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 μ mol/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 μ mol/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 hydrocarbons³⁻⁹ perhaps by increasing their covalent binding to hepatic proteins.^{10,11} 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 intermediates,^{4,6,7,11,13-17} or both. Other effects on cellular function or metabolism, however, cannot be excluded.^{6,7,11}

The metabolism of styrene takes place mainly in the liver but also occurs in extrahepatic tissues.¹⁸⁻²¹ The biotransformation is stimulated by styrene itself^{22,23} and phenobarbital^{21,24} 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),^{28,29} 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 hydratase^{37,38} 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-phenylethanols and to 4-vinylphenol are minor pathways.²¹ In man about 90% of the styrene uptake is eliminated as mandelic and phenylglyoxylic acids.^{39,40}

Acetone has been shown to enhance the hepatic

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Fig 1 Concentration of styrene 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. Mean values (n=5) and standard deviations are shown.

metabolism of various drugs in vitro⁴¹⁻⁴⁴ and in vivo.^{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.⁴⁷ A dose dependent depression of hepatic glutathione level has been observed after inhalation exposure to styrene.⁴⁸ The covalent binding of SO to rat liver macromolecules has been shown to be dependent on the glutathione content of the liver.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/m³ (293 mg/m³) of styrene on one occasion and to a mixture of 2.89 (SD 0.04) mmol/m³ (301 mg/m³) of styrene and 21.3 (SD 0.2) mmol/m³ (1240 mg/m³) 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/m³ (300 mg/m³) for styrene and 20.7 mmol/m³ (1200 mg/m³) 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 isotachopheresis. For details of the exposure conditions and the analytical methods used in the deter-

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 50 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

Time period (min)	VE BTPS* (l/min) (n=6)	Amount given (mmol) (n=6)	Uptake (mmol) (n=6)	Uptake in % of given amount (n=6)	Styrene concentration in arterial blood (μM) (n=5)	Styrene glycol concentration in arterial (μM) (n=5)
0-30St	19.6 ± 1.2	1.54 ± 0.09	1.09 ± 0.05	71.0 ± 1.6	15.6 ± 1.0	1.41 ± 0.28
St + Ac	19.0 ± 2.2	1.53 ± 0.19	1.03 ± 0.08	68.8 ± 2.7	16.1 ± 0.9	1.24 ± 0.21
30-60 St	20.8 ± 0.8	1.66 ± 0.07	1.10 ± 0.03	67.1 ± 2.0	17.4 ± 1.5	2.08 ± 0.43
St + Ac	19.2 ± 1.5	1.57 ± 0.13	1.03 ± 0.05	66.2 ± 2.3	19.1 ± 0.9	2.12 ± 0.31
60-90 St	20.3 ± 0.9	1.62 ± 0.08	1.09 ± 0.06	67.2 ± 2.1	17.4 ± 0.7	2.72 ± 0.47
St + Ac	19.1 ± 1.7	1.62 ± 0.15	1.06 ± 0.07	66.1 ± 2.0	19.4 ± 1.0	2.97 ± 0.79
90-120 St	21.2 ± 1.3	1.68 ± 0.11	1.09 ± 0.06	66.7 ± 2.3	17.9 ± 0.6	3.08 ± 0.56
St + Ac	20.2 ± 1.5	1.71 ± 0.13	1.10 ± 0.06	64.9 ± 1.9	20.2 ± 0.9	3.20 ± 0.67
0-120 St	20.5 ± 0.9	6.50 ± 0.30	4.38 ± 0.17	67.7 ± 1.9		
St + Ac	19.4 ± 1.7	6.42 ± 0.60	4.22 ± 0.26	66.6 ± 2.2		

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

mination of styrene and its metabolites the reader is referred to the report by Wigaeus *et al.*⁴⁹

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.⁵⁰ 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%.⁵⁰

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_t , of styrene (calculated from $Cl_t = \text{dose}/AUC_{\infty}$)⁵² 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 α ⁵³ (fig 2). The half life ($t_{1/2}$) 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 $t_{1/2}$ 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 $t_{1/2}$ of 3.1 (SD 0.5) h was observed for acetone in blood.

