

# Exposure to styrene

## Uptake, distribution, metabolism and elimination in man

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WIGAEUS E, LÖF A, BJURSTRÖM R, BYFÄLT NORDQVIST M. Exposure to styrene: Uptake, distribution, metabolism and elimination in man. *Scand j work environ health* 9 (1983) 479–488. Eight male subjects were exposed for 2 h to about 2.88 mmol/m<sup>3</sup> (300 mg/m<sup>3</sup>) of styrene vapor during light physical exercise. The uptake of styrene averaged 4.4 mmol, or 68 % of the amount supplied. The arterial blood concentration of styrene reached a relatively stable level of about 20 µmol/l after 75 min of exposure. The calculated value of blood clearance was 1.7 (SD 0.3) l/min, and the extraction ratio about 0.2. The half-time for the elimination phase was 41 (SD 7) min, and the calculated volume of distribution 99 (SD 13) l. The concentration of styrene in the subcutaneous adipose tissue was about 50 µmol/kg 30–90 min after exposure. The concentration of nonconjugated styrene glycol in blood increased linearly during exposure and was about 15 % of the styrene concentration in blood at the end of exposure. It was eliminated with a half-time of 72 (SD 13) min. Within 28 h after exposure 58 % of the total uptake of styrene was recovered in the urine as mandelic and phenylglyoxylic acid. Their excretion half-times (0–20 h) were 3.6 (SD 0.4) and 8.8 (SD 1.3) h, respectively. Styrene-7,8-oxide was detected and quantified in blood in a complementary study.

**Key terms:** arterial blood, exercise, kinetics, mandelic acid, phenylglyoxylic acid, styrene glycol, styrene-7,8-oxide, subcutaneous adipose tissue, urine.

Styrene (ethenylbenzene, vinylbenzene, phenylethylene) is mainly used in the production of plastics and resins (42). Occupational exposure to styrene occurs mainly via inhalation, and a relative uptake of about 60–70 % of the amount inspired has been reported (2, 6, 15, 17, 18, 30, 41). The Swedish hygienic standard for the 8-h time-weighted average concentration is 1.06 mmol/m<sup>3</sup> (110 mg/m<sup>3</sup>) with a recommended short-time value of 2.88 mmol/m<sup>3</sup> (300 mg/m<sup>3</sup>).

The metabolism of styrene is comparably well understood [see the work of Pantarotto et al (32)]. The main metabolic end products in man, mandelic and phenylglyoxylic acid, have been measured in urine after experimental and occupational

exposure in order to establish appropriate bases for the biological monitoring of styrene exposure (7, 16, 20, 21, 22, 23, 24, 25, 45). However, the precursors of mandelic and phenylglyoxylic acid, the biologically active styrene-7,8-oxide (1,2-epoxyethylbenzene, phenyloxirane) (32, 43) and its hydrated product styrene glycol (1-phenyl-1,2-ethanediol, phenylethylglycol) (33), had not been identified in man when the present investigation was initiated. Styrene-7,8-oxide (referred to as styrene oxide in the present communication) has been demonstrated as a styrene metabolite in vitro with the use of rat liver microsomes (26) and detected in trace amounts in vivo in the lungs and liver of mice pretreated with an inhibitor of epoxide hydratase (32). Styrene glycol has been detected after cleavage with  $\beta$ -glucuronidase in urine from animals treated with styrene (13, 31). Recently styrene glycol was determined in human blood after inhalation exposure to styrene (44). The main purpose of the present study

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was to detect and, if possible, quantify styrene oxide and styrene glycol in human blood after inhalation exposure to styrene and, further, to study the uptake, distribution, metabolism, and elimination of styrene in man.

## Subjects, material and methods

The volunteers were eight healthy men with an average age of 27 (range 23–34) years, an average weight of 71 (range 60–80) kg, and an average height of 179 (range 174–191) cm. The subjects were rather similar in bodily constitution. One was quite lean, and none were obese. They had not been occupationally exposed to solvents. None had suffered from any disease having a detrimental effect on the function of the respiratory and circulatory organs.

The subjects were exposed in pairs in an exposure chamber for 2 h to a styrene concentration of 2.84 (SD 0.03) mmol/m<sup>3</sup> (296 mg/m<sup>3</sup>) (Merck, analytical grade) during light physical exercise, work load of 50 W, on a bicycle ergometer. The volume of the chamber was 12 m<sup>3</sup>, and the air change 10 times/h. Solvent vapor was generated essentially as described elsewhere (2).

The styrene concentration in the chamber was continuously monitored with an

infrared analyzer (Miran 1 A). The exhaled air for each individual was collected during 4 min at regular time intervals, and the uptake during each 30-min period was calculated from the mean value of two sampling periods during this time. The methods for determining the styrene concentration in the inspiratory and expiratory air and the measurements of the solvent uptake were basically the same as described elsewhere (2). Heart rate was measured manually two to three times during each period of exhaled air sampling.

