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Blood styrene and urinary metabolites in styrene polymerisation

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ABSTRACT The results of the analysis of blood and urine samples for styrene and its metabolites in 491 workers in a styrene polymerisation plant in the United States are reported. The levels of exposure to styrene were estimated to be less than 10 ppm, but nevertheless styrene and metabolites were detectable in more than 50% of workers in polymerisation jobs, within 4 h of exposure. Workers involved in the manufacture and purification of styrene from ethyl benzene also had detectable blood styrene and urinary metabolites in 83% of recently exposed subjects. The relationship between styrene in blood and in subcutaneous fat and urinary metabolites as pharmacokinetic variables is discussed.

Various indices of human exposure to organic solvents have been investigated in recent years in recognition of the fact that air sample analysis, even at best, may be an inadequate measure of individual environmental exposure. This results from the frequent difficulty of obtaining an accurate or representative personal sample and of the many changes in levels of exposure during actual working conditions over short periods of time. At the same time the non-uniformity of human responses, even to similar exposure levels, has been noted. Individual variation in absorption, distribution, storage, metabolism and excretion can be marked.

Efforts to assess individual exposure quantitatively have dealt with measurement of unaltered materials (such as styrene, benzene, dichloromethane, trichloroethylene and various anaesthetics) in expired air and blood, and of urinary metabolites including trichloroacetic acid (for trichloroethylene), phenols (for benzene), hippuric acid (for toluene), and mandelic or phenylglyoxylic acid (for styrene) (Riley *et al.*, 1966; Stewart *et al.*, 1968; Stewart *et al.*, 1970; Hunter and Blair, 1972; Sherwood, 1972; Corbett, 1973; Szadkowski *et al.*, 1973). For many of these compounds, under either controlled experimental or measured occupational exposures, the biological exposure indices correlate well with the concentration of the compound in air during exposure. Several aspects of these questions have been reviewed by Astrand (1975).

The need to monitor low exposures to styrene appears to be important, as carcinogenic risk is currently being assessed, partly because of the effects seen in workers with another vinyl monomer, vinyl chloride (Creech and Makk, 1975), the mutagenic behaviour of styrene oxide (Milvy and Garro, 1976), and animal studies with various vinyl monomers: trichloroethylene (*Chemical and Engineering News*, 1976), vinyl chloride (Maltoni and Lefemine, 1975), vinylidene chloride (Maltoni, 1977; Lee, 1977) and acrylonitrile (Murray *et al.*, 1976).

In particular, styrene (phenylethylene, vinyl benzene) inhalation response has been well characterised by breath and blood levels during controlled experimental exposures at 50–200 ppm, which approximates to the TLV* (Stewart *et al.*, 1968; Astrand *et al.*, 1974). Thus breath and blood concentrations measured within 6 h of exposure can be correlated with ambient exposure levels greater than 50 ppm, and urinary metabolites, measurable for at least 16 h after exposure can also be correlated with previous exposure. Recently, measurement of subcutaneous fat concentrations of styrene in 25 occupationally exposed subjects from the group to be described in this paper showed that styrene could be detected in fat for as long as three days after exposure (Wolff *et al.*, 1977).

The use of urinary metabolites as an exposure index has special merit in industrial hygiene evaluation, because it does not require an invasive

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*TLV=Threshold Limit Value of the American Conference of Governmental Industrial Hygienists

technique and because the analytical method is fairly simple and has been extensively studied. Nevertheless, the usefulness of urinary metabolite measurements for styrene is limited by the fact that observed levels, which reach a maximum at 8–12 h after exposure, become insignificant within 16–24 h (Bardodej and Bardodejova, 1970). For any estimation of body burdens more than 16–24 h after exposure, an alternative exposure index is necessary.

Further, although urine analysis is valuable as a screening method for exposures in excess of 50 ppm (time-weighted average), insensitivity of present methods limits its use at lower levels of exposure (Schaller *et al.*, 1976). Even at low exposures, however, urinary metabolite determinations may be potentially useful for identifying sporadic exposures such as those incurred from accidental spills or other unusual exposures which would not be recognised by air sampling (time-weighted average). The analytic method of choice is now gas chromatography, which allows detection of at least 10 mg/l of mandelic acid, and eliminates a confounding background level of 90–250 mg/l with the formerly used colorimetric method (Engstrom and Rantanen, 1974).

The absorption–elimination measurements of styrene exposure, established under controlled laboratory conditions, have been applied to a limited number of industrial situations, with excellent results. Thus, the data of Gotell *et al.* (1972) for 17 occupationally exposed subjects were entirely analogous to previously reported exposure criteria derived from laboratory experiments. Initial breath concentrations and decay curves for styrene correlated well with exposure data derived from personal air sampling. Urinary metabolites were similarly related to exposure, although some evidence of a levelling effect at higher exposure (> 150 ppm) was presented. In another study among 21 Japanese workers, urinary metabolites were also representative of exposure levels (Ohtsuji and Ikeda, 1970), and two Swedish studies of 36 and 47 workers showed a good curvilinear relationship between end-of-shift urinary metabolites and time-weighted average styrene exposure (Harkonen *et al.*, 1974; Engstrom *et al.*, 1976). The correlation at levels of exposure less than 100 ppm appeared to be linear, and the authors in both studies suggested that for an end-of-shift urine (which should represent a metabolite concentration near its peak) a urinary metabolite concentration of less than 2300 mg/g creatinine or 3000 mg/l would indicate an exposure below the current TLV of 100 ppm.