The volume of distribution, $V_d\beta$, of styrene (calculated from $V_d\beta = t_{1/2}\beta \times Cl_t/\ln 2$)⁵² 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 intraindividual variations were large, however, especially in the combined study. This may be

Table 2 Calculated values of blood clearance (Cl_b), volume of distribution ($V_d\beta$), and half lives ($t_{1/2}$) 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

	St	St + Ac
Cl_b	1.9 ± 0.1 l/min	1.6 ± 0.1 l/min
$V_d\beta$	102 ± 6 l	84 ± 18 l
$t_{1/2}$ styrene (10–120 min)	39 ± 3 min	35 ± 4 min
$t_{1/2}$ SG (2.5–120 min)	71 ± 6 min	66 ± 2 min
$t_{1/2}$ MA (0–20 h)	3.6 ± 0.2 h	4.0 ± 0.5 h
$t_{1/2}$ PGA (0–20 h)	8.8 ± 0.6 h	8.6 ± 0.9 h

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) $\mu\text{mol/kg}$ 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) $\mu\text{mol/l}$ in the single exposure study and 3.2 (SD 1.5) $\mu\text{mol/l}$ in the combined study (fig 3). SO could not be detected in any of the samples in this experiment. After some analytical modifications⁴⁹ a complementary study with four subjects was performed and 0.05 (SD 0.03) $\mu\text{mol/l}$ of SO and 2.1 (SD 0.3) $\mu\text{mol/l}$ 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 $t_{1/2}$ (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 condi-

Fig 2 Semilogarithmic plot of concentration of styrene in arterial blood for five subjects 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. Mean values (n=5) are shown.

tions was observed. The $t_{1/2}$ 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_b 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.^{54,55} Styrene is mainly cleared by biotransformation.^{39,40} Since the liver, with its high activity of styrene metabolising enzymes^{18–21} 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_b values obtained in this study can (apart from some

Fig 3 Concentration of styrene glycol in arterial blood for five subjects during and after exposure to 2.81 mmol/m^3 (293 mg/m^3) of styrene ($\circ$) and to a combination of 2.89 mmol/m^3 (301 mg/m^3) of styrene and 21.3 mmol/m^3 (1240 mg/m^3) of acetone ($\bullet$) 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/m^3 (293 mg/m^3) of styrene ($\circ$) and to a combination of 2.89 mmol/m^3 (301 mg/m^3) of styrene and 21.3 mmol/m^3 (1240 mg/m^3) of acetone ($\bullet$) 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 contribu-

tion 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 (E_h) $\rightarrow 1$. The claim that in rats styrene at low levels is metabolised in a high affinity perfusion limited pathway⁵⁶ 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.^{57–59} The saturating exposure level of styrene vapour was estimated to be between 200 and 600 ppm (8.2 and 24.5 mmol/m^3) in rats.⁵⁸ Dose dependent urinary excretion of MA and PGA after styrene administration has also been shown.⁶⁰ There are indications in published reports of dose dependent urinary excretion of MA and PGA in occupationally exposed workers also.^{61,62} 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/m^3) were not dose dependent.⁴⁰ 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.⁶³ Stimulating agents are not able to enhance the metabolic rate of high affinity chemicals occurring at low concentrations

(below K_m , app), however, implying a perfusion limited metabolic clearance.⁵⁶ The extrahepatic metabolism of styrene is not necessarily perfusion limited, however, even at low concentrations and the activities of styrene mono-oxygenase and epoxide hydratase in the extrahepatic tissues of many species are lower, and therefore easier to saturate, than those in the liver.¹⁸⁻²¹ Consequently the stimulation of extrahepatic drug metabolising enzymes might increase the rate of clearance of styrene. The lung may be of particular importance since it receives all of the cardiac output⁶⁴; changes in tissue perfusion might also modify tissue extraction and hence clearance.