Arterial blood samples were withdrawn from a catheter introduced into a brachial artery. For the analysis of styrene in blood, samples of 1.0 ml were equilibrated with air at 37°C for 20 min in citrate-treated headspace bottles (volume 22.5 ml, Perkin Elmer). Headspace air was analyzed for styrene by gas chromatography (3% SE-30 on Chromosorb GAW, 2.0 m, 145°C, nitrogen flow 25 ml/min, flame ionization detector, Perkin Elmer F33). Individual calibration curves were obtained through the addition of 2 µl of the solutions (N = 8) of different concentrations of styrene in dimethylsulfoxide (Merck, analytical grade) to blood sampled before exposure (2 µl of dimethylsulfoxide was added to all samples taken during and after exposure). The calibration curves were linear ( $r = 0.995$ –

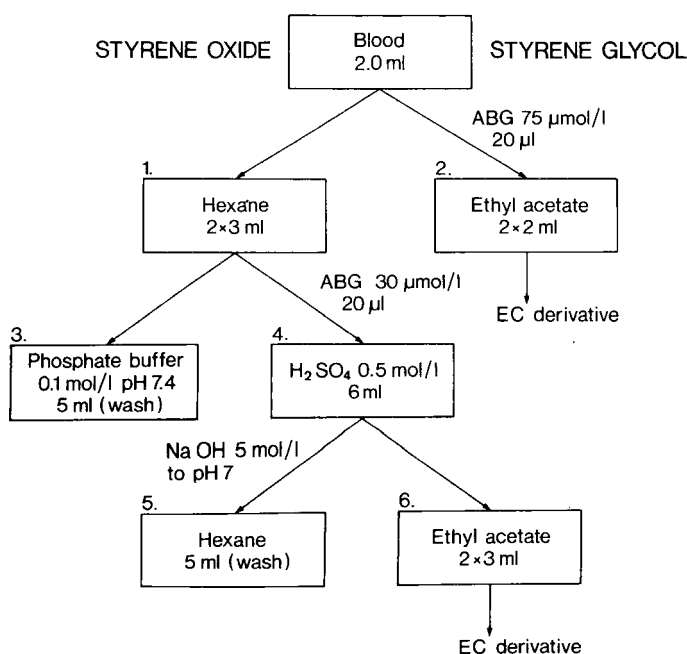


**Fig 1.** A scheme for the extraction of styrene oxide and styrene glycol from blood. Allylbenzene glycol (ABG) was added as the internal standard. The ethyl acetate extracts were treated with pentafluorobenzoyl chloride to make derivatives suitable for electron capture (EC) detection. (H<sub>2</sub>SO<sub>4</sub> = sulfuric acid, NaOH = sodium hydroxide)

0.999) for the blood concentration range in question (0–32.5  $\mu\text{mol/l}$ ).

Needle biopsies of subcutaneous adipose tissue were taken (in duplicate when possible) from the gluteal region immediately before and 0.5 and 1.5 h after exposure as described elsewhere (15). Immediately after the sampling the specimens were weighed and stored at  $-20^{\circ}\text{C}$  until analyzed within the same day. The mean weight of the 26 specimens analyzed was 34.0 (range 8.3–108.2) mg. The contents of styrene in subcutaneous adipose tissue were determined as described elsewhere (15). The amount of body fat of the respective subjects was estimated by means of anthropometric measurements (11).

For the analysis of styrene oxide and nonconjugated styrene glycol in blood an extraction procedure was developed (fig 1). Each blood sample (2 ml) was extracted with hexane (fig 1, step 1) and ethyl acetate (fig 1, step 2) in sequence to remove styrene oxide and styrene glycol, respectively. Allylbenzene glycol [synthesized according to Duverger–van Bogaert et al (12)] was added as the internal standard to the hexane extracts and to the remaining aqueous phase. Acidification converted styrene oxide to styrene glycol (fig 1, step 4), which was derivatized with pentafluorobenzoyl chloride (Aldrich, 98 %) and analyzed by a gas chromatographic technique based on electron capture detection (1.5 % SE-30 on Chromosorb GAW-DMCS, 2.0 m,  $125^{\circ}\text{C}$ , nitrogen flow 60 ml/min, Carlo Erba FTV 2350) (12). The peak areas of derivatized styrene glycol and allylbenzene glycol were integrated (Varian Vista 401 Chromatography Data System). Calibration curves were obtained through the addition of 10  $\mu\text{l}$  of a solution, in toluene (Merck, analytical grade), of styrene oxide (Fluka AG, Buchs SG,  $\geq 97$  % purity, distilled) or styrene glycol (Aldrich, 97 % purity) to blood before extraction. The blood concentration range so obtained was 0–1.1 and 0–9.7  $\mu\text{mol/l}$  for styrene oxide and styrene glycol, respectively. The area ratio of styrene glycol/allylbenzene glycol was plotted against the concentration of styrene oxide and styrene glycol, respectively ( $y = 1.45x - 0.26$ ,  $N = 21$ ,  $r = 0.992$  for styrene glycol and  $y = 3.18x + 0.10$ ,  $N = 10$ ,  $r = 0.994$  for styrene oxide).