Urinary mandelic acid concentrations were recently reported for a group of 58 German styrene polymerisation workers exposed to less than 8 ppm styrene (Thiess *et al.*, 1976). Mandelic acid in urine samples obtained immediately after work was below 105 mg/l in all cases and below 50 mg/l in all but two, with 38 above 10 mg/l.

Considering the variety of methods involved, the reported values of urinary mandelic acid correlate remarkably well with exposure levels (Fig. 1). Blood styrene concentrations previously reported are summarised in Fig. 2, where an estimated blood styrene of less than 0·1 ppm can be extrapolated for exposure levels of 10 ppm styrene.

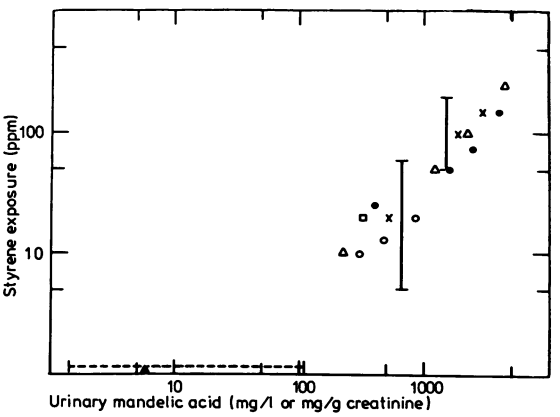

Fig. 1 *Correlation of styrene exposures with reported concentrations of urinary mandelic acid.* ○ *Ohtsuji and Ikeda, 1970;* ● *Harkonen* et al., *1974;* × *Gotell* et al., *1972;* I *Ikeda* et al., *1972;* △ *Engstrom* et al., *1976;* ▲ *Slob, 1973;* □ *Bardodej and Bardodejova, 1970;*- - - - - - - *Thiess* et al., *1970.*

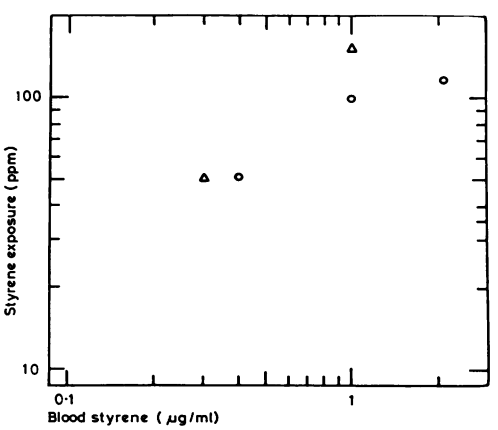

Fig. 2 *Relation of styrene exposure to styrene concentration in venous blood.* △ *Astrand* et al., *1974;* ○ *Stewart* et al., *1968.*

Materials and methods

During a clinical field survey of 491 workers in a styrene polymerisation plant in December 1975, we had the opportunity to compare the concentration of styrene in blood with urinary metabolites and, in 25 cases, in subcutaneous fat. These workers were exposed to a variety of styrene concentrations and had widely different work experience.

The styrene plant in which the workers investigated in this study were employed produced styrene-butadiene rubber during the 1940s, including synthesis of styrene monomer from coke oven by-products. After 1951 production of this plant was exclusively directed to the manufacture and polymerisation of styrene. Since 1969 styrene has been produced by catalytic dehydrogenation of ethyl benzene obtained elsewhere. The subjects were volunteers from the local union, with job categories as indicated in Fig. 3; of 668 union members enrolled, 51 were listed as retired.

A detailed occupational and exposure history was taken, and the facts pertinent to this study included present job category and most recent exposure. The job categories were separately classified according to intensity of styrene exposure, with heaviest exposures among styrene polymerisation operators and production workers. Other job categories were ranked according to assessed work histories as having had continuous, intermittent or practically no exposure (Table 1). General exposures were also related to measurements made during a National Institute for Occupational Safety and Health industrial hygiene field survey (Maier and Ruba, 1974). The most recent exposures (date and hour) were recorded.

Blood samples (10 ml) were taken in pre-heparinised glass vacutainer tubes and frozen until analysed. Urine samples were collected in four-ounce polyethylene jars and frozen until analysed.