The $t_{1/2}$ of styrene in blood is dependent on Cl and V_d : ($t_{1/2} = \ln 2 \times V_d/Cl$).⁵² In rats V_d of styrene is independent of the dose (12.5–90.3 $\mu\text{mol/kg}$) whereas the Cl is dose dependent, giving a longer $t_{1/2}$ with increasing doses.⁵⁸ The calculated mean values of V_d , Cl , and $t_{1/2}$ were all somewhat lower in the combined study compared with the single exposure study (table 2).

In this study the solvent concentrations, work load, or exposure time, or a combination of these, were not adequate enough to cause a noticeable interference of Cl . The minimal blood acetone concentration capable of inducing carbon tetrachloride hepatotoxicity in rats has been estimated to be about 5 mmol/l⁶—that is, only about eight times higher than the blood concentration obtained in this study. Pretreatment of rats with high doses of acetone (60 mmol/kg intraperitoneally) has been shown to affect the hepatic microsomal drug metabolising activity within 15 minutes.⁴⁵ At lower doses (2.2 mmol/kg intraperitoneally) acetone did not modify the total urinary excretion of mandelic and phenylglyoxylic acid after an equimolar dose of styrene as compared with the excretion after administration of styrene alone.²⁷ The possible modification of the formation of SO from styrene and the subsequent hydration to SG, however, is of greater toxicological importance.

No modification of either the formation or the oxidation, or both, of SG or the excretion of MA and PGA was seen in the combined exposure. An increased concentration of SG in blood and a delayed excretion of MA in the urine have been noted after the co-administration of ethanol (p.o) and styrene, as compared with styrene inhalation alone.²⁵

The present study did not show any significant interactions of acetone on the uptake, distribution, metabolism, or elimination, or a combination of these, of styrene during acute co-exposure to acetone at the recommended Swedish short term exposure limit concentrations and light physical work.

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References

- Tossavainen A. Styrene use and occupational exposure in the plastics industry. *Scand J Work Environ Health* 1978;4:7–13.
- Kjellberg A, Wigaeus E, Åstrand I, Engström J, Ljungqvist E. Long-term effects of exposure to styrene in a polyester plant. *Arbete och Hälsa* 1979;18:1–25. (Summary in English.)
- Cornish HH, Adefuin J. Potentiation of carbon tetrachloride toxicity by aliphatic alcohols. *Arch Environ Health* 1967;14:447–9.
- Côté MG, Traiger GJ, Plaa GL. Effect of isopropanol-induced potentiation of carbon tetrachloride on rat hepatic ultrastructure. *Toxicol Appl Pharmacol* 1974;30:14–25.
- Traiger GJ, Plaa GL. Chlorinated hydrocarbon toxicity. Potentiation by isopropyl alcohol and acetone. *Arch Environ Health* 1974;28:276–8.
- Hewitt WR, Miyajima H, Côté MG, Plaa GL. Acute alteration of chloroform-induced hepato- and nephrotoxicity by n-hexane, methyl n-butyl ketone, and 2, 5-hexanedione. *Toxicol Appl Pharmacol* 1980;53:230–48.
- MacDonald JR, Gandolfi AJ, Sipes IG. Acetone potentiation of 1,1,2-trichloroethane hepatotoxicity. *Toxicol Lett* 1982;13:57–69.
- Plaa GL, Hewitt WR, du Souich P, Caillé G, Lock S. Isopropanol and acetone potentiation of carbon tetrachloride-induced hepatotoxicity: single versus repetitive pretreatments in rats. *J Toxicol Environ Health* 1982;9:235–50.
- Folland DS, Schaffner W, Grinn HE, Crofford OB, McMurray DR. Carbon tetrachloride toxicity potentiated by isopropyl alcohol. Investigation of an industrial outbreak. *JAMA* 1976;236:1853–6.
- Sipes IG, Stripp B, Krishna G, Maling HM, Gillette JR. Enhanced hepatic microsomal activity by pretreatment of rats with acetone or isopropanol. *Proc Soc Exp Biol Med* 1973;142:237–40.
- MacDonald JR, Gandolfi AJ, Sipes IG. Covalent binding of ¹⁴C-1,1,2-trichloroethane to hepatic proteins following acetone pretreatment. *Drug Chem Toxicol* 1982;5:233–47.
- Traiger GJ, Plaa GL. Relationship of alcohol metabolism to the potentiation of CCl_4 hepatotoxicity induced by aliphatic alcohols. *J Pharmacol Exp Ther* 1972;183:481–8.
- Glende EA Jr, Recknagel RO. Biochemical basis for the in vitro pro-oxidant action of carbon tetrachloride. *Exp Mol Pathol* 1969;11:172–85.
- Plaa GL, Traiger GJ. Mechanism of potentiation of CCl_4 -induced hepatotoxicity. Pharmacology and the future of man. Vol 2. *Proceedings of the 5th International Congress of Pharmacology, San Francisco, 1972*. Basel: Karger, 1973:100–13.
- Traiger GJ, Plaa GL. Effect of aminotriazole on isopropanol- and acetone-induced potentiation of CCl_4 hepatotoxicity. *Can J Physiol Pharmacol* 1973;5:291–6.
- Maling HM, Stripp B, Sipes IG, Highman B, Saul W, Williams MA. Enhanced hepatotoxicity of carbon tetrachloride, thioacetamide, and dimethylnitrosamine by pretreatment of rats with ethanol and some comparisons with potentiation by isopropanol. *Toxicol Appl Pharmacol* 1975;33:291–308.
- Sipes IG, Slocumb ML, Holtzman G. Stimulation of microsomal dimethylnitrosamine-N-demethylase by pretreatment of mice with acetone. *Chem Biol Interactions* 1978;21:155–66.