With the described method styrene oxide could not be detected in the blood of

any of the subjects. The lower limit for detection was estimated to be 0.06  $\mu\text{mol/l}$ . To improve the sensitivity of the styrene oxide determination, the washing procedure of the hexane extract (fig 1, step 3) was omitted; this modification changed the calibration curve markedly ( $y = 3.99x + 0.01$ ,  $N = 10$ ,  $r = 1.000$ ). Extractions of blood, to which styrene glycol had been added, had shown that no detectable amounts of styrene glycol were extractable with hexane (fig 1, step 1) at concentrations appropriate for this study. At higher concentrations, 6–36  $\mu\text{mol/l}$ , 0.1–0.3 % of the added styrene glycol was recovered in the hexane extract. Because of the low styrene oxide concentrations expected, the method was further modified. The blood volume was doubled to 4 ml, and the amount of the internal standard was reduced from 0.6 to 0.3 nmol. By these modifications the sensitivity of the method was further improved ( $y = 17.52x + 0.00$ ,  $N = 10$ ,  $r = 0.992$ ) and the lower limit for detection was estimated to be 0.003  $\mu\text{mol/l}$ . A complementary study was undertaken in which four of the subjects were reexposed under the same conditions, and styrene glycol and styrene oxide were analyzed in venous blood collected 5–30 min after exposure.

All urine from the subjects was collected up to 39 h after exposure. Samples were taken before exposure, half an hour after, and about 3 and 6 h after exposure, and then at individual time intervals. The urine was collected in bottles and frozen as soon as possible. Urine volume and specific gravity were measured before the samples were frozen. Separations and determinations of mandelic and phenylglyoxylic acid in the urine were accomplished by isotachopheresis according to a method described elsewhere (39) after the following modifications (personal communication from J Sollenberg): The leading electrolyte consisted of 5mM trichloroacetic acid in 0.4 % hydroxypropylmethylcellulose (Methocel 90 HG, Dow Chemical) adjusted to pH 3.35 with solid  $\beta$ -alanine, the terminating electrolyte was 10 mM acetylsalicylic acid adjusted to pH 3.40 with solid sodium hydroxide, and a 230-mm capillary tube was used along with a migration current of 200  $\mu\text{A}$ . The detection limit for mandelic and phenylglyoxylic acid in the urine was 0.1 mmol/l.

## Results

The total uptake of styrene during 2 h of exposure at 50 W [The heart rate averaged 92 (SD 4) beats/min, indicating light to moderate physical work (3).] was 4.4 (SD 0.5) mmol or 68 % of the amount supplied (table 1).

The styrene concentration in arterial blood (seven subjects because of the failure of catheter introduction into one subject) increased during the first 75 min of exposure and then approached a steady-state level (fig 2). At the termination of exposure the mean concentration was 21.2 (SD 6.9)  $\mu\text{mol/l}$  (table 1).

The styrene concentration in alveolar air at the end of exposure was estimated to be about 0.34  $\mu\text{mol/l}$  from the regression line between relative uptake versus the alveolar concentration as the percentage of the concentration in the inspiratory air ( $y =$

$-0.72x + 74.6$ ,  $r = -0.93$ ) (10). The in vivo quotient between blood and alveolar air at the end of exposure was a mean of 62, which was in close agreement with the measured in vitro partition coefficient, 61 (SD 15) at 37°C. The quotient between blood and inspiratory air was significantly lower, a mean of 7.5.

The in vivo steady-state partition coefficient between arterial blood and inspiratory air,  $N_{\text{eff}}$ , can be calculated from the following equation (5):

$$N_{\text{eff}} = N/[1 + E_t(\dot{Q}_t/\dot{V}_A)N].$$

$N$  is the thermodynamic blood:air partition coefficient at 37°C, 61 (according to this study);  $\dot{Q}_t$  is the total cardiac output, 9 l/min at 50 W (1); and  $\dot{V}_A$  is the alveolar ventilation, 17 l/min at 50 W (1).  $E_t$  is the systemic extraction and can be calculated from the difference in solvent concentration between arterial and mixed venous

**Table 1.** Experimental results from 2 h of exposure to a styrene concentration of about 2.88 mmol/m<sup>3</sup> (300 mg/m<sup>3</sup>) during physical exercise with a work load of 50 W. The pulmonary ventilation ( $\dot{V}_E$ ), the amount of styrene given and taken up, and the relative uptake of styrene are given during each 30-min period. The arterial blood concentration of styrene and styrene glycol are given at the end of each 30-min period.