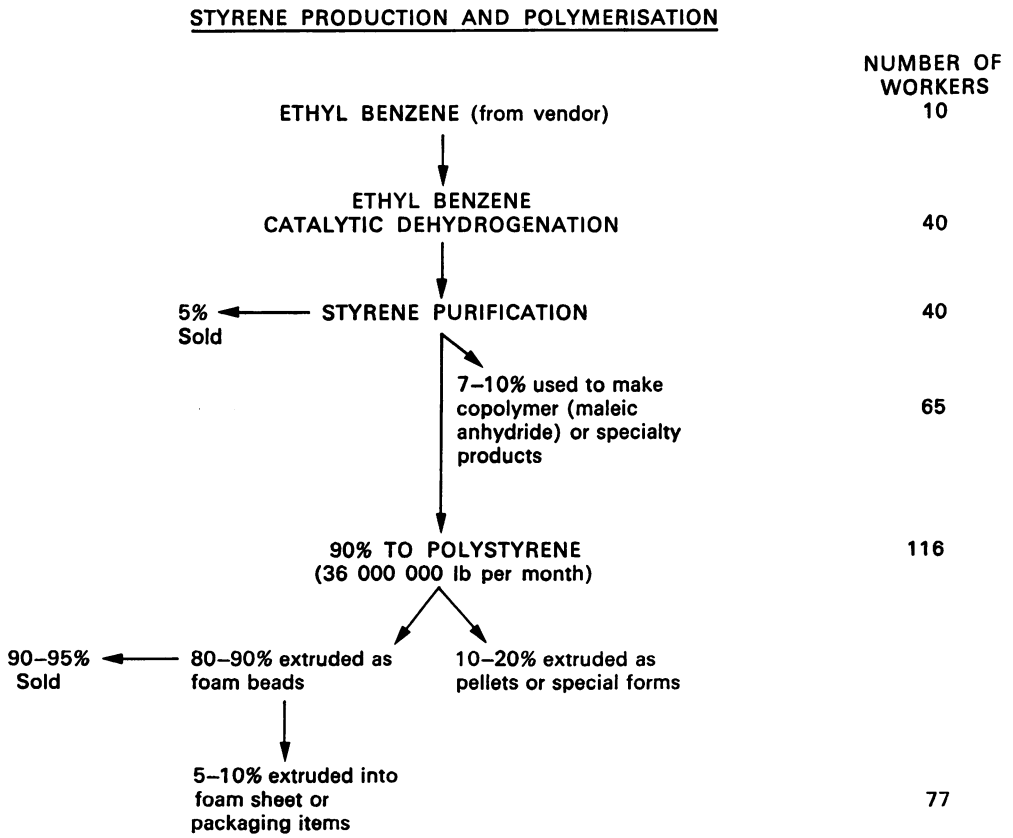

Fig. 3 *Job classifications of styrene production and polymerisation workers according to union seniority list (July, 1975). Other production workers are classified as transportation and storage (59), utility (49) and maintenance (134).*

Table 1 *Job classification of styrene polymerisation workers*

Group	Job description	Number of workers	Total in subdivision
I	Retired: no current exposure	47	47
II	Low or no exposure		
	Unclassified, machinists (1), garage mechanic (2)	15	
	Coal handler and boiler house workers	21	
	Janitor	8	44
III	Maintenance: no direct exposure		
	Craneman, storeroom, insulator, painter, boilermaker	18	
	Electrician	15	
	Utility, effluent and coal handler	14	47
IV	Products handlers		
	Polystyrene handler	16	
	Transportation	15	
	Packaging operator	25	
	Warehouse materials	7	
	Polystyrene sheet extrusion	41	
	Polystyrene extrusion (beads)	14	
	Latex-specialty	29	146
V	Maintenance: direct exposure		
	Carpenter	6	
	Instrument maintenance	9	
	Labourer	13	
	Liquid handler	10	
	Millwright	19	
	Pipefitter	29	
	Rigger	3	
	Welder	11	100
VI	Styrene manufacture and purification	29	29
VII	Polymerisation workers	78	78
Total			491

ANALYTICAL METHODS

Urinary metabolites of styrene

Urinary mandelic (MA) and phenylglyoxylic (PGA) acids were measured as their methyl esters by gas chromatography using the method of Buchet *et al.* (1974). This method recently has been shown to yield secondary methylation products, probably involving the alpha-keto moiety, from phenylglyoxylic acid (Schaller *et al.*, 1976) and therefore the values for PGA may be underestimated by 10–20%, as judged by the occasional occurrence of other peaks in standard runs. However, in this investigation freshly prepared standards in the range of 15–150 mg/l were run daily, and both mandelic and phenylglyoxylic acid produced linear calibration curves by peak height or by electronically integrated area. All samples showing the presence of 15 mg/l or more were analysed in duplicate and the values averaged. The average standard deviation from the mean of these measurements was 24%, with the absolute deviation generally less than 10 mg/l for values below 50 mg/l.

Urinary concentrations were expressed as mg/g creatinine, in an effort to normalise values for the dilution of urine. The mean of 153 creatinine measurements was 1·56 ± 0·8 g/l creatinine. Gas chromatographic analyses were performed using a Perkin-Elmer 3920 gas chromatograph with dual flame ionisation detectors and Columbia Scientific electronic integrator, using a 10 ft × 1/8 in DEGS on chromosorb column at 120°C, N_2 flow rate 30 cc/min.

Styrene in whole blood

The styrene concentration in blood was determined by spectrofluorometric assay following cyclohexane extraction. Blood (0·5 ml) was combined with an equal volume of freshly distilled cyclohexane in a 16 × 150 mm test tube, and after brief centrifugation (1800 *g*) the cyclohexane layer was transferred by means of a Pasteur pipette into a quartz cuvette (0·3 × 0·3 cm), which was measured at 250 nm in a MPF4 Hitachi spectrofluorometer. With sufficiently pure cyclohexane, the fluorescence spectrum of the extracted sample was virtually identical to that of styrene dissolved in cyclohexane. The extraction efficiency of styrene in cyclohexane added to blood was 95 ± 10%, and the fluorescence intensity was proportional to the styrene concentration range of interest here. The sensitivity limit was 1–3 parts per billion, at which level the fluorescence spectrum was still recognisably that of styrene, with a prominent sharp line at 293 nm and moderately resolved peaks at 303, 307 and 317 nm. A more complete description of this assay will be given elsewhere.

Results

STYRENE POLYMERISATION PLANT

At the time of the examinations polystyrene accounted for over 90% of the production in this plant, with the remainder of the styrene being used in specialty products including butadiene–styrene copolymer and maleic anhydride–styrene copolymer. Most of the polystyrene was made into expandable polystyrene beads by means of pentane impregnation, and then shipped elsewhere for extrusion into polystyrene foam. Some extrusion of polystyrene was done at the plant (Fig. 3), and less than 10% of the polystyrene was sold in pellet (non-foam) form.

