* Concentration of styrene in the subcutaneous adipose tissue of seven subjects in the first 24 h after 2 h of exposure to about 210 mg/m³ in inspiratory air (at rest and during exercise at intensities up to 150 W). Mean values and standard errors are stated.

There was a linear relationship between the amount of styrene taken up per kilogram of body weight and the concentrations of styrene in alveolar air 0.5 and 2 h after exposure. The correlation coefficient was 0.68 and 0.80, respectively. No correlation was found between the amount taken up and concentrations in alveolar air later in the decay phase.

The mean weight of the 48 adipose tissue specimens from the subjects was 54.6 mg (range 8.8—137.7 mg). The whole amount of evaporated styrene from the specimen was obtained in the first 30-min syringe in 31 of the 41 specimens taken after exposure. A small part, i.e., 4.8 ± 1.8 % (mean ± SD) of the total evaporated amount was obtained in the next 30 min in 10 cases.

The mean concentration in the double specimens, taken from six subjects 2 to 4 h after exposure, amounted to 3.9 mg/kg. The concentrations ranged from 1.2 to 8.1 mg/kg. The intrapair mean difference was 2.2 mg/kg, and the standard deviation of the difference was 1.9 mg/kg. Student's t-test disclosed no significant difference between the concentrations in samples 2 and 1 ($p > 0.4$).

The mean weight of the six subspecimens of white adipose tissue from one rat analyzed by gas chromatography was 36.6 mg (range 15.2—62.6 mg). According to this gas chromatographic method the mean value for the styrene concentration amounted to 20.8 mg/kg (SD = ± 3.4 mg). The concentration in the two subspecimens assayed by liquid scintillation counting was 19.7 and 20.2 mg/kg, respectively.

In fig. 3 the mean values are given for the styrene concentrations in adipose tissue of subjects at different times during the first 24 h after exposure. The concentrations were on about the same level

*Table 4.* Styrene concentration in adipose tissue of four subjects up to 13 d after 2 h of exposure to 210 mg/m³ in inspiratory air (at rest and during exercise).

| Subject and anthropometric data | Styrene concentration in adipose tissue (mg/kg) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 30 min | 2 h | 4 h | about 21 h | 7 d | 8 d | 9 d | 11 d | 13 d |
| 1. Height 178 cm Weight 76.6 kg Body fat 15.2 kg | 4.9 | 6.6 | 2.3 | 3.5 | 0.6 | | 0.4 | | 0.07 |
| 2. Height 176 cm Weight 75.5 kg Body fat 14.8 kg | 2.0 | 1.5 | 2.6 | 1.6 | | 0.3 | | 0.1 | |
| 3. Height 190 cm Weight 84.9 kg Body fat 17.7 kg | 1.3 | 1.2 | 2.4 | 2.8 | 1.0 | | | | |
| 4. Height 177 cm Weight 86.6 kg Body fat 12.8 kg | 0.3 | 2.1 | 2.2 | 1.3 | 0.2 | | | | |

during the whole period, i.e., 4.5—3.5 mg/kg.

The height, weight, estimated amount of body fat and the styrene concentration in the adipose tissue of four subjects are listed in table 4. Styrene was detectable in adipose tissue up to 13 d after the 2-h exposure.

In fig. 4 the logarithms for the concentrations 1 to 13 d after exposure are plotted against time. In the two cases with three and five observations, respectively, the concentrations displayed an exponential decline. If this was the case for all four subjects, the half-life for the concentrations in adipose tissue was 2.2 to 4 d. The subject who had the largest estimated amount of body fat had the longest styrene half-life.

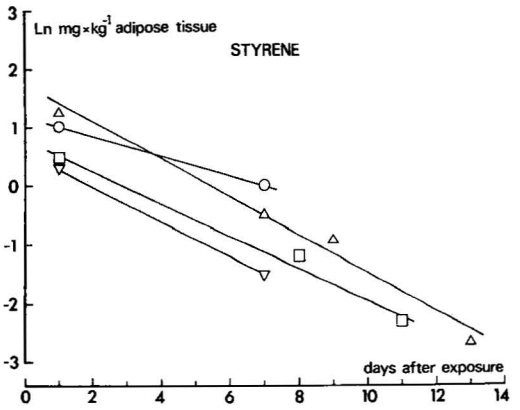


*Fig. 4.* Concentration of styrene in subcutaneous adipose tissue of four subjects from 1 to 13 d after 2 h of exposure to about 210 mg/m³ in inspiratory air (at rest and during exercise at intensities up to 150 W).

