Neurotoxic effects of styrene: further evidence

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Abstract

The relation of exposure to styrene to measures of nervous system function was investigated in 70 men working in four factories in the Montreal area of Quebec. Mild sensory nerve conduction deficits were found, the proportion with such deficits rising from 23% in those exposed to less than 50 ppm to 71% in those exposed to more than 100 ppm. Reaction time was slower for those with a larger body burden, as indicated by area under the excretion curve, and for those who failed to clear the metabolite during the weekend. No slowing in conduction time was found among a small group of five men exposed to more than 100 ppm for less than four weeks. There was some evidence that both central and peripheral slowing recovered when workers were removed from exposure. Uptake, storage, or elimination of styrene was influenced by the physical demands of the work, skinfold thickness, cumulative exposure, and alcohol consumption. Nevertheless, only the wearing of a mask and current consumption of alcohol were associated with a lower risk of sensory conduction deficit. While there was no clear indication that neurotoxic effects were related to individual differences in the capacity to metabolise high concentrations of styrene, measurement of urinary metabolites may be helpful in identifying those at highest risk.

Styrene monomer is widely used in the manufacture of fibreglass products, an industry that includes many small enterprises with little investment capital. In recent years the permitted concentrations of styrene in the breathing zone of workers have been reduced in some countries to 50 ppm¹ or even lower values.² The substance is highly volatile, however, and extraction of vapour may be difficult; in these circumstances workers may still be exposed at or above the older limit of 100 ppm.

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Styrene is taken up through the lung³ and to a lesser extent through exposed skin.⁴ A small proportion is excreted unchanged either by exhalation or in the urine.⁵ Most is metabolised with approximately 85% eliminated as mandelic acid and 10% as phenylglyoxylic acid.⁶ Previous studies have shown that the uptake is increased by physical effort,⁷ that styrene accumulates in subcutaneous tissue,⁸ and that metabolism is competitively inhibited by concurrent intake of alcohol.⁹

The neurotoxic properties of styrene have been examined in people exposed at work and in exposure chamber experiments. Such investigations suggest that the substance may affect the function of both the central^{10,11} and peripheral nervous system.¹¹⁻¹⁴

Abnormalities have been reported in electroencephalographic recordings of workers exposed to high levels of styrene¹¹; the proportion of abnormal readings increased with the concentration of urinary metabolite (mandelic acid) measured during the previous five weeks. Behavioural measures, particularly reaction time, have been adversely affected in subjects with a high concentration of mandelic acid in urine some 15¹⁵ to 60 hours¹⁶ after exposure. In studies of small groups of workers previously exposed to styrene it appears that the slowing in the functioning of the central nervous system may be completely reversible.^{16,17}

Effects of styrene on the peripheral nervous system are less clear. No slowing of nerve conduction velocity was reported in a study of 20 rats exposed to styrene for five days a week for 11 weeks.¹⁸ Only motor conduction velocities were measured, however.

The effect on the peripheral nervous system of exposed workers has also been investigated. Sensory nerve conduction velocity was measured in three of these studies¹¹⁻¹³ and in each study conduction velocity was slower than for the referent group; however, no study showed a statistically significant deficit on this measure. In a fourth study sensory conduction velocity was not assessed but the investigators concluded, on the basis of neurological examination, symptom report, and measures of motor velocity, that styrene was toxic to the peripheral nervous system.¹⁴

No evidence has been reported on the rate of onset or reversibility of any effect on the peripheral nervous system; Triebig *et al* followed up the same workers some 12 months after initial study and found

no further deterioration of function.¹³

The correlation observed between urinary metabolites and changes in the central nervous system has not been fully explained. It is not clear whether high concentrations of metabolites (particularly mandelic acid) reflect differences in environmental exposure or differences in the uptake, storage, or metabolism of the substance: either mechanism could increase the effective body burden and hence the neurotoxicity. Any increased burden might presumably affect peripheral as well as central nervous function, although this has not been shown.

The present study was set up to (a) investigate the relation of exposure to styrene to nervous system functioning, (b) determine the relation between functioning and biological parameters of uptake and excretion, and (c) seek evidence on the time course of any observed effects on the nervous system.

