Uptake, distribution, metabolism, and elimination of styrene in man. A comparison between single exposure and co-exposure with acetone

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ABSTRACT Six male subjects were exposed for two hours during light physical exercise to 2.81 mmol/m³ (293 mg/m³) of styrene on one occasion and to a mixture of 2.89 mmol/m³ (301 mg/m³) of styrene and 21.3 mmol/m³ (1240 mg/m³) of acetone on another (combination study). About 68% of the dose (somewhat more than 4 mmol) of styrene was taken up. The arterial blood concentration of styrene reached a relatively stable level after about 75 minutes of exposure of about 18 and 20 μmol/l after the single and combined exposure, respectively. Calculated values of mean blood clearance were 1.9 l/min in the styrene study and 1.6 l/min in the combination study; the half life of styrene in blood was about 40 minutes in both studies. The concentration of non-conjugated styrene glycol increased linearly during exposure and reached about 3 μmol/l at the end of exposure and was eliminated with a half life of about 70 minutes. Styrene-7,8-oxide was detected and quantified in the blood in a complementary study. The half lives for the excretion of mandelic and phenylglyoxylic acid in the urine were about four and nine hours, respectively, in both studies.

Styrene is one of the most widely used raw materials in the modern polymer industry and the most extensive and intensive exposure occurs in the reinforced plastics industry.¹ Laminators are also often exposed to acetone used as a cleaning agent.²

Exposure of experimental animals and man to isopropanol or its keto metabolite acetone has been shown to potentiate the effects of hepatic and renal toxins such as chlorinated hydrocarbons³⁴⁵⁶⁷⁸⁹ perhaps by increasing their covalent binding to hepatic proteins.¹⁰¹¹ Acetone is largely responsible for the remarkable potentiating ability of isopropanol,¹² which may be important when considering occupational exposure where exposure to a variety of chemical agents can alter the toxic effects of any one of them.

The mechanism of potentiation of hepatotoxicity has been postulated to be either non-specific membrane changes which render the cell more susceptible to toxic injury⁴ or increased bioactivation of the toxicants to reactive intermediates⁵⁶⁷¹¹¹³−¹⁷ or both. Other effects on cellular function or metabolism, however, cannot be excluded.⁶⁷¹¹

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The metabolism of styrene takes place mainly in the liver but also occurs in extrahepatic tissues.¹⁸−²¹ The biotransformation is stimulated by styrene itself²²²³ and phenobarbital²¹²² and is suppressed by the coadministration of ethanol, toluene, and trichloroethylene.²⁵−²⁷ The first step in the major metabolic pathway is the formation of styrene-7,8-oxide (SO),²⁸²⁹ a reaction preferentially catalysed by the microsomal cytochrome P-450 system. It is now generally accepted that the toxicity of styrene may be mainly ascribed to its biotransformation to the more reactive compound SO which is known to be mutagenic³⁰−³⁵ and capable of covalent binding to macromolecules in vivo (M Byfält Nordqvist et al, in preparation).³⁶ SO is hydrated to styrene glycol (SG) which is catalysed by epoxide hydratase³⁷³⁸ with subsequent metabolism to mandelic, phenylglyoxylic, benzoic, and hippuric acids; SO is also conjugated with glutathione (GSH) with urinary elimination of mercapturic acids.²¹ Conjugation of SG with glucuronic acid and styrene metabolism to 1- and 2-phenylethanol and to 4-vinylphenol are minor pathways.²¹ In man about 90% of the styrene uptake is eliminated as mandelic and phenylglyoxylic acids.³⁹⁴⁰

Acetone has been shown to enhance the hepatic
metabolism of various drugs in vitro\textsuperscript{41–44} and in vivo.\textsuperscript{10,17,45,46} Significantly reduced hepatic glutathione concentrations and slightly increased hepatic cytochrome P-450 levels have been shown in rats after inhalation exposure to acetone alone and to a mixture of acetone and styrene.\textsuperscript{47} A dose dependent depression of hepatic glutathione level has been observed after inhalation exposure to styrene.\textsuperscript{48} The covalent binding of SO to rat liver macromolecules has been shown to be dependent on the glutathione content of the liver.\textsuperscript{36}

The purpose of this study was to investigate if the uptake, distribution, metabolism, or elimination, or a combination of these, of styrene in man after an acute exposure was modified by simultaneous exposure to acetone at the recommended Swedish short term exposure limit concentrations.