| Time period<br>(min) | $\dot{V}_E$ BTPS <sup>a</sup><br>(l/min)<br>(N = 8) |     | Given amount<br>(mmol)<br>(N = 8) |      | Uptake<br>(mmol)<br>(N = 8) |      | Uptake in % of<br>given amount<br>(N = 8) |     | Styrene concentration<br>in arterial blood<br>( $\mu\text{mol/l}$ )<br>(N = 7 <sup>b</sup> ) |     | Styrene glycol<br>concentration in<br>arterial blood<br>( $\mu\text{mol/l}$ )<br>(N = 7 <sup>b</sup> ) |      |
|----------------------|-----------------------------------------------------|-----|-----------------------------------|------|-----------------------------|------|-------------------------------------------|-----|----------------------------------------------------------------------------------------------|-----|--------------------------------------------------------------------------------------------------------|------|
|                      | Mean                                                | SE  | Mean                              | SE   | Mean                        | SE   | Mean                                      | SE  | Mean                                                                                         | SE  | Mean                                                                                                   | SE   |
| 0–30                 | 19.8                                                | 0.9 | 1.56                              | 0.07 | 1.10                        | 0.05 | 70.9                                      | 1.4 | 17.3                                                                                         | 1.5 | 1.27                                                                                                   | 0.21 |
| 30–60                | 20.6                                                | 0.8 | 1.64                              | 0.07 | 1.10                        | 0.04 | 67.3                                      | 1.5 | 19.8                                                                                         | 2.0 | 1.99                                                                                                   | 0.34 |
| 60–90                | 20.6                                                | 0.8 | 1.65                              | 0.06 | 1.11                        | 0.05 | 67.3                                      | 1.6 | 19.5                                                                                         | 1.5 | 2.69                                                                                                   | 0.34 |
| 90–120               | 21.5                                                | 1.0 | 1.71                              | 0.09 | 1.13                        | 0.06 | 66.1                                      | 1.8 | 21.2                                                                                         | 2.3 | 3.02                                                                                                   | 0.41 |
| 0–120                | 20.6                                                | 0.8 | 6.56                              | 0.26 | 4.44                        | 0.18 | 67.9                                      | 1.5 |                                                                                              |     |                                                                                                        |      |

<sup>a</sup> 37°C, ambient pressure, saturated with water.

<sup>b</sup> Failure of catheter introduction in one subject.

**Table 2.** Calculated individual values of clearance ( $Cl_t$ ), half-time in blood ( $t_{1/2\beta}$ ), and volume of distribution ( $V_d\beta$ ) of styrene from a 2-h inhalation exposure to a styrene concentration of about 2.88 mmol/m<sup>3</sup> (300 mg/m<sup>3</sup>) during physical exercise with a work load of 50 W.

|                                                                | Individual values |     |     |     |     |     |     | Mean | SE  |
|----------------------------------------------------------------|-------------------|-----|-----|-----|-----|-----|-----|------|-----|
|                                                                | A                 | B   | C   | D   | E   | F   | G   |      |     |
| $Cl_t$ (l/min) <sup>a</sup>                                    | 2.1               | 2.2 | 1.4 | 1.5 | 1.3 | 1.8 | 1.8 | 1.7  | 0.1 |
| $Cl_t$ (l $\times$ kg <sup>-1</sup> $\times$ h <sup>-1</sup> ) | 1.8               | 1.6 | 1.2 | 1.5 | 1.0 | 1.6 | 1.6 | 1.5  | 0.1 |
| $t_{1/2\beta}$ (min)                                           | 34                | 30  | 46  | 42  | 46  | 40  | 49  | 41   | 3   |
| $V_d\beta$ (l) <sup>b</sup>                                    | 101               | 95  | 94  | 89  | 86  | 102 | 126 | 99   | 5   |
| $V_d\beta$ (l/kg)                                              | 1.5               | 1.2 | 1.3 | 1.5 | 1.1 | 1.5 | 1.9 | 1.4  | 0.1 |

<sup>a</sup>  $Cl_t = \text{dose}/\text{AUC}_\infty$ ; dose = calculated total uptake ( $\mu\text{mol}$ ),  $\text{AUC}_\infty$  = area under the blood versus concentration time curve during and after exposure ( $\mu\text{mol} \times \text{min/l}$ ).

<sup>b</sup>  $V_d\beta = t_{1/2\beta} \times Cl_t/\ln 2$ .

blood divided by the arterial concentration,  $(C_a - C_v)/C_a$  (5). The mixed venous concentration at the end of exposure was  $16.5 \mu\text{mol/l}$ , estimated from the Fick equation  $[U_L = (C_a - C_v) \times \dot{Q}_t]$ , where  $U_L$  is the respiratory uptake. The resulting value of  $E_t$  was 0.22. With the use of these values in the aforementioned equation, the in vivo steady-state partition coefficient between arterial blood and inspiratory air,  $N_{\text{eff}}$ , was calculated to be 7.5, which is the same as the observed quotient.