Material and methods

STUDY GROUP

Seventy five workers were recruited from four factories manufacturing boats or vehicle panels in the Montreal region of Quebec. Eligible workers were determined by environmental surveys at each of the factories, using both static and personal sampling. All workers exposed to more than 50 ppm and a sample of the less exposed were approached. Only one group (a foreman and two assistants exposed to less than 20 ppm) did not wish to participate and were replaced by a similar group from the same factory. Five of the volunteers were women who have been excluded from the analysis reported below. The seventy men had a mean age of 28.9 years (range 18–52) and had been exposed for periods of a few weeks to more than 20 years.

PERIODS OF EXPOSURE

Workers at all four factories were investigated during a period of "continuous exposure" defined as a period in which a worker had been employed at similar levels (± 10 ppm) of exposure for at least 30 days. At one factory (factory B), further measures were carried out during (a) a period of new exposure—that is, after a holiday of three or four weeks (depending on seniority)—and (b) for those laid off or reassigned to non-exposed jobs after a period of at least 67 days from last exposure.

MEASURES OF NERVOUS SYSTEM FUNCTION

Peripheral

Nerve conduction velocity was measured, using a TECA electromyograph, in the median (motor and sensory), ulnar (motor and sensory), and sural (sensory) nerves. These measurements were made for all workers during a period of continuous exposure and for those at factory B who were laid off or re-assigned to non-exposed work. Indices of

function derived from these measures are described below.

Central

Reaction time was measured over a ten minute testing period using a portable simple unprepared reaction time test¹⁹ previously used in studies of the neurotoxic effects of solvents.^{16 20 21} Measures were carried out before exposure, at the start of the shift on Monday, in groups of not more than six workers. These measurements were made for all subjects during a period of continuous exposure and for those at factory B on return from holiday. The mean of all the reaction times (about 80) recorded by each man during a ten minute test session is taken as his reaction time score.

SYMPTOMS

As all workers were francophone the symptom questionnaire used was a translation into French of an extended version of a symptom questionnaire developed in Sweden.²² It includes questions on the presence of symptoms in both the peripheral and central nervous system. The questionnaire was completed by the worker at home. He also recorded information on medical history, previous employment, and leisure activities.

FACTORS AFFECTING UPTAKE, STORAGE, AND EXCRETION

Physical demands

Three observers rated the physical demands of each job such as sprayer, roller, or finisher. This was done separately for each of the four factories, a total of 32 different jobs. Each job was rated on a three point scale; little, moderate, or strenuous physical activity. The same rating was recorded by at least two of the three observers for each of the jobs and this agreed rating was taken as the physical effort required.

Protective clothing

The workers recorded, on a five point scale, the frequency with which they wore a mask or gloves, or both (from "all of the time" to "not at all"). The masks and gloves used were of widely different types and no attempt was made to code their probable effectiveness. Two workers used fresh air respirators; these were excluded from the analysis of biological measures of exposure.

Skinfold thickness

Subcutaneous fat was estimated with a Holtain skinfold caliper over the triceps of the non-dominant arm.

OTHER FACTORS

Other factors recorded were those thought to affect liver (or kidney) function and hence metabolism or

elimination. These were (1) age, (2) present alcohol consumption (number of drinks a week), (3) cumulative alcohol consumption (total "drink-years"), and (4) an index of cumulative exposure computed from the titles of jobs since joining the company. In the absence of environmental records for past exposures this index was weighted by job exposures estimated at the time of the study.

MEASUREMENTS OF CURRENT EXPOSURE TO STYRENE *Environmental surveys*

Typical exposure at each of 32 workplaces within the four factories was determined by surveys using static pumps and personal monitoring before and after neurophysiological testing.

Personal monitoring

During one eight hour workshift each worker wore a diffusion badge changed at four hour intervals. The mass of styrene in micrograms absorbed on this badge estimated the amount of styrene impinging on the respiratory zone of the worker in a single day.

Twenty four hour urine sample

Each urine sample passed during the 24 hour monitored period (first urine Monday to first urine Tuesday) was collected in a separate container and labelled with the time and date. Concentration of mandelic acid was measured for each sample, using gas chromatography (detection limit of 150 mg/l). Volumes less than 15 ml and samples with creatinine less than 0.3 g were excluded.