The major production processes in this plant were semi-automated, so that styrene and ethyl benzene were usually maintained within a closed system. However, workers experienced direct exposure to these chemicals when obtaining quality control samples or when, in the case of polymerisation operators, chemicals had to be added through ports in the reactor vessel.

Maintenance procedures which require repair of leaks in styrene or ethyl benzene pipelines also

involved intermittent high exposure for maintenance workers.

EXPOSURE INFORMATION OBTAINED FROM JOB HISTORY

The exposure categories summarised in Table 1 were derived by ranking the subject's present job according to estimated exposure intensity, with those jobs having average styrene exposure of more than 5 ppm in air being ranked as high (groups V, VI, VII), and those jobs in areas with finished polystyrene products, as medium or low (group IV). Those jobs having potential styrene contact on an infrequent or intermittent basis were placed in category V. These exposure estimates were consistent with data available from a NIOSH air sampling study performed in 1974 (Maier and Ruba) and, further, with air sampling data obtained in early 1976 by the company (Table 2). During the period of the examinations reported here the plant was operating below capacity, which could have resulted in lower styrene exposure than usual.

The workers had ceased to be exposed to styrene for varying intervals before examination. According to previously reported metabolic elimination rates, the urinary and blood exposure levels were expected to be time-dependent, and therefore a time factor of 'most recent exposure' was used to aid interpretation of urinary and blood measurements. An uneven distribution within 'most recent exposure' intervals sometimes occurred. Pipefitters, a subgroup of V, had only 2 of 25 in the 0–16 h group, which is pertinent in evaluating their exposure data, especially as this job category was judged to have significant styrene exposure.

URINARY METABOLITE AND BLOOD STYRENE DETERMINATIONS

The majority of 477 completed urinary and 364 blood analytical measurements were undetectable or below significantly elevated levels (Table 3). In the entire group 31 urinary mandelic acid determinations were higher than 20 mg/g creatinine (average 53 ± 33 SD), and 42 were above 15 mg/g (43 ± 33 SD), suggesting an average exposure level of about 5 ppm by analogy with Figure 1. There were 28

Table 2 *Concentration of styrene in air of a polymerisation plant*

Manufacturing area	*Mean* concentrations, ppm reported by*			
	NIOSH†		*Company‡*	
	Styrene	*Ethyl benzene*	*Styrene*	*Ethyl benzene*
Styrene polymerisation	17 (15)	<1	19 (22)	3 (8)
	2·5§ (6)		17 (13)	
Styrene purification	4 (9)	<1	22 (13)	19 (13)
Ethyl benzene cracking	3 (8)	3	3	6
Latex-specialty area	<5	<1		

*Mean of several measurements, n, in parentheses.
Range and standard deviation were large, 100–200% of the mean.
†1–2 hour samples.
‡8-hour time-weighted averages.
§Maleic anhydride–styrene copolymerisation area.

Table 3 *Relationship of urinary metabolites and blood styrene in various concentration ranges*

Urinary metabolites			*Blood styrene*	
Concentration range (mg/g creatinine)	*MA* (*no. of determinations in each range*)	*PGA*	*Concentration range (ppb)*	*Number of determinations*
0 (ND)	341	344	0–1	114
2 (trace)	77	77	> 1–2	130
4–8	2	3	> 2–3	48
> 8–12	11	9	> 3–4	29
> 12–16	10	14	> 4–6	26
> 16–20	5	3	> 6–10	4
21–28	8	10	> 10–14	3
29–36	5	3	> 14–20	6
37–52	9	8	> 20–30	1
60–80	3	3	40–50	1
> 80–100	3	2	70–80	1
> 100–120	2	—	> 80–90	1
140–160	1	—		
Total	477	476		364

MA = mandelic acid; PGA = phenylglyoxylic acid; ND = not detected.

blood styrene determinations above 5 ppb, and 13 above 10 ppb (average 28 ± 24 SD). The lower limit of detection for MA and PGA was about 10 mg/l, and the values of urinary creatinine ranged from 0·2 to 3·8 g/l (average 1·56), which accordingly raised or lowered the concentration of urinary metabolites. Blood styrene levels exceeding 5 ppb were considered to be significantly raised, because this value was consistently higher than background contamination or control sera determinations. Recently exposed subjects in high exposure categories (groups VI, VII, Tables 4 and 5) had the highest prevalence of increased urinary metabolites (> 5 mg/g creatinine), blood styrene (> 5 ppb), and instances in which both values were simultaneously raised.

The preponderant number of cases with detectable urinary metabolites and blood styrene was observed among polymerisation workers (group VII) and styrene production workers (group VI) who had been exposed within 4 h of examination.

Maintenance workers (group V) also had a higher prevalence of increased urinary metabolites and blood styrene, especially those with very recent exposure, such as millwrights (2/6), labourers (3/5), and liquid handlers (1/4); however, pipefitters removed for more than 16 h from exposure (5/26) also had increased metabolite levels, reflecting their previous relatively higher (peak) exposures (Table 6).

In several other subgroups the frequency of detectable urinary metabolites or blood styrene was low. Thus, among 47 retired workers no instance of raised blood styrene was observed and in two cases urinary metabolites were observed at 10 mg/l, the limit of detection. Similarly, in the miscellaneous category of low or non-exposed subjects (group II, Table 4) a low prevalence of raised blood styrene and no detectable urinary metabolites were observed. No history of administration of medication was recorded that could explain the occurrence of MA and PGA in urine of retired persons.