$E_t$  can also be expressed as the total blood clearance divided by the cardiac output,  $Cl_t/\dot{Q}_t$  (35).  $Cl_t$  averaged  $1.7$  (SD 0.3)  $\text{l/min}$  ( $1.5 \text{ l/kg} \times \text{h}$ ) (table 2) when calculated from the total dose (uptake) of styrene divided by the total area under the blood concentration versus time curve,  $\text{dose}/\text{AUC}_x$  (35). ( $\text{AUC}_x$  was calculated by the trapezoidal rule during exposure and integrated according to Simpson's rule during the elimination phase). This procedure gave an  $E_t$  of 0.19.

The elimination of styrene from blood

after exposure is shown in fig 2 and 3. The decay was considered biphasic during the time studied. Individual semilogarithmic plots of blood concentration versus time were treated by the method of residuals (28) so as to resolve the curves into a linear terminal phase of slope  $\beta$ , and a linear initial phase of slope  $\alpha$  (fig 3). The half-time ( $t_{1/2}$ ) for the rapid distribution phase (0–5 min,  $\alpha$ ) was 1.9 (SD 0.8) min and that of the elimination phase (10–120 min,  $\beta$ ) was 40.8 (SD 7.0) min (table 2). The volume of distribution,  $V_d\beta$ , of styrene was 99 (SD 13)  $\text{l}$  ( $1.4 \text{ l/kg}$ ) (table 2), according to the equation  $V_d = t_{1/2} \times Cl_t/\ln 2$  (35).

The concentration of styrene in subcutaneous adipose tissue was 53 (SD 17) and 49 (SD 19)  $\mu\text{mol/kg}$  30 and 90 min after exposure, respectively. The quotient between the concentration of styrene in adipose tissue 30 min after exposure to that in arterial blood at the end of exposure was 3.1 (SD 1.1). The mean amount of adipose tissue in the subjects, 12 (SD 7)  $\text{kg}$ , was estimated from the total amount of body fat on

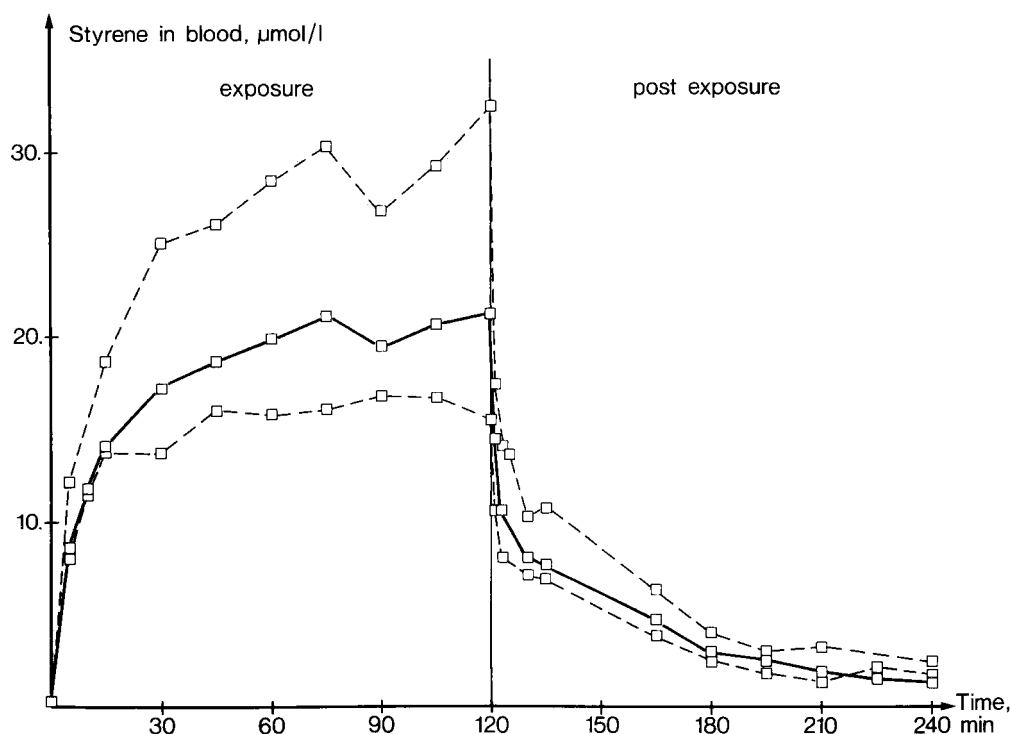


**Fig. 2.** The concentration of styrene in the arterial blood of seven subjects ( $\square$ ) during and after exposure to a styrene concentration of about  $2.88 \text{ mmol/m}^3$  ( $300 \text{ mg/m}^3$ ) for 2 h during physical exercise with a work load of 50 W. The solid line represents the mean value ( $N = 7$ ), and the two broken lines show the two subjects with the overall highest and lowest blood concentration.