- ¹⁸ Ryan AJ, James MO, Ben-Zvi Z, Law FCP, Bend JR. Hepatic and extrahepatic metabolism of ¹⁴C-styrene oxide. *Environ Health Perspect* 1976;17:135-44.
- ¹⁹ Salmons M, Pacheca J, Cantoni L, Belvedere G, Mussini E, Garattini S. Microsomal styrene monooxygenase and styrene epoxide hydrolase activities in rats. *Xenobiotica* 1976;6:585-91.
- ²⁰ Cantoni L, Salmons M, Facchinetti T, Pantarotto C, Belvedere G. Hepatic and extrahepatic formation and hydration of styrene oxide in vitro in animals of different species and sex. *Toxicol Lett* 1978;2:179-86.
- ²¹ Pantarotto C, Salmons M, Szczawinska K, Bidoli F. Gas chromatographic-mass spectrometric studies on styrene toxicity. In: A'lbaiges I, ed. *Analytical techniques in environmental chemistry*. Oxford: Pergamon Press, 1980:245-79.
- ²² Parkki MG, Marniemi J, Vainio H. Action of styrene and its metabolites styrene oxide and styrene glycol on activities of xenobiotic biotransformation enzymes in rat liver in vivo. *Toxicol Appl Pharmacol* 1976;38:59-70.
- ²³ Sandell J, Parkki MG, Marniemi J, Aitio A. Effects of inhalation and cutaneous exposure to styrene on drug metabolizing enzymes in the rat. *Res Commun Chem Pathol Pharmacol* 1978;19:109-18.
- ²⁴ Ohtsuiji H, Ikeda M. The metabolism of styrene in the rat and the stimulatory effect of phenobarbital. *Toxicol Appl Pharmacol* 1971;18:321-8.
- ²⁵ Wilson HK, Robertson SM, Waldron HA, Gompertz D. Effect of alcohol on the kinetics of mandelic acid excretion in volunteers exposed to styrene vapour. *Br J Ind Med* 1983;40:75-80.
- ²⁶ Ikeda M, Ohtsuiji H, Imamura T. In vivo suppression of benzene and styrene oxidation by co-administered toluene in rats and effect of phenobarbital. *Xenobiotica* 1972;2:101-6.
- ²⁷ Ikeda M, Hirayama T. Possible metabolic interaction of styrene with organic solvents. *Scand J Work Environ Health* 1978;4:41-6.
- ²⁸ Leibman KC, Ortiz E. Styrene epoxide—an intermediate in microsomal oxidation of styrene to its glycol. *Pharmacologist* 1968;10:203.
- ²⁹ Leibman KC, Ortiz E. Epoxide intermediates in microsomal oxidation of olefins to glycols. *J Pharmacol Exp Ther* 1970;173:242-6.
- ³⁰ Loprieno N, Abbondandolo A, Baralf R, et al. Mutagenicity of industrial compounds: styrene and its possible metabolite styrene oxide. *Mutat Res* 1976;40:317-24.
- ³¹ Milvy P, Garro AJ. Mutagenic activity of styrene oxide (1,2-epoxyethylbenzene), a presumed styrene metabolite. *Mutat Res* 1976;40:15-8.
- ³² Vainio H, Pääkkönen R, Rönholm K, Raunio V, Pelkonen O. A study on the mutagenic activity of styrene and styrene oxide. *Scand J Work Environ Health* 1976;3:147-51.