Table 4 *Urinary metabolites, blood styrene and styrene in body fat among styrene polymerisation workers*

Group	*Job classification*	*Time since last exposure*	*MA**	*PGA**	*Blood styrene > 5 ppb*	*Both increased†*	*Styrene in subcutaneous fat (ppm)*
I	Retired	>36 h n=47	2/47	2/47	0/37	0/37	0/1
II	Low or no exposure	0–4 h	0/11	0/11	1/9	0/1	
		> 4–16	0/11	0/11	0/11	0/11	
		> 16–36	0/2	0/2	1/2	0/2	
		> 36 h	0/18	0/18	1/16	0/16	
		n=44	0/42	0/42	3/38	0/38	
III	Maintenance with no direct exposure	0–4 h	1/13	1/13	1/8	0/7	1/1
		> 4–16	1/6	1/6	0/4	0/4	
		> 16–36	1/7	1/7	0/7	0/6	0/1
		> 36 h	1/15	1/15	1/14	1/13	0/1
		n=47	4/41	4/41	2/33	1/30	0/3
IV	Products handlers	0–4 h	3/55	3/55	0/39	0/39	
		> 4–16	1/37	1/37	2/29	0/28	
		> 16–36	0/15	0/15	0/10	0/10	0/1
		> 36 h	0/37	0/37	0/33	0/28	0/1
		n=146	4/144	4/144	2/111	0/105	0/2
V	Maintenance, with direct exposure	0–4 h	6/25	4/25	1/21	0/18	1/1
		> 4–16	0/4	0/4	0/3	0/3	
		> 16–36	2/24	1/24	0/20	0/17	
		> 36 h	4/47	4/47	0/38	0/38	2/7
		n=100	12/100	9/100	1/82	0/78	3/8
VI	Styrene manufacture and purification	0–4 h	10/12	10/12	3/11	2/9	2/2
		> 4–16	3/6	3/6	2/6	2/6	
		> 16–36	1/4	1/4	1/3	1/3	
		> 36 h	1/6	1/6	0/5	0/5	0/1
		n=29	15/28	15/28	6/25	5/23	2/3
VII	Polystyrene polymerisation workers	0–4 h	18/35	19/35	12/23	7/22	4/4
		> 4–16	3/12	3/12	0/10		
		> 16–36	0/8	0/8	1/6	0/6	
		> 36 h	0/22	0/22	1/17	0/16	3/4
		n=78	22/77	22/77	14/56	7/54	7/8

*MA and PGA > 5 mg/g creatinine.
†Simultaneously increased urinary metabolites and blood styrene.

Table 5 *Urinary metabolite and blood styrene concentration among styrene polymerisation workers recently exposed (4 h)*

Groups	*Urinary metabolites raised*		*Blood styrene > 5 ppb*	*Both raised*
	MA (> 5 mg/gC†)	*PGA*		
Low or unexposed (II, III)	1/23	1/23	2/17	0/16
Estimated 1–5 ppm average exposure* (IV, V)	9/80	7/80	1/60	0/57
Estimated > 5 ppm average exposure* (VI, VII)	28/47	29/47	15/35	9/31
Total	38/150	37/150	18/112	9/104

*Based on data of Maier and Ruba, 1974.
†gC = g creatinine.

Table 6 *Subgroups with significant exposure indicated by urinary metabolites and/or blood styrene concentration**

Workers	*Group*	*Total in group*	*Urinary metabolites > 5 mg/gC†*	*Blood styrene > 5 ppb*	*Both raised*
Labourer, millwright, instrument maintenance	V	41	5/11	1/11	0/11
Latex-specialty and extrusion	IV	41	2/18	0/12	0/12
Packaging operator	IV	26	1/11	1/11	1/11
Electrician	III	16	1/2	0/2	0/1
Liquid handler	V	9	1/4	0/3	0/2
Pipefitter (> 16 hours only)	V	28	5/16	1/20	1/19

*Subjects having worked within the past four hours, except for pipefitters.
†gC = g creatinine.

CORRELATION OF STYRENE EXPOSURE WITH URINARY METABOLITES AND BLOOD STYRENE

Relative exposure by job category

The occurrence of increased urinary metabolites and blood styrene paralleled the estimated exposure intensities of job categories (Table 5), with the highest incidence among styrene production and polymerisation workers. The findings for group V are consistent with significant but intermittent exposure, because of the nature of maintenance work which calls for frequent but not continuous repairs in high styrene exposure areas. The absolute values of the urinary metabolites are consistent with this concept (Tables 3, 9, 10).

Within exposure groups III, IV and V (Table 6), certain job categories were found to account for all but one case of raised urinary or blood metabolites in the larger groups. These included specific maintenance workers and those handling finished polystyrene.

The polymerisation process could be subdivided into four separate areas (Tables 7 and 9), and no significant differences among these subgroups are evident, possibly because of the small number of workers studied.

There were a number of cases with trace urinary metabolites, those near the limit of detection (approx. 7–15 mg/l) or values which were reduced to below 10 mg/g when corrected for creatinine concentration (Table 8). For example, 20% of 92 products handlers (group IV) showed only traces of urinary metabolites for those exposed within 16 h. In the same group, 6% had raised urinary metabolites (Table 4). In 11 cases, blood styrene levels were found at trace concentrations (3–4·9 ppb), including nine with raised urinary metabolites.