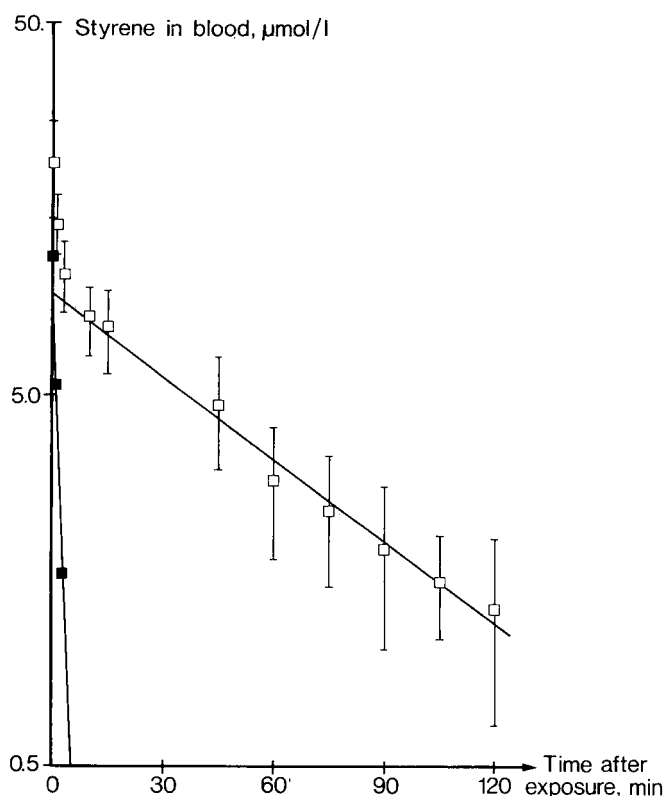


**Fig 3.** Semilogarithmic plot of the mean concentration ( $\pm$  SD) of styrene in the arterial blood of seven subjects ( $\square$ ) after the end of exposure to a styrene concentration of about  $2.88 \text{ mmol/m}^3$  ( $300 \text{ mg/m}^3$ ) for 2 h during physical exercise with a work load of 50 W. The method of residuals was used to resolve the curve into a linear terminal phase and a linear initial phase ( $\blacksquare$ ). Individual regression lines were used to calculate the mean half-time of the initial and terminal phase, 1.9 (SD 0.8) and 40.8 (SD 7.0) min, respectively.

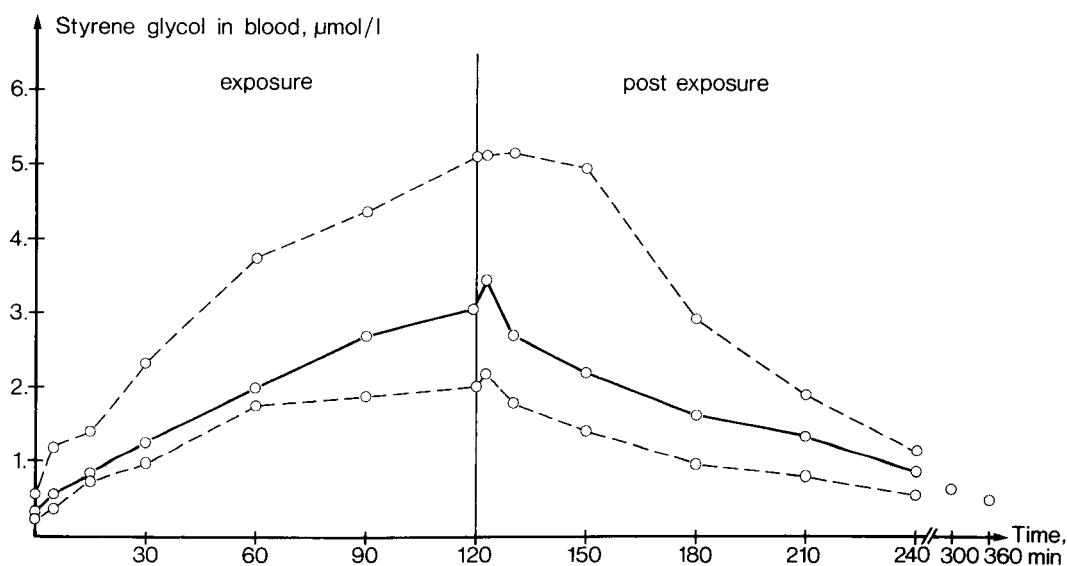


**Fig 4.** The concentration of styrene glycol in the arterial blood of seven subjects during and after exposure to a styrene concentration of about  $2.88 \text{ mmol/m}^3$  ( $300 \text{ mg/m}^3$ ) for 2 h during physical exercise with a work load of 50 W. The solid line represents the mean value ( $N = 7$ ), and the two broken lines show the two subjects with the overall highest and lowest blood concentration. The mean concentration of styrene glycol in the venous blood is shown at 300 and 360 min.

the assumption that the average fat contents of adipose tissue is 80 % (19). If it is assumed that the concentration of styrene in a sample of subcutaneous adipose tissue is representative of the mean concentration in all the adipose tissue, the content of styrene in the adipose tissue can be calculated. Thus the amount of styrene in the adipose tissue was estimated to be 0.6 (SD 0.4) mmol 30 min after exposure; this level represented 13 (SD 8) % of the total uptake.