**Subjects and methods**

The volunteers were six healthy men with an average age of 26 (range 23–34), an average weight of 69 kg (range 60–80) and an average height of 177 cm (range 172–180). They had no occupational exposure to solvents and none had suffered from any disease having a detrimental effect on the function of the respiratory and circulatory systems.

The subjects were exposed in pairs in an exposure chamber for two hours to 2·81 (SD 0·03) mmol/m\(^3\) (293 mg/m\(^3\)) of styrene on one occasion and to a mixture of 2·89 (SD 0·04) mmol/m\(^3\) (301 mg/m\(^3\)) of styrene and 21·3 (SD 0·2) mmol/m\(^3\) (1240 mg/m\(^3\)) of acetone on another (combination study). The two exposures were at least one month apart. The recommended Swedish short term exposure limit value is 2·88 mmol/m\(^3\) (300 mg/m\(^3\)) for styrene and 20·7 mmol/m\(^3\) (1200 mg/m\(^3\)) for acetone. The exposures were performed during light physical exercise (work load of 50 W) on a bicycle ergometer.

The uptake of styrene was measured using the Douglas bag technique. Unchanged styrene and the styrene metabolites styrene glycol (SG) and styrene oxide (SO) were analysed by gas chromatography in arterial blood sampled during and after exposure (sampling times are shown in fig 1). The styrene concentration in subcutaneous adipose tissue was determined by gas chromatography in biopsy specimens taken 30 and 90 minutes after exposure. The concentrations of mandelic and phenylglyoxylic acid (MA and PGA) in the urine, in samples taken up to about 25 hours after exposure, were analysed by isotachophoresis. For details of the exposure conditions and the analytical methods used in the deter-
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Table 1 Experimental results from two hours of exposure to about 2.81 mmol/m³ (293 mg/m³) of styrene (St) and 2.89 mmol/m³ (301 mg/m³) of styrene and 21.3 mmol/m³ (1240 mg/m³) of acetone (St + Ac) in combination during physical exercise with a work load of 30 W. The pulmonary ventilation (VE), the amount of styrene given and taken up, and the relative uptake of styrene are given during each 30 minute period. The arterial blood concentration of styrene and styrene glycol are given at the end of each 30 minute period. Mean values and standard errors of means are given.