Mandelic acid concentrations corrected for creatinine were used to estimate:

The *maximum* (log) concentration—that is, the specimen with the highest concentration of mandelic acid during the 24 hour period. Where no mandelic acid was detected, the probable concentration was estimated on the assumption of log normality.

Time from start of shift to maximum concentration of urinary mandelic acid (computed as the mid-point between two samples). Where mandelic acid was not detected in any sample, no maximum could be determined and this value was treated as missing.

The *slope* of the excretion curve, computed by fitting a regression line to the maximum and later points. Where mandelic acid was not detected in any sample, no slope could be determined and this value was treated as missing.

The *area* under the excretion curve (computed from the three parameters described above). The area may be interpreted as an index of the amount of styrene that has been added to the body burden by exposure during a single workshift. Where no mandelic acid was detected during the 24 hour period this value was taken as zero.

End of shift concentration of mandelic acid (corrected for creatinine) was estimated from urine samples

collected up to one hour before or two hours after the end of shift. Such a sample could be identified for 45 workers. The end of shift mandelic acid has been recommended as a biological indicator of styrene exposure.

Weekend clearance of mandelic acid

A single specimen of urine was collected on rising on the Monday of the reaction time test. The presence of mandelic acid in this sample indicates incomplete clearance of styrene (or its metabolites) from the previous week.

In workers with incomplete clearance at the start of the 24 hour urine sample the base line for the maximum and area described above was taken as measured concentration of mandelic acid in the first sample; the measures thus reflect additional, not total, body burden.

TESTING PROTOCOL

Questionnaires and urine containers were distributed on Friday before the reaction time test and a training session on the test was completed. On Monday the reaction time test was carried out before work started and nerve conduction measured later the same day. One week later 24 hour urine and workshift monitoring were completed.

EXCLUSIONS AND MISSING DATA

Six workers had been exposed to their current level of styrene (± 10 ppm) for less than four weeks and these men have been excluded from the main analysis reported below. Three workers agreed to take part in the study but refused nerve conduction measurement. Two left the company between measures of the reaction time and nerve conduction. Thus 59 men were available for analysis of effects on the peripheral nervous system and 64 for that of the central nervous system.

Results

RELATION OF BIOLOGICAL TO ENVIRONMENTAL LEVELS OF EXPOSURE

Correlations were computed between each of the biological measures of exposure estimated from the 24 hour urine sample and the mass of styrene from the diffusion badge. The coefficients were $r = 0.64$ ($p < 0.001$) with log maximum concentration, $r = 0.12$ ($p = 0.20$) with time to maximum concentration, $r = -0.36$ ($p < 0.01$) with slope of the excretion curve, and $r = 0.72$ ($p < 0.001$) with area under the excretion curve. Thus those with higher exposure had higher maxima, shallower excretion curves, and larger areas under the curve than those with less environmental exposure. Those with higher exposure were also more likely to have mandelic acid detected in a start of shift Monday urine sample ($r = 0.31$, $p < 0.01$).

Table 1 shows the effect of potential modifiers of this relation between exposure and biological indices. Under "uptake" it may be seen that those in jobs requiring much physical effort reached a higher maximum concentration than expected from the environmental measurements. Under "storage" a substantial correlation is seen between skinfold thickness and time to maximum excretion. Cumulative alcohol consumption and cumulative exposure were more associated than age alone with slow clearance, reflected in the presence of mandelic acid in a post-weekend urine sample. Current alcohol consumption was associated with a lower maximum concentration.

NERVOUS SYSTEM FUNCTIONING

Peripheral nervous system

Table 2 shows correlations between nerve conduction velocity and exposure measures. No relation was observed between motor conduction velocity in either ulnar or median nerve and any of the exposure measures. Sensory conduction velocity in each of the three nerves measured was slower for those with higher current exposure. This was so whether the exposure estimate used was that obtained from the environmental survey—that is, "typical" exposure—mass absorbed on a passive diffusion badge on a single day or the area under the mandelic acid excretion curve. The correlations with age are also shown in table 2; none reached statistical significance.