- ³³ de Meester C, Poncelet F, Roberfroid M, Rondelet J, Mercier M. Mutagenicity of styrene and styrene oxide. *Mutat Res* 1977;56:147-52.
- ³⁴ Stoltz DR, Withey RJ. Mutagenicity testing of styrene and styrene epoxide in salmonella typhimurium. *Bull Environ Contam Toxicol* 1977;17:739-42.
- ³⁵ Busk L. Mutagenic effects of styrene and styrene oxide. *Mutat Res* 1979;67:201-8.
- ³⁶ Marniemi J, Suolinna EM, Kaartinen N, Vainio H. Covalent binding of styrene oxide to rat liver macromolecules in vivo and in vitro. In: Ullrich V, Roots I, Hildebrand A, Estabrook RW, Conney A, eds. *Microsomes and drug oxidations. Proceedings of the 3rd International Symposium Berlin, 1976*. Oxford: Pergamon Press, 1977:698-703.
- ³⁷ Oesch F, Jerina DM, Daly J. A radiometric assay for hepatic epoxide hydrolase activity with (7-³H) styrene oxide. *Biochim Biophys Acta* 1971;227:685-91.
- ³⁸ Oesch F, Thoenen H. Epoxide hydrolase in human liver biopsy specimens: assay and properties. *Biochem Pharmacol* 1974;23:1307-17.
- ³⁹ Caperos JR, Humbert B, Droz PO. Styrene exposure, II. Percentage studies of absorption, excretion, and metabolism by human subjects. *Int Arch Occup Environ Health* 1979;42:223-30. (Summary in English.)
- ⁴⁰ Guillemain MP, Bauer D. Human exposure to styrene, III. Elimination kinetics of urinary mandelic and phenylglyoxylic acids after single experimental exposure. *Int Arch Occup Environ Health* 1979;44:249-63.
- ⁴¹ Anders MW. Acetone enhancement of microsomal aniline para-hydroxylase activity. *Arch Biochem Biophys* 1968;126:269-75.
- ⁴² Vainio H, Hänninen O. Enhancement of aniline p-hydroxylation by acetone in rat liver microsomes. *Xenobiotica* 1972;2:259-67.
- ⁴³ Cinti DL. Agents activating the liver microsomal mixed function oxidase system. *Pharm Ther* 1978;2:727-49.
- ⁴⁴ Moldéus P, Gergely V. Effect of acetone on the activation of acetaminophen. *Toxicol Appl Pharmacol* 1980;53:8-13.
- ⁴⁵ Clark H, Powis G. Effect of acetone administered in vivo upon hepatic microsomal drug metabolizing activity in the rat. *Biochem Pharmacol* 1974;23:1015-9.
- ⁴⁶ Kitada M, Kamataki T, Kitagawa H. Enhancement in vivo of drug oxidations following administration of benzphetamine, acetone, metyrapone and dimethylsulfoxide. *Jpn J Pharmacol* 1978;28:213-21.
- ⁴⁷ Vainio H, Zitting A. Interaction of styrene and acetone with drug biotransformation enzymes in rat liver. *Scand J Work Environ Health* 1978;4:47-52.
- ⁴⁸ Vainio H, Järvisalo J, Taskinen E. Adaptive changes caused by intermittent styrene inhalation on xenobiotic biotransformation. *Toxicol Appl Pharmacol* 1979;49:7-14.