<table>
<thead>
<tr>
<th>Time period (min)</th>
<th>VE BTPS*</th>
<th>Amount given (mmol)</th>
<th>Uptake (mmol)</th>
<th>Uptake in % of given amount (n=6)</th>
<th>Styrene concentration in arterial blood (μM)</th>
<th>Styrene glycol concentration in arterial (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-30St</td>
<td>19.6 ± 1.2</td>
<td>1.54 ± 0.09</td>
<td>1.09 ± 0.05</td>
<td>71.0 ± 1.6</td>
<td>15.6 ± 1.0</td>
<td>1.41 ± 0.28</td>
</tr>
<tr>
<td>St + Ac</td>
<td>19.0 ± 2.2</td>
<td>1.53 ± 0.19</td>
<td>1.03 ± 0.08</td>
<td>68.8 ± 2.7</td>
<td>16.1 ± 0.9</td>
<td>1.24 ± 0.21</td>
</tr>
<tr>
<td>30-60 St</td>
<td>20.8 ± 0.8</td>
<td>1.66 ± 0.07</td>
<td>1.10 ± 0.03</td>
<td>67.1 ± 2.0</td>
<td>17.4 ± 1.5</td>
<td>2.08 ± 0.43</td>
</tr>
<tr>
<td>St + Ac</td>
<td>19.2 ± 1.5</td>
<td>1.57 ± 0.13</td>
<td>1.03 ± 0.05</td>
<td>66.2 ± 2.3</td>
<td>19.1 ± 0.9</td>
<td>2.12 ± 0.31</td>
</tr>
<tr>
<td>60-90 St</td>
<td>20.3 ± 0.9</td>
<td>1.62 ± 0.08</td>
<td>1.09 ± 0.06</td>
<td>67.2 ± 2.1</td>
<td>17.4 ± 0.7</td>
<td>2.72 ± 0.47</td>
</tr>
<tr>
<td>St + Ac</td>
<td>19.1 ± 1.7</td>
<td>1.62 ± 0.15</td>
<td>1.06 ± 0.07</td>
<td>66.1 ± 2.0</td>
<td>19.4 ± 1.0</td>
<td>2.97 ± 0.79</td>
</tr>
<tr>
<td>90-120 St</td>
<td>21.2 ± 1.3</td>
<td>1.68 ± 0.11</td>
<td>1.09 ± 0.06</td>
<td>66.7 ± 2.3</td>
<td>17.9 ± 0.6</td>
<td>3.08 ± 0.56</td>
</tr>
<tr>
<td>St + Ac</td>
<td>20.2 ± 1.5</td>
<td>1.71 ± 0.13</td>
<td>1.10 ± 0.06</td>
<td>64.9 ± 1.9</td>
<td>20.2 ± 0.9</td>
<td>3.20 ± 0.67</td>
</tr>
<tr>
<td>0-120 St</td>
<td>20.5 ± 0.9</td>
<td>6.50 ± 0.30</td>
<td>4.38 ± 0.17</td>
<td>67.7 ± 1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>St + Ac</td>
<td>19.4 ± 1.7</td>
<td>6.42 ± 0.60</td>
<td>4.22 ± 0.26</td>
<td>66.6 ± 2.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Temperature of 37°C, ambient pressure, saturated with water.

minoration of styrene and its metabolites the reader is referred to the report by Wigaeus et al.46

Acetone in the inspiratory air was analysed using the same gas chromatographic equipment as for styrene. The analysis of acetone in blood was performed using a headspace technique similar to that described elsewhere.47 Individual calibration curves were obtained by adding 2 μl of standard solutions of styrene and acetone in dimethylsulphoxide (DMSO) to 1 ml of blood. A constant volume of 1 ml blood and 2 μl of DMSO was used throughout the experiment. The concentration of acetone in subcutaneous adipose tissue was analysed by a gas chromatographic "purge-and-trap" method.51

Student's t test for dependent observations was used for statistical analysis and a probability of 0.05 was taken as the criterion of significance.

Results

The total and relative uptakes of styrene were similar during the single and combined exposures, 4.4 (SD 0.4) and 4.2 (SD 0.6) mmol, and 68 (SD 5) and 67 (SD 5) per cent (table 1). The uptake of acetone was about 20 mmol, calculated from the amount supplied and assuming a relative uptake of 45%.49

The styrene concentration in the arterial blood (five subjects only because of the failure to introduce the catheter in one subject) increased during the first 75 minutes and then approached a steady state (fig 1). At the termination of exposure the mean concentration was 18 (SD 1) and 20 (SD 2) μmol/l in the single and combined exposures, respectively; this difference was not statistically significant. The acetone concentration in blood increased linearly with time (y = 5.0x + 63.0; r = 0.99; n = 11) over the whole exposure period and reached 649 (SD 77) μmol/l at the end of the exposure.

The total blood clearance, Cl, of styrene (calculated from Cl = dose/AUCₚ)² was 1.9 (SD 0.3) l/min with the single exposure and 1.6 (SD 0.3) l/min with the combined exposure (table 2). This difference was not statistically significant.