Measures of the three sensory nerves cannot be considered independent and a mean deviation score

$$\sum_{i=1-3} \frac{(X_i - \bar{X}_i)/3}{SD_i}$$

was computed, where X_i represents the sensory

nerve conduction velocity (SNCV) in the median nerve, X_2 in the ulnar nerve, and X_3 in the sural; $\bar{X}_i$ represents the mean SNCV in the median nerve for the sample, and SD_i the standard deviation of the sensory nerve conduction velocity measured in this nerve. This mean is shown as the standardised sensory score (SSS) in table 2.

Means of sensory conduction velocity and the SSS score are shown by exposure group in table 3.

The conduction velocities recorded were compared with the range of normal values in use at the Montreal Neurological Institute. From this it appeared that only two workers had one or more "abnormal" conduction measurements—that is, more than two standard deviations above the mean—on these externally derived normal values. The distribution of minor sensory nerve conduction deficits was then examined: these were taken as values more than one standard deviation from the group mean. As would be expected a large proportion (24/59 workers) were identified as having at least one sensory deficit. Of these, 19 had slowing on only one nerve, three on two nerves, and two on all three sensory nerves. All five workers with two or more deficits had been exposed to at least 50 ppm of styrene.

The proportion with deficits increased with environmental exposure. Seven men among 30 (23%) exposed to 50 ppm or less had some deficit in sensory nerve conduction velocity. The proportion doubled to 46.7% (7/15) for those exposed to 51–100 ppm and increased again to 71% (10/14) among men exposed to more than 100 ppm ($\chi^2 = 9.45$; $p < 0.01$). Below 50 ppm there was no evidence of an increased risk with exposure: 33% (5/15) of those exposed to 20 ppm or less had a deficit compared with only two of the 15 workers (13%) exposed to between 21 and 50 ppm.

Table 1 Potential modifiers of the relation between exposure and biological indices; partial correlations having adjusted for environmental measures of exposure†

Factors affecting	Biological parameters of exposure				
	Maximum (MA conc) (n = 54)	Time to maximum (n = 45)	Slope of excretion curve (n = 46)	Area under excretion curve (n = 54)	MA detected (Monday work start) (n = 57)
Uptake:					
Physical demands	0.29*	-0.03	-0.02	0.16	-0.01
Mask	-0.12	-0.12	0.16	-0.19	-0.04
Gloves	0.05	-0.15	-0.04	0.14	0.10
Storage:					
Skinfold	-0.20	0.44***	0.07	-0.22	-0.06
Metabolism/elimination:					
Age	-0.08	-0.05	0.08	-0.05	0.20
Present alcohol	-0.23*	-0.05	-0.14	-0.18	-0.08
Cumulative alcohol	-0.02	0.01	-0.08	-0.01	0.28*
Cumulative exposure	0.27*	0.08	-0.17	0.12	0.38**

* $p < 0.05$ (one tailed); ** $p < 0.01$ (one tailed); *** $p < 0.001$ (one tailed).

†Partial correlation after adjustment for exposure (personal monitoring) on the day of 24 hour urine sample.

MA = Mandelic acid.

Table 2 Correlation of nerve conduction velocity with exposure and age

Exposure estimates	Motor NCV		Sensory NCV			Standardised sensory score (SSS) (n = 59)
	Median (n = 58)	Ulnar (n = 59)	Median (n = 59)	Ulnar (n = 58)	Sural (n = 57)	
Workplace (environmental survey)	-0.01	+0.11	-0.22*	-0.22*	-0.35**	-0.35**
Personal monitoring (diffusion badge)	-0.02	+0.08	-0.27*	-0.34**	-0.39**	-0.44**
Biological monitoring (area under excretion curve)	-0.02	-0.02	-0.11	-0.30*	-0.34**	-0.33**
Age	-0.20	-0.05	0.02	-0.08	0.13	0.03

* $p < 0.05$ (one tailed); ** $p < 0.01$ (one tailed).
NCV = Nerve conduction velocity.