- ⁴⁹ Wigaeus E, Löf A, Bjurström R, Byfält Nordqvist M. Exposure to styrene. Uptake, distribution, metabolism and elimination in man. *Scand J Work Environ Health* 1983;9:479-88.
- ⁵⁰ Wigaeus E, Holm S, Åstrand I. Exposure to acetone. Uptake and elimination in man. *Scand J Work Environ Health* 1981;7:84-94.
- ⁵¹ Holm S, Lundgren E. A purge-and-trap method for the analysis of acetone in biological tissues. *Anal Biochem* 1984;136:157-60.
- ⁵² Rowland M, Tozer TN. *Clinical pharmacokinetics: concepts and application*. Philadelphia: Lea & Febiger, 1980.
- ⁵³ Mayersohn M, Gibaldi M. Mathematical methods in pharmacokinetics. *American Journal of Pharmaceutical Education* 1971;35:19-28.
- ⁵⁴ Rowell LB. Human cardiovascular adjustments to exercise and thermal stress. *Physiol Rev* 1974;54:75-159.
- ⁵⁵ Åstrand I. Effect of physical exercise on uptake, distribution and elimination of vapors in man. In: Fiserova-Bergerova E, ed. *Modeling of inhalation exposure to vapors: uptake, distribution and elimination*. Vol 2. Boca Raton, Florida: CRC Press Inc, 1983:107-30.
- ⁵⁶ Andersen ME. Saturable metabolism and its relationship to toxicity. *CRC Crit Rev Toxicol* 1981;9:105-50.
- ⁵⁷ Withey JR, Collins PG. Pharmacokinetics and distribution of styrene monomer in rats after intravenous administration. *J Toxicol Environ Health* 1977;3:1011-20.
- ⁵⁸ Ramsey JC, Young JD. Pharmacokinetics of inhaled styrene in rats and humans. *Scand J Work Environ Health* 1978;4:84-91.
- ⁵⁹ Withey JR. The toxicology of styrene monomer and its pharmacokinetics and distribution in the rat. *Scand J Work Environ Health* 1978;4:31-40.
- ⁶⁰ Ikeda M, Imamura T. Evaluation of hippuric, phenylglyoxylic and mandelic acids in urine as indices of styrene exposure. *Internationale Archiv für Arbeitsmedizin* 1974;32:93-101.
- ⁶¹ Göttel P, Axelson O, Lindelöf B. Field studies on human styrene exposure. *Work Environ Health* 1972;9:76-83.
- ⁶² Engström K, Härkönen H, Kalliokoski P, Rantanen J. Urinary mandelic acid concentration after occupational exposure to styrene and its use as a biological exposure test. *Scand J Work Environ Health* 1976;2:21-6.

⁶³ Savolainen H, Pfäffli P. Effects of chronic styrene inhalation on rat brain protein metabolism. *Acta Neuropath (Berl)* 1977;40:237-41.

⁶⁴ Roth RA, Wiersma DA. Role of the lung in total body clearance of circulating drugs. *Clin Pharmacokinet* 1979;4:355-67.

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Toxocaral antibodies in personnel occupationally concerned with dogs

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