The rate of elimination of styrene from the blood after exposure is shown in figs 1 and 2 and was considered to show a biphasic decay. Individual semilogarithmic plots of blood concentration versus time were treated by the method of residuals so as to resolve the curves into a linear terminal phase of slope β, and a linear initial phase of slope α² (fig 2). The half life (t₁/₂) for the rapid distribution phase (0–5 min, α) was 1.9 (SD 0.9) min and 1.1 (SD 0.1) min, and the t₁/₂ of the elimination phase (10–120 min, β) was 38.8 (SD 7.4) min and 35.0 (SD 10.2) min in the single and combined studies respectively (table 2). These differences were not statistically significant. A monoexponential decline (0–120 min) with a t₁/₂ of 3.1 (SD 0.5) h was observed for acetone in blood.

The volume of distribution, Vₐβ, of styrene (calculated from Vₐβ = t₁/₂β × Cl/ln 2)² was 102 (SD 14) l in the single exposure study and 84 (SD 39) l in the combined study. This difference was not statistically significant.

The concentration of styrene in the subcutaneous adipose tissue 30 and 90 minutes after exposure was 62 (SD 17) and 53 (SD 17) μmol/kg in the single exposure study and somewhat higher, 75 (SD 58) and 91 (SD 55) μmol/kg, in the combined study. The intrindividual variations were large, however, especially in the combined study. This may be
Table 2  Calculated values of blood clearance (Cl), volume of distribution (Vd), and half lives (t1/2) of styrene, styrene glycol (SG) in blood and mandelic acid (MA), and phenylglyoxylic acid (PGA) in urine after two hours of inhalation exposure to about 2-81 mmol/m³ (293 mg/m³) of styrene (St) and 2-89 mmol/m³ (301 mg/m³) of styrene and 21-3 mmol/m³ (1240 mg/m³) of acetone (St+Ac) in combination during physical exercise with a work load of 50 W. Mean values and standard errors of means are given.

<table>
<thead>
<tr>
<th></th>
<th>St</th>
<th>St + Ac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl</td>
<td>1-9±0-1 l/min</td>
<td>1-6±0-1 l/min</td>
</tr>
<tr>
<td>Vd</td>
<td>102±6 l</td>
<td>84±18 l</td>
</tr>
<tr>
<td>t1/2 St</td>
<td>39±3 min</td>
<td>35±4 min</td>
</tr>
<tr>
<td>(10-120 min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>t1/2 SG</td>
<td>71±6 min</td>
<td>66±2 min</td>
</tr>
<tr>
<td>(2-5-120 min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>t1/2 MA</td>
<td>3-6±0-2 h</td>
<td>4-0±0-5 h</td>
</tr>
<tr>
<td>(0-20 h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>t1/2 PGA</td>
<td>8-8±0-6 h</td>
<td>8-6±0-9 h</td>
</tr>
<tr>
<td>(0-20 h)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

because single samples only were analysed for their styrene content in the combined study whereas duplicate samples were taken and the mean values used in the single exposure study. The mean acetone concentration in the subcutaneous adipose tissue was 225 (SD 67) and 233 (SD 57) μmol/kg at 30 and 90 minutes after exposure. The ratio of styrene in adipose tissue 30 minutes after exposure to that in arterial blood at the end of exposure was 3-5 (SD 0-5) in the single study and 3-9 (SD 3-7) in the combined study. The corresponding ratio for acetone was 0-35 (SD 0-08).

The arterial blood concentration of non-conjugated SG increased continuously during exposure and reached 3-1 (SD 1-2) μmol/l in the single exposure study and 3-2 (SD 1-5) μmol/l in the combined study (fig 3). SO could not be detected in any of the samples in this experiment. Some analytical modifications44 a complementary study with four subjects was performed and 0-05 (SD 0-03) μmol/l of SO and 2-1 (SD 0-3) μmol/l of SG were detected in venous blood collected 5-30 minutes after exposure.