Reaction time

Reaction time scores were strongly correlated with age ($r = 0.58$, $p < 0.001$) and observed minus expected (O-E) scores were computed to allow for this ($E = 215.8 + 1.8 \text{ age}$). This age adjusted reaction time score was not related to estimates of environmental exposure ($r = 0.09$, $p > 0.05$), or to values obtained by personal monitoring of a single workshift ($r = 0.07$, $p > 0.05$). It was, however, related to the area under the excretion curve ($r = 0.28$, $p < 0.02$) and to the presence of mandelic acid in the urine at the start of the Monday shift ($r = 0.22$, $p < 0.05$).

Symptoms

No correlation was found between the number of symptoms either overall (r_s) or within symptom type and exposure to styrene, whether this was reflected in environmental estimates ($r_s = -0.18$, $p > 0.05$), personal monitoring ($r_s = -0.17$, $p > 0.05$), or the area under the excretion curve ($r_s = 0.02$, $p > 0.05$).

No individual symptom was significantly ($p < 0.05$) related to any of these measures in the expected direction. Pain and tingling in the hands had the highest correlation (with area under the curve) of any symptom; $r = 0.18$, $p = 0.08$, one tailed. Eight workers complained of this symptom.

RELATION OF NEUROPHYSIOLOGICAL SCORES TO BIOLOGICAL PARAMETERS OF EXPOSURE

A prior hypothesis of this study was that subjects slower to excrete the solvent would be at more risk of neurological damage than others with similar environmental exposure. Partial correlations between neurophysiological measures and parameters of the excretion curve, having allowed for exposure, are shown in table 4.

The slope of the excretion curve was unrelated to neurophysiological deficit after allowance for environmental exposure.

The time to maximum excretion had a weak relation to slowing in conduction velocity, those with later peaks having a lower standardised score ($p = 0.07$) and being more likely to have a deficit (> 1 SD from the mean) on one or more nerves ($p = 0.09$). This difference was not explained by skinfold thickness, the partial correlations having allowed for this factor being almost identical (SSS, $r = -0.17$; deficit, $r = 0.23$) with those shown in table 4. The relation of time of maximum excretion to deficit was particularly evident in those exposed to more than 100 ppm. The 10 men with sensory nerve slowing at these exposures had a mean time to maximum excretion of 9.2 hours; four men with high exposures but without nervous

Table 3 Mean sensory conduction velocity and standardised sensory score (SSS) by exposure in the workplace

Workplace exposure (environmental survey)	Sensory conduction velocity				SSS
		Median	Ulnar	Sural	
≤ 50 ppm (n = 30)	Mean	45.6	45.8	42.7	0.33
	SD	4.7	4.5	3.1	0.60
51-100 ppm (n = 15)	Mean	43.5	44.6	39.9	-0.17
	SD	4.3	4.7	3.2	0.66
> 100 ppm (n = 14)	Mean	42.5	41.4	39.0	-0.53
	SD	5.2	5.5	3.0	0.81
Overall (n = 59)	Mean	44.3	44.4	41.1	0.00
	SD	4.7	4.8	3.1	0.67
Difference between groups	F	2.4	3.9*	8.0***	8.3***

* $p < 0.05$ (two tailed); *** $p < 0.001$ (two tailed).

Table 4 Partial correlation of neurophysiological scores with biological indices, having adjusted for environmental measures of exposure†

Neurophysiological measure	Biological parameters of exposure				
	Maximum (MA conc) (n = 54)	Time to maximum (n = 46)	Slope of excretion curve (n = 45)	Area under excretion curve (n = 54)	MA detected (Monday work start) (n = 57)
Sensory nerve conduction:					
SSS	-0.15	-0.22	0.09	-0.19	-0.10
SNCV deficit	0.17	0.20	-0.06	0.26*	0.05
Reaction time (adjusted for age)	0.24*	0.10	-0.05	0.33**	0.32**

*p < 0.05 (one tailed); **p < 0.01 (one tailed).

†Partial correlations after adjustment for exposure (personal monitoring) on the day of 24 hour urine sample.

SNCV = Sensory nerve conduction velocity.

system deficit had a mean time of 6.1 hours ($t = 2.59$, $p < 0.05$).