Subgroups of IV, latex-specialty, with a 40% incidence of trace urinary metabolites, and extrusion workers with 25%, showed traces of urinary metabolites without concomitant detectable blood styrene concentrations. However, in these groups there were also instances of blood styrene concentrations between 3–4·9 ppb. With the data of Table 6, these findings suggest low-level exposure for those subgroups.

Trace level metabolites were also observed in 15 of 91 maintenance workers (Table 8, group V) examined more than 16 h after last exposure. Among 47 recently exposed polymerisation workers, there were 10 cases of trace urinary metabolites and six cases of trace blood styrene, the latter with five coincidentally raised urinary metabolites. The combined number of trace and elevated urinary metabolites for polymerisation workers exposed within 16 h was 13/47; the comparable number for blood styrene was 18/33. For maintenance workers (group V) more than 16 h after exposure, 21/71 urinary metabolites and 5/58 blood styrenes were either trace or elevated.

Table 7 *Urinary metabolites, blood styrene and styrene in body fat among styrene workers recently exposed (≤ 4 h)*

Compound	*Urinary metabolites > 5 mg/gC*		*Blood > 5 ppb*	*Both raised*	*Fat styrene*
	MA	*PGA*			
Maleic anhydride–styrene copolymer	3/4	3/4	2/4	2/4	2/2
'Expandable' polystyrene	0/2	0/2	1/2*	0/2	
Polystyrene area I	8/19	8/19	8/12**	4/12	
Polystyrene II	5/7	6/7	1/4	1/4	1/1
Total	16/32	17/32	12/22	7/22	3/3

*'Expandable' polystyrene: one had raised blood styrene without raised urinary MA or PGA. 'Expandable' polystyrene is polymer beads made by using pentane as a blowing agent.
**In polystyrene area I there were four individuals with raised blood styrene, without raised urinary MA or PGA.

Table 8 *Urinary metabolites or blood styrene detectable as trace amounts (approx. 5–10 mg/l and 3–5 ppb respectively) among workers in a styrene polymerisation plant*

Group	*Job classification*	*Time since last exposure (h)*	*Number with trace of*		*Blood styrene 3–4·9 ppb*	*Total in subgroup*
			MA	*PGA*		
I	Retired	> 36	3	4	3†	47
II	Miscellaneous, unexposed	< 16	2	2	1*	22
		> 16	2	2	2*	20
III	Maintenance, incidental or no exposure	< 16	3	3	3*	19
		> 16	3	3	5†	22
IV	Products handling	< 16	19	18	11‡	92
		> 16	4	4	1*	52
V	Maintenance with direct exposure	< 16	4	4	4§	29
		> 16	15	15	5¶	91
VI	Styrene manufacture and purification	< 16	—	—		18
		> 16	1	1		10
VII	Polystyrene polymerisation workers	< 16	10	10	6**	47
		> 16	5	5	2*	30
Total		< 16	38	37	6	227
		> 16	33	34	2	
		Total	71	71	8	272

*None of this group had concomitant raised urinary metabolites.
†One had concomitant raised urinary metabolites.
‡Nine had concomitant raised urinary metabolites.
§All had concomitant raised urinary metabolites.
¶Three of this group had concomitant raised urinary metabolites.
**Five had concomitant raised urinary metabolites.

Table 9 *Average concentrations of raised urinary metabolites and blood styrene measurements among styrene polymerisation workers**

Job classification	*Average urinary metabolite concentrction*		*Number of subjects*	*Average blood styrene (ppb)*	*Number of subjects*
	MA (mg/g creatinine)	*PGA*			
Styrene polymerisation					
Area I	35 ± 28	26 ± 20	(11)	31 ± 30	(9)
Area II	17 ± 14	18 ± 11	(7)		
Styrene manufacture**	42 ± 18	20 ± 10	(6)		
Styrene purification	61 ± 44	51 ± 32	(6)	29 ± 31	(4)
Maleic anhydride–styrene copolymerisation	27 ± 1	27 ± 8	(2)	7 ± 2	(2)
Labourer	17 ± 7	13 ± 13	(3)		
Millwright	47 ± 6	16 ± 19	(2)		
Painters	21 ± 5	15 ± 4	(2)		

*For subgroups with more than one subject having urinary MA or PGA greater than 5 mg/g creatinine and blood styrene greater than 5 ppb.
**For styrene manufacture workers, MA–PGA ratios are different from those of polymerisation workers ($P < 0.01$).

Table 10 *Mean values of raised urinary metabolites and blood styrene related to time since exposure*

Time since exposure (h)	*N*	*Urinary MA, PGA (mg/gC)***		*Blood styrene (ppb)***
In subjects with urinary metabolic concentration > 5 mg/gC*				
< 0·7	17	41 ± 32	31 ± 23	15 ± 24 (n = 11)
1–7	14	34 ± 28	23 ± 22	6 ± 5 (n = 10)
2–4	6	20 ± 15	18 ± 10	3 ± 1 (n = 4)
7–26	10	20 ± 10	17 ± 6	4 ± 2
42–48	4	12 ± 4	7 ± 4	4 ± 3
In subjects with blood styrene ⩾ 5 ppb				
<0·7	7	34 ± 39	32 ± 32	34 ± 32
1–1·7	6	35 ± 41	30 ± 34	10 ± 4
2–4	4	1 ± 1	1 ± 1	25 ± 30
7–26	5	6 ± 8	9 ± 13	6 ± 1
42–48	2	6 ± 6	6 ± 6	28 ± 31

*gC = g creatinine.
**Mean ± SD.