The blood concentration of nonconjugated styrene glycol increased continuously during exposure (fig 4) and reached 3.0 (SD 1.1)  $\mu\text{mol/l}$  (table 1). The elimination of styrene glycol from blood is shown in fig 4 and 5. A monoexponential decline (2.5–120 min) was observed with a half-time of 72.1 (SD 13.3) min.

In the complementary study with four subjects a styrene oxide concentration of 0.05 (SD 0.03)  $\mu\text{mol/l}$  and styrene glycol concentration of 2.1 (SD 0.3)  $\mu\text{mol/l}$  were detected in venous blood collected 5–30 min after exposure.

The average cumulative amount of mandelic and phenylglyoxylic acid excreted within a mean of 28 (SD 5) h after exposure was 2.6 (SD 0.4) mmol or 58 (SD 10) % of the total uptake. Mandelic acid constituted 59 (SD 10) % of the amount of the acids determined. The peak concentration of both acids appeared in the urine collected about 3 h after exposure. The concentration ratio between mandelic and phenylglyoxylic acid was 3.9 (SD 1.7) in the urine sampled about 0.5 h after exposure and 0.6 (SD 0.4)

the next morning, about 20 h after exposure. The half-time, 0–20 h after exposure, was 3.6 (SD 0.4) h for the elimination of mandelic acid and 8.8 (SD 1.3) h for phenylglyoxylic acid.

## Discussion

The arterial blood concentration of styrene reached a relatively stable level of about 20  $\mu\text{mol/l}$  after 75 min of exposure. After 2 h of exposure to styrene 88 % of the steady-state concentration in relatively well perfused tissues should have been reached when one considers that the half-time was 41 min. The fact that steady state was almost obtained is supported by the agreement between the theoretical and the experimental  $N_{\text{eff}}$ . In a study by Ramsey et al (34) the venous blood concentration of styrene, after 2 h of exposure to about 3.27 mmol/m<sup>3</sup> at rest, was about 90 % of the concentration after 6 h of exposure. The mean half-time for the rapid elimination phase of 41 min in this study is in close agreement with that obtained by Teramoto & Horiguchi in styrene workers (41) and by Ramsey et al in volunteers (34) (40 and 35 min, respectively). The  $V_d$  determined by Ramsey et al was a mean of 1,350 ml/kg, and their data gave a  $\text{Cl}_t$  average of 1.6 l/kg  $\times$  h, ie, the same as obtained in the present study (table 2). The  $\text{Cl}_t$  value for styrene is about the same or slightly higher than the total blood flow through the liver [about 1.6 l/min at rest or during a work load of

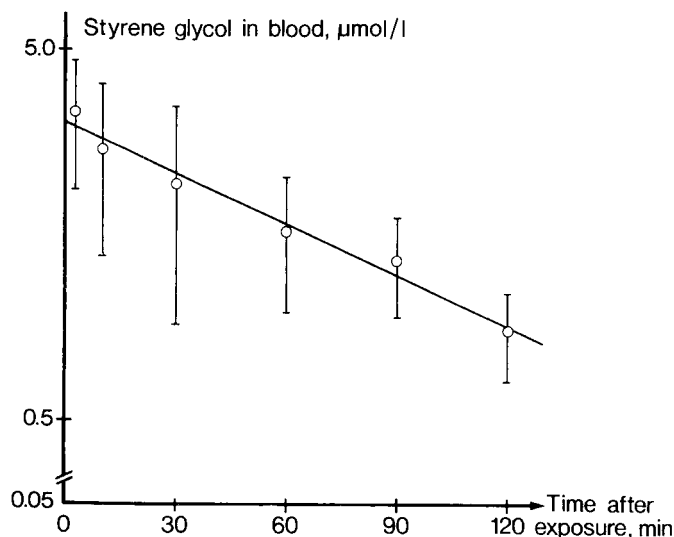


**Fig 5.** Semilogarithmic plot of the mean ( $\pm$  SD) concentration of styrene glycol in the arterial blood after the end of exposure to a styrene concentration of about 2.88 mmol/m<sup>3</sup> (300 mg/m<sup>3</sup>) for 2 h during physical exercise with a work load of 50 W. Individual regression lines were used to calculate the mean half-time of 72.1 (SD 13.3) min.