This late peak suggests that highly exposed workers may not have reached peak elimination by the end of shift. In the sample as a whole, end of shift concentration of mandelic acid was negatively related to sensory nerve conduction deficit (SSS, $r = 0.40$, $p < 0.01$; deficit, $r = -0.34$, $p < 0.05$) having allowed for exposure during the shift. In the highly exposed men with peripheral nervous system deficit the end of shift samples had mean concentrations of mandelic acid that were less than half (1.50 g/g creatinine) those of the highly exposed workers (MA = 3.49 g/g creatinine) who were not found to have slowing of the peripheral nervous system ($t = 1.89$, $p < 0.05$, one tailed).

Maximum concentration of urinary metabolite was not related, overall, to slowing of the peripheral nervous system (table 4) but those with higher concentrations were more likely to have a slow reaction time ($p < 0.05$).

The area under the excretion curve, having allowed for environmental exposure, was significantly related to both slowing in reaction time ($p < 0.01$) and to sensory conduction (deficit, $p < 0.05$; SSS, $p = 0.08$). The relation between slow clearance of metabolites (mandelic acid detected on Monday morning) and slowing of reaction time was strongly evident ($p < 0.01$) when differences in environmental exposure had been taken into account. This single measure of clearance showed no significant relation to measures of nerve conduction velocity. Further work, however, suggests that measures of Monday morning mandelic acid on successive weeks may be a useful predictor of peripheral nervous system effect (D Gautrin, unpublished data).

RELATION OF NEUROPHYSIOLOGICAL SCORE TO FACTORS AFFECTING UPTAKE, STORAGE, OR ELIMINATION

Table 5 shows the relation of each of the potential modifiers of uptake, storage, or elimination to nerve conduction velocity (standardised sensory score and

deficit) and to reaction time. Having accounted for environmental exposure those wearing a mask were less likely to have a sensory nerve deficit. Current consumption of alcohol was also related to lower than expected nerve conduction deficit, having accounted for exposure to styrene. Such a result is consistent with the lower maximum concentration for current alcohol drinkers shown in table 1.

Table 6 shows the relation of wearing a mask and consumption of alcohol to sensory nerve conduction velocity deficit. The smaller proportions with deficit among those wearing masks or drinking alcohol are present at all levels of exposure to styrene.

None of the other factors considered was related to the neurophysiological measures, only cumulative exposure to alcohol showing some trend towards slow reaction time. In particular there was no evidence that cumulative exposure increased the likelihood of deficit.

It has been shown that the area under the excretion curve was significantly related to both slowing in sensory conduction velocity and to reaction time after allowance for environmental exposure (table 4).

Table 5 Partial correlation of neurophysiological scores with potential modifiers, having adjusted for environmental measures of exposure†

Factors affecting	Sensory NCV		Reaction time (adjusted for age) (n = 64)
	SSS (n = 59)	SNCV deficit (n = 59)	
Uptake:			
Physical demands	0.06	-0.04	-0.13
Mask	0.12	-0.24*	-0.06
Gloves	0.08	-0.00	-0.17
Storage:			
Skinfold	-0.09	0.03	0.04
Metabolism/elimination:			
Age	-0.05	-0.10	—
Present alcohol	0.21	-0.22*	0.13
Cumulative alcohol	-0.03	-0.01	0.20
Cumulative exposure	-0.14	0.06	0.04

*p < 0.5 (one tailed).

†Partial correlations after adjustment for workplace exposure (environmental survey).

Table 6 Proportion with sensory nerve conduction velocity deficit among those wearing masks or drinking alcohol

	Mask		Alcohol	
	Seldom or never	At least half the time	Seldom or never	At least once a week
Workplace exposure (environmental study):				
< 50 ppm	31.6% 19	0.0% 9	45.5% 11	10.5% 19
51-100 ppm	50.0% 10	40.0% 5	50.0% 6	44.4% 9
> 100 ppm	80.0% 5	66.7% 9	83.3% 6	62.5% 8
Overall	44.1% 34	34.8% 23	56.5% 23	30.6% 36

The size of the partial correlation of area with conduction velocity was much reduced after adjustment for the wearing of a mask and current consumption of alcohol and the correlation no longer reached statistical significance ($r = 0.17$, $p = 0.11$). No such reduction was seen in the partial correlation with reaction time and neither current alcohol consumption nor the consumption of alcohol in the previous 24 hours reduced the relation between Monday morning excretion of mandelic acid and slowing in reaction time (partial correlation, accounting for alcohol = 0.34).