## DISCUSSION

The present study confirms previous results, i.e., that styrene is highly soluble in blood and tissues. As in previous investigations entailing constant exposure in the laboratory, the styrene concentration in arterial blood rose continuously. No equilibrium developed between arterial blood and alveolar air during 2 h of exposure, and the quotient rose successively. The present study also disclosed that the uptake in the lungs increased sharply as exercise intensity increased.

Since the in vitro blood/air and fat/air partition coefficients and the degree of metabolism are of the same magnitude for styrene and xylene (10), a comparison of the present results and findings in previous experimental exposure to xylene (1, 10) becomes a subject of interest. The subjects referred to in the xylene experiment were exposed exactly like the subjects in the present styrene series, i.e., during 2 h and both at rest and during increasing exercise intensities. The subjects in the xylene series were exposed to 435 mg/m³ in inspiratory air, whereas the subjects in the styrene series were exposed to 210 mg/m³, i.e., a concentration half as great. The uptake in the organism of xylene and styrene, respectively, was proportionally of about the same size, averaging about 1,000 and 500 mg, respectively. The decline in the concentration in adipose tissue was slow for both substances without any significant change in the mean values for the groups in the first 24 h. After about 1 d the mean concentration in adipose tissue amounted to 8.1 mg/kg for the cited xylene group and to 3.5 mg/kg in the styrene series. Thus, the ratio between the concentrations in adipose tissue was on about the same level as the ratio between the exposure levels.

The total amount of styrene in the fat depots of the body was calculated on the basis of the concentrations in individual specimens and on the assumption that these specimens were representative of the fat depots. In addition, it was supposed that the fat content in the adipose tissue averaged 80 % (13). The rough calculation on the basis of these assumptions gave a mean value of 40 mg of styrene in adipose tissue about 24 h after exposure, i.e., about 8 % of the styrene taken up in the organism. The corresponding value for the group of subjects in the xylene experiment was only 5 %. However, the fat depots of the xylene group were on the average half as large as in the styrene group.

The observation that the elimination of the solvent through respiration increased sharply even in light physical exercise means that leisure-time physical exercise can be recommended to people exposed to solvents. However, this activity is probably of modest significance after exposure to styrene since most of the sub-

stance is eliminated by means of biotransformation. On the other hand, physical exercise should be capable of reducing elimination time considerably after exposure to solvents eliminated mainly via the lungs.

Seppäläinen and Härkönen reported abnormal electroencephalographic findings in 24 % of 96 workers subjected to long-term exposure to styrene (19). The workers were examined at least 20 h after concluded exposure. The authors felt that the changes could not be regarded as acute effects after this period of time. The present study, in which the styrene concentrations in adipose tissue failed to undergo any major change the first 24 h after exposure, gives cause for questioning this conclusion. Some indirect exposure of internal organs, such as the nervous system, still continues after 24 h by means of release from adipose tissue. The half-lives of styrene and its metabolites in the central nervous system of man are unknown. Extrapolations cannot be made from adipose tissue in respect to the concentrations and half-lives of styrene in the fat-rich central nervous system of man. The latter has a different lipid composition and probably a different tissue/blood coefficient than adipose tissue, not to mention differing blood perfusion conditions. In rats, however, Savolainen and Pfäffli have observed a strong correlation between the concentrations of styrene monomer in perinephric adipose tissue and in brain after long-term exposure (18). In man there is cause to suspect that the elimination of styrene from the central nervous system is slower than is the case for a solvent with a lower fat/blood coefficient.

It was surprising to find styrene in adipose tissue up to as much as 13 d after a brief exposure at a concentration of 50 ppm in inspiratory air, i.e., the current Swedish threshold limit value. Industrial exposure is often of longer duration and can produce concentrations in inspiratory air corresponding to the threshold limit value. The concentrations in adipose tissue might therefore be higher in many employees in factories in which styrene is processed than in the subjects of this study. There is reason to suspect that elimination after industrial exposure takes much longer than two weeks. Therefore, the need for field studies appears to be justified.

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