50 W (1)]. The high clearance indicates that styrene during rest and light physical exercise is metabolized in a high-affinity perfusion-limited pathway in man. This phenomenon has earlier been proposed for the metabolism of styrene in rats (4). Besides almost complete hepatic extraction ( $E_h \rightarrow 1$ ) the obtained  $Cl_t$  values probably represent some extrahepatic metabolism (9, 32, 36, 37), some continued uptake by adipose and other poorly perfused tissues, and a small contribution from the expiration of unchanged styrene after exposure (15, 34).  $E_t$  was found to be about 0.2 in this study with light or moderate physical work. This value is lower than the  $E_t$  calculated from the data published by Ramsey et al (assuming  $\dot{Q}_t = 7.4$  l/min), which was 0.3 (34). The difference can be explained by the smaller fraction of  $\dot{Q}_t$  perfusing the liver during physical exercise compared with rest (1); it stresses the importance of hepatic perfusion in the elimination of styrene.

The adipose tissue : blood concentration ratio at equilibrium can be estimated according to the relation  $\lambda_{\text{adipose tissue}} = 0.7 \lambda_{\text{oil}} + 0.3 \lambda_{\text{blood}}$ , where  $\lambda$  is the partition coefficient tissue : air (40). With the oil : air and blood : air partition coefficients obtained by Sato & Nakajima, 5,465 and 52, respectively (38), the adipose tissue : blood partition coefficient would be about 74. The comparably low ratio between the styrene concentration in subcutaneous adipose tissue and arterial blood of about 3 at the end of exposure indicates that styrene in subcutaneous adipose tissue is far from equilibrium with styrene in arterial blood after 2 h of exposure. According to the long half-time of styrene in subcutaneous adipose tissue, 2.2–5.2 d (14, 15), it would take at least 7 d of continuous exposure to reach 90 % of steady state. Calculations of the total amount of styrene in the adipose tissue are very rough estimates based on the assumption that the concentrations in the biopsies from the gluteal region are representative of all adipose tissue. The blood perfusion in adipose tissue is not uniform [Blood perfusion ratios between perirenal and subcutaneous adipose tissue of 1.7 at rest and 3.2 at a work load of about 100 W have been reported (8).], and therefore the distribution of a solvent is probably not either. However the calcu-

lated relative uptake of about 13 % in adipose tissue after exposure is of about the same size as the 8 % reported after exposure to styrene during stepwise increased work load (15).

The concentration of nonconjugated styrene glycol in blood continued to rise after the styrene concentration had leveled off; this finding was to be expected from the obtained values of half-time in blood, 72 min for styrene glycol and 41 min for styrene. The epoxide hydratase activity in tissues of different species is much higher than the monooxygenase activity, and the formed styrene oxide can be expected to be readily converted to styrene glycol also in man (9, 27, 29). If so, the half-time of styrene glycol determines the time to reach a steady-state level of styrene glycol in blood, and it would take about 4 h of continuous exposure to reach 90 % of steady state (cf 2.2 h for styrene). Recently a styrene glycol concentration of about 1  $\mu\text{mol/l}$  was detected in human blood after 4 h of styrene exposure at 2.11  $\text{mmol/m}^3$  at rest (44).

The detection of styrene oxide in human blood might be of toxicologic importance. Styrene oxide was also detected in the venous blood (duplicate samples taken before lunch) of workers in a styrene polymerization plant. The styrene oxide and styrene glycol concentration in blood from these workers ( $N = 5$ ) was 0.03 (SD 0.01) and 1.6 (SD 1.2)  $\mu\text{mol/l}$ , respectively (unpublished results).

The half-time of mandelic and phenylglyoxylic acid in urine, 3.6 and 8.8 h (0–20 h after exposure), respectively, is in agreement with the half-time of 3.9 (0–20 h) and 10.5 h (0–50 h), respectively, reported by Guillemin & Bauer (22). In the present study the elimination rate of mandelic acid seemed to decrease 15–20 h after exposure. Because of the low concentrations (close to the detection limit) and the few individual values after 20 h of exposure, we were unable to identify a slower elimination phase, which has been reported to have a half-time of 24.7 h (30–60 h) (22). Due to the biphasic elimination of mandelic and phenylglyoxylic acid, the half-time obtained in different experimental studies is influenced by the duration of the exposure. Since the ratio between mandelic and phenylglyoxylic acid varies with

time, the duration of exposure also influences the ratios obtained at different times after exposure. Guillemin & Bauer reported a ratio of 3.0 after a 4- to 8-h long exposure and 0.8 14 h after the exposure (22). In the present study the intraindividual differences in this ratio were large; they varied between 2.6 and 7.9 at the end of exposure and between 0.2 and 1.4 20 h after exposure. Using only the mandelic or phenylglyoxylic acid concentration for biological monitoring is therefore not advisable. The sum of both acids in the urine the next morning has been advocated to give a good measure of styrene uptake during the previous day (22). In our study with a rather narrow uptake range (3.9–5.3 mmol) there was no correlation between the total uptake of styrene and the cumulative excretion of mandelic and phenylglyoxylic acid, neither at the end of exposure ( $r = 0.05$ ) nor the next morning, about 20 h after exposure ( $r = -0.17$ ).

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