ONSET AND REVERSAL OF NERVOUS SYSTEM EFFECTS

Peripheral nervous system

Onset—Five workers had been exposed to more than 100 ppm of styrene for less than four weeks and so were excluded from the main analysis. None of these was found to have any deficit in sensory conduction velocity. Fourteen workers had been exposed to more than 100 ppm for longer than four weeks. Of these, ten had one or more deficits. The mean SSS for the newly exposed workers was 0.56 (SD = 0.22) and for those employed more than one month – 0.53 (SD = 0.81). This difference is unlikely to be due to chance ($t = 2.90$, $p < 0.02$). No clear pattern for the rate of onset was seen beyond one month; three out of four of those exposed for one to three months had a sensory nerve deficit; the figures for four to six months were three out of four and from seven to 12 months four out of six.

Reversal—Eleven male workers from factory B were laid off or re-assigned to work without exposure to styrene. Of these, only two had been exposed to high levels (> 100 ppm) both at the time of initial assessment and continuously to lay off. Six had been exposed to less than 50 ppm throughout and three to less than 50 ppm at the time of initial test but to more than 50 ppm between the initial assessment and lay off. The mean change in SSS was compared for the two highly exposed to lay off and the six exposed at low levels. There was no significant difference in the

SSS score of the two groups of workers (high exposure = 0.17, low exposure = 0.56, $p = 0.34$) during continuous exposure; the difference was, however, in the expected direction with the more exposed having the lower score. Inspection of the change in SSS between continuous exposure and after lay off showed that the previously highly exposed men had improved their score more (0.57, SD = 0.75) than the less exposed whose score had declined somewhat (-0.95 , SD = 0.45). Despite the small numbers, this difference is statistically significant ($t = 3.59$, $p < 0.02$) and results from a mean improvement of about 2 m/sec on each of the three sensory nerves for these two workers laid off after high exposure.

Reaction time

Onset—Five workers had been exposed to more than 100 ppm for less than four weeks at the time of their initial reaction time measurement. Their mean RT score, adjusted for age, was 23 m/sec slower than expected. The mean (O-E) score for those employed for at least one month at those high levels was 9 m/sec better than expected ($t = 1.86$, $p = 0.06$).

Reversal—Twenty eight workers from factory B completed the reaction time test during continuous exposure and again after their holiday. The extent of change in reaction time between the two occasions was not significantly related to environmental measures or to the area under the excretion curve during continuous exposure; the largest correlation was with area ($r = 0.13$, $p = 0.27$). Nevertheless, the five workers at this factory in whom mandelic acid had been detected preshift at the time of the initial reaction time test had a slower reaction time than other workers during continuous exposure; this group also improved more when measured after the holiday (table 7). The mean age of the two groups was similar (mandelic acid detected, 36.8 years, none detected, 35.9 years).

Table 7 Mean reaction time during continuous exposure and on return from holiday (factory B only) by mandelic acid present in preshift urine

		<i>Reaction time</i>		
		<i>(i)</i> <i>Continuous exposure</i>	<i>(ii)</i> <i>Post-holiday</i>	<i>(i)-(ii)</i>
<i>Mandelic acid detected</i>	No (n = 23)	Mean 272.0	278.0	- 6.0
		SD 29.7	28.9	25.2
Yes (n = 5)	Mean 309.9	290.6	+ 19.6	
	SD 23.8	40.6	22.3	
Overall (n = 28)	Mean 278.8	280.0	- 1.5	
	SD 28.9	31.0	24.8	
Difference between groups		<i>t</i> = 2.6*	<i>t</i> = 0.8	<i>t</i> = 2.1*

* $p < 0.05$ (two tailed).