A monoexponential decline of SG in blood was observed (fig 4). The mean t1/2 (2-5-120 min) was 71 (SD 14) minutes in the single exposure study and 66 (SD 4) minutes in the combined study (table 2), a non-significant difference.

The cumulative urinary excretion of MA and PGA within a mean of 25 (SD 2) hours after exposure represented 51 (SD 8) per cent of the total uptake in the single exposure study. The corresponding value was 52 (SD 13) per cent within 24 (SD 4) hours in the combined study. The excretion rates of MA and PGA were considered to decline monoexponentially within 20 hours of exposure, and no difference between the two exposure conditions was observed. The t1/2 for the elimination of MA was 3-6 (SD 0-5) hours in the single study and 4-0 (SD 1-2) hours in the combined study; for the elimination of PGA, the corresponding half lives were 8-8 (SD 1-5) and 8-6 (SD 2-1) hours (table 2).

Discussion

The calculated Cl, values for styrene of 1-9 and 1-6 l/min are of the same order as the total blood flow through the liver (about 1-6 l/min) during rest or light physical exercise.35 35 Styrene is mainly cleared by biotransformation.39 40 Since the liver, with its high activity of styrene metabolising enzymes39 40 and its large mass, invariably contains more degradative enzymes than extrahepatic tissues the liver probably also plays a predominant part in vivo in the total clearance of styrene. Therefore the Cl, values obtained in this study can (apart from some
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**Fig 3** Concentration of styrene glycol in arterial blood for five subjects during and after exposure to 2.81 mmol/m³ (293 mg/m³) of styrene (○) and to a combination of 2.89 mmol/m³ (301 mg/m³) of styrene and 21.3 mmol/m³ (1240 mg/m³) of acetone (●) for two hours during physical exercise with a work load of 50 W. The two last values represent venous blood. Mean values (n=5) and standard deviations are shown.

**Fig 4** Semilogarithmic plot of concentration of styrene glycol in arterial blood after end of exposure to 2.81 mmol/m³ (293 mg/m³) of styrene (○) and to a combination of 2.89 mmol/m³ (301 mg/m³) of styrene and 21.3 mmol/m³ (1240 mg/m³) of acetone (●) for two hours during physical exercise with a work load of 50 W.

continued accumulation in adipose tissue) be mainly ascribed to hepatic clearance with a minor contribution only from metabolism in extrahepatic tissues. Thus at low exposures styrene seems to be effectively extracted from the blood perfusing the liver—that is, the hepatic extraction ratio (Eh)→1. The claim that in rats styrene at low levels is metabolised in a high affinity perfusion limited pathway seems to be valid also in man.

With higher exposures the elimination kinetics of styrene from the blood have been found to be dose dependent, indicating a saturation of the rate limiting step. The saturating exposure level of styrene vapour was estimated to be between 200 and 600 ppm (8-2 and 24-5 mmol/m³) in rats. Dose dependent urinary excretion of MA and PGA after styrene administration has also been shown. There are indications in published reports of dose dependent urinary excretion of MA and PGA in occupationally exposed workers also. Guillemin and Bauer, however, found that the rates of elimination of MA and PGA during short term (4-8 h) experimental exposure to 50-200 ppm (2-0-8-2 mmol/m³) were not dose dependent. The decreased half life of MA excretion shown in workers exposed to higher concentrations indicates a possible enzyme induction by styrene itself resulting in an increased rate of breakdown. A decreased styrene accumulation in rat tissues after prolonged exposure also suggests metabolic adaptation. Stimulating agents are not able to enhance the metabolic rate of high affinity chemicals occurring at low concentrations.
We are very grateful to Professor I Åstrand for encouraging and valuable discussions. We are also grateful to Ms E Gullstrand, Ms E Lundgren, Ms E-M Nydahl, Ms C Uggla, and Ms K Wiberg for their skilful technical help, and Ms M-B Cedervall for her patient typing of this manuscript.

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