Exposure information from concentration of blood styrene and urinary metabolites

Higher mean concentrations of urinary metabolites were observed among styrene manufacture and purification workers (Table 9). Styrene manufacture workers also had mean mandelic acid concentrations approximately twice that of phenylglyoxylic acid (different from other MA–PGA ratios, $P < 0.01$).

An end-of-shift urine could represent a metabolite concentration in the maximum concentration range, only if the subject were exposed within the first few hours of work. The data of Table 10 show a trend toward decreasing concentration of urinary metabolites with increasing time away from the work environment.

Among 42 workers with urinary metabolite values greater than 15 mg/g creatinine, the mean mandelic acid concentration was 43 ± 33 mg/g creatinine, suggesting an average exposure concentration of about 5 ppm.

Regression analysis showed no consistent statistically significant relationships between urinary mandelic acid and blood styrene concentrations, a result not unexpected on the basis of the metabolic model (Fig. 4). The correlation between MA and PGA was highly significant (Fig. 5).

For 25 of these workers with subcutaneous fat biopsies (Wolff *et al.*, 1977), styrene in blood and fat and urinary metabolites are now reported in Table 11. Among workers recently exposed (for less than 8 h), 8/8 had detectable styrene in fat tissue, 2/6 had blood styrene greater than 5 ppb, and 5/8 had increased urinary metabolites. Of the 11 exposed 20–72 h previously, five had detectable styrene in fat, with no instance of raised blood styrene, and two had detectable urinary metabolites.

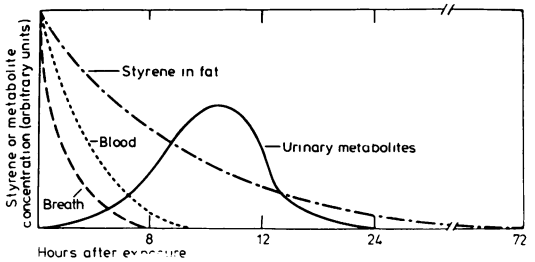

Fig. 4 *Metabolic model for styrene elimination.*

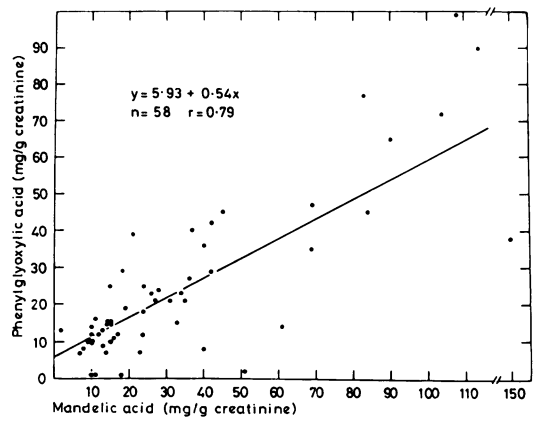

Fig. 5 *Correlation of urinary phenylglyoxylic and mandelic acid among 58 styrene polymerisation workers.*

Discussion

Our findings indicate that blood styrene concentrations and urinary metabolite determinations are expedient biological measures of exposure to styrene, even at low concentrations of styrene in air. Blood styrene measurement, using a new analytical method, is useful with very recent exposures, and determination of both factors in cases of high exposure may be used by comparison with the metabolic model (Fig. 4) to estimate previous maximum exposure. Estimation of the fat concentration of organic materials has become possible in selected cases by use of the non-surgical needle biopsy technique to obtain fat for analysis. However, it is not practical for routine daily or weekly screening of working populations.

The present study is unique in its use of biological exposure measurements in such a large working population (491). Blood styrene and urinary metabolite measurements suggested that more than half of the styrene production and polymerisation workers in this plant were exposed to styrene during

Table 11 *Comparison of fat and blood styrene concentration with urinary metabolites*

Time off (h)	*Estimated* exposure*	*Styrene in fat (ppm)*	*Styrene in blood (ppb)*	*Urinary MA/PGA (mg/g creatinine)*
0	— III	0·1	NA	0
0	M IV	0·2	NA	0
3	M VI	0·3	4·2	69/35
3	H VII	0·4	8·2	26/33
4	H VII	0·8	3·5	13/9
4 [2]*	H VII	0·1	1·6	0/0
4 [0·5]	H VII	0·3	3·5	42/29
8 [1·5]	M VI	1.2	15·4	114/90
20	H VII	—**	1·4	0
48	H V	—**	4·7	18/0
48 (96)*	H V	—**	1·6	0/0
48	M IV	—	1·9	0
48	— III	0·6	2·8	trace
48	— III	—	2·3	0
60	M VII	0·1	NA	0
60	M VII	0·1	1·9	0
72	M VII	0·3	1·2	0
72	— V	0·6	1·4	0
72	L V	—	2·1	0
96	M IV	—	0·7	0
96	M IV	—	0·7	0

*Time since last exposure in parentheses. Time first examined (urine and blood) in square brackets. Roman numerals correspond to categories in Tables. H = high; M = medium; L = low.
**Small amount of fat reduced sensitivity; therefore unreliable indication of absence of styrene in fat.
NA = not assessed.

the previous eight hours, although even the highest urinary mandelic acid and blood styrene levels (152 mg/g creatinine, 85 ppb) indicated that the TLV had not been exceeded. Average exposures were estimated to be approximately 5 ppm. Thiess *et al.* (1976) have reported similar urinary metabolite concentrations among a group of 158 workers with styrene exposures below 8 ppm in air. The prevalence of elevated biological exposure indices was well correlated with previously estimated exposure intensities for various subgroups. Thus, more than 50% of polymerisation and production workers, as groups, had increased urinary metabolites and more than 40% had raised blood styrene levels. With the number of trace level biological exposure measurements, these frequencies among polymerisation workers became 75·6% and 54·5% for urinary metabolites and blood styrene level indices, respectively.