Discussion

The first objective of this study was to confirm, in a sample including highly exposed workers, whether or not styrene had effects on either central or peripheral nervous functioning. It appears that exposure to styrene is indeed associated with slowing in sensory, but not motor, nerve conduction velocity. This slowing is not severe when compared with clinical norms but the proportion with mild slowing increases with intensity of exposure. On each of three nerves measured, workers exposed above 100 ppm had a mean conduction velocity that was approximately 92% of that in men exposed to 50 ppm or less; this reduction in capacity could not be attributed to age. Effects on the central nervous system were also seen; the mean reaction time was slower in those workers whose urine still contained mandelic acid despite the weekend break from exposure. In these workers the mean reaction time was 309.9 m/sec, 10% slower than that expected for age.

These results are similar to those reported previously. Rosen found a slowing of approximately 8% in sensory conduction velocity when he compared the most exposed group with normal controls.¹² Cherry *et al* found a slowing of 10% in Monday morning reaction time when exposed workers were compared with non-exposed.²⁰ The present study, however, has sufficient numbers to demonstrate that the association with styrene is unlikely to be due to chance.

The second objective was to examine the relation between the rate of uptake and clearance of styrene and neurotoxicity.

It was previously suggested that failure to clear styrene during the weekend might result from individual differences in the capacity to metabolise styrene, and that those slow to clear would, for this reason, be at increased risk of damage to the nervous system.¹⁶

The results presented here are, to some degree, consistent with this hypothesis. Firstly, as previously observed those with mandelic acid in their urine on Monday morning had a slower reaction time.¹⁶ Secondly, the time of peak excretion was somewhat later in those with slowing in sensory nerve conduction velocity, particularly for those exposed to more than 100 ppm. On the other hand, the lack of any relation between the slope of the excretion curve (having allowed for exposure) and neurotoxic effect argues against the notion that saturation of the enzyme reactions involved in styrene metabolism was more frequent among those with nervous system deficit than in those without.

It is clear both from this study and from earlier reports that several potentially measurable factors, such as physical effort or body fat, influence the relation between environmental measures of styrene and both body burden and pattern of excretion. In

the present study allowance for such factors did not fully explain the relation between pattern of elimination and neurotoxic effect. It may be that more precise estimates of these factors, or inclusion of others, would help to answer the question as to whether or not individual differences in metabolism are important. In practical terms it is evident that risk assessment for each worker or group of workers cannot adequately take account of every potential modifying factor. Even where a factor, such as alcohol intake (which presumably operates through P-450 enzyme induction²³) may reduce risk it may not be politic (or useful) to attempt to assess its prevalence. Biological monitoring of exposure, as has long been recognised, can provide an integrated estimate of the net result of these different influences on the body burden of the worker, without exact knowledge of the contribution made by each factor.

The third objective was to investigate the time course of neurotoxic effect and to this end the work force at the largest factory was followed up for 12 months.

Nerve conduction measurements on five newly exposed workers suggest that no measurable slowing takes place in the first few weeks of high exposure. Removal from exposure appears to be associated with increased sensory conduction velocity, but this result was based on only two highly exposed workers. Such changes would, however, be consistent with improvements in function seen some months after removal from exposure to solvents known to affect the peripheral nervous system—for example, methyl-n-butylketone.²⁴

The time course of effects on the central nervous system appears to be more acute; those with recent high exposure had reaction times that were much the same (or slower) than those exposed for many weeks. Any slowing in reaction time associated with delayed excretion of mandelic acid appears to be largely reversed within four weeks and possibly within a few days.

Thus although achievement of the third objective was limited by small numbers with changing exposure during the working year, it does appear that the time course of the effects on the peripheral and central nervous systems may differ, with effects on the central nervous system apparently reflecting acute overload during the previous week and effects on the peripheral nervous system being associated with repeated high exposures over a period of at least four weeks.

Taken together, these results have implications for monitoring those at risk. Strict observance of an environmental exposure limit of 50 ppm or below, with use of a suitable mask, would reduce to a minimum the proportion of workers at risk of neurotoxic effect either from high intake or slow elimination. With higher exposure limits there may

be greater need for biological monitoring. The results of this study suggest that choice of this monitor needs special care. End of shift urine samples may consistently underestimate the risk of those with highest exposure. Where exposure guidelines of more than 50 ppm are in place, monitoring of delayed elimination should provide a better indication of those at risk of the mild and probably reversible neurotoxic effects of styrene shown in this paper.

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