Raised and trace exposure indices at low prevalence in other subgroups appeared to occur within several specific work categories (Tables 6 and 8), although it is not clear that low levels or occasional exposures are confined to these groups. For example, four of 18 packaging operators had detectable or trace urinary metabolites. Although this group was estimated to have low exposure, the presence of styrene monomer in finished polystyrene products is well documented, and more detailed investigation of such workers may be of interest. Maintenance workers involved in repair work in areas of styrene use also appear to have significant though intermittent exposure (Table 4). Among pipefitters especially, relatively high exposure was suggested by the prevalence of 8/26 (five raised and three trace) detectable urinary metabolite levels (average concentration 12 mg/gc) for workers removed from exposure for more than 16 h. Production and polymerisation workers with known high exposures had 2/40 such determinations.

The intensity of exposure, suggested by urinary metabolite concentration (Table 9), appears to be greater for styrene manufacture and purification than for polymerisation workers, as in the former groups MA concentration was significantly higher. Furthermore, among styrene manufacture workers, the average concentration of urinary mandelic acid was more than twice that of phenylglyoxylic acid. It may be that workers in this area had a higher exposure to ethyl benzene than in other areas; this may have less efficient metabolic conversion to PGA than to MA. However, the data of Bardodej and Bardodejova (1970), which showed that MA and PGA comprised 64% and 25% of ethyl benzene metabolism and 85% and 10% of styrene metabolism respectively, do not support this hypothesis. Nevertheless, complex exposures are known to result in metabolic effects different from exposure to either substance alone, at least for toluene–benzene and toluene–styrene exposures (Ikeda *et al.*, 1972), and this may be a factor here as well.

Styrene exposure can be characterised by the interrelation of styrene concentration in breath and blood with urinary metabolite concentration and concentration in fat, as shown in Figure 4.

Several instances of simultaneously raised blood styrene and urinary metabolites were observed among the polymerisation workers, a finding that apparently underlines their higher exposure (see Tables 5, 6, 7 and 8). However, several cases were also observed in which blood styrene was raised without a concomitant increase in urinary metabolites (Tables 5 and 8). This may be attributable to very recent exposure, resulting in high blood styrene, before significant excretion of urinary metabolites. Thus, from the metabolic model (Fig. 4), the preferential fat/blood (130) and blood/breath (32) partition (Rees, 1974) causes a rapid decline first in breath, then in blood concentration of styrene. After an initial lag, urinary excretion of mandelic and phenylglyoxylic acids achieves a maximum concentration at 8–16 h after exposure. This metabolite elimination presumably coincides with a substantial depletion of fat-stored styrene, because urinary

metabolites have been shown to account for 95% of the styrene absorbed in human subjects (Bardodej and Bardodejova, 1970).

The preferential use of urinary metabolites as a sensitive indicator of styrene exposures is suggested by the more frequent occurrence of raised metabolite levels than of raised blood styrene among this group of workers. Indeed, although the ambient exposures are not available to provide firm evidence for this, the metabolic model (Fig. 4) supports this conclusion. Styrene is extremely fat-soluble, so that the amount absorbed will be preferentially concentrated in the adipose tissue rather than the blood or breath. Thus, blood and breath concentrations are low in comparison. However, metabolism of styrene peaks within 8–12 h and efficiently eliminates styrene from the fat tissue, as 95% of that absorbed can be accounted for by urinary metabolites (Bardodej and Bardodejova, 1970). Accordingly, blood concentrations become insignificant (Table 8) within 4–8 h, and in this group of workers were less often detected than were urinary metabolites. Urinary metabolites, however, are excreted over a 8–20 h period with a rather broad maximum (representing a large concentration range) which allows detection in a small urine sample over a fairly long time interval after exposure.

The metabolic model suggests that fat and blood concentrations should be proportional, since an equilibrium exists. In the group examined there were 10 cases with both blood styrene and fat styrene determinations, and here a significant correlation was found (r = 0·78; Fig. 6). However, in contrast to the predictive model (Fig. 4), blood levels are correlated even with urinary mandelic acid in this same small subgroup (r = 0·66), suggesting that this correlation is an artefact attributable to the small number of cases involved. As expected, within a larger group with raised urinary MA, the correlation was poor (r = 0·12, n = 42).

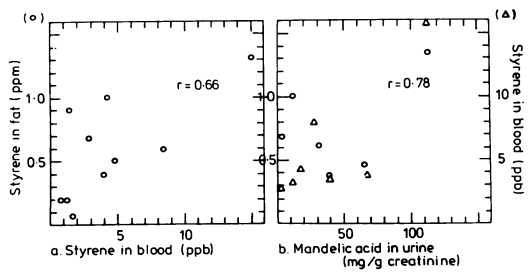

Fig. 6 *Relation of styrene in fat to* (a) *styrene in blood and* (b) *urinary metabolites. Relation of styrene in blood to urinary metabolites* (b, △).

The partition coefficient of styrene (blood/oil) has been predicted as 130 on the basis of laboratory work (Rees, 1974). Among the subgroup having blood and fat levels, the ratio of fat to blood concentration of styrene ranged from 49–429 with a mean of 150. While many volume and blood flow factors are neglected, this approximation agrees well with the predicted value.

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