British Journal of Industrial Medicine 1986;43:692–697

Quantitative relation of urinary phenol levels to breathzone benzene concentrations: a factory survey

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ABSTRACT Urine samples were collected from 64 men and 88 women in shoe factories and printing plants at the end of a seven hour day shift in the latter half of a week in spring. Urine samples were also taken from 43 men and 88 women in the same factories but who were not exposed to solvents. Exposure to benzene during the shift was monitored by passive dosimeters. Both phenol in urine and benzene in activated carbon were analysed with FID gas chromatographs. The urinary concentrations of phenol were linearly related to the time weighted average concentrations of benzene in the breathzone air; the variation was so small that those exposed to 10 ppm benzene could be separated from the non-exposed at least on a group basis when the phenol concentration was corrected either for creatinine concentration or for specific gravity. The urinary phenol concentrations corresponding to 10 ppm benzene were 47·5 mg/l (as observed), 57·9 mg/g creatinine, or 46·6 mg/l (specific gravity 1·016).

Benzene is considered to be a cause of human leukaemia[1] and occupational as well as non-occupational exposure to this chemical has been a focus of keen attention. Among the biological indicators of exposure to benzene, urinary phenol has been most frequently used although it may be inferior to benzene in blood in its sensitivity and specificity,[2 3] probably because urine is apparently much more readily available from factory workers than venous blood, and this is especially so in the case of the general population. The existing data on the quantitative relation between exposure to benzene and urinary phenol excretion are, however, usually hampered by the technical limitations prevailing at the time of the study such as the low specificity of urinary phenol determination, the inability to measure average exposure during work, the small number of subjects, or a combination of these factors.[2]

The present study was initiated to establish the quantitative exposure excretion relation, using passive samplers to determine the time weighted average exposure intensity of exposure to benzene and FID gas chromatography for the specific measurement of phenol in urine. The results of studies with similar methodology have been described for toluene hippuric acid/o-cresol[4] and tetrachloroethylene total trichloro compounds.[5]

Accepted 21 January 1986

Materials and methods

EXAMINEES

The factory survey was conducted in the latter half of a week in spring. The workers exposed to benzene were 64 men (aged 26·9 ± 8·4 years as arithmetic mean ± arithmetic standard deviation (AM ± ASD)) and 88 women (29·9 ± 8·5 years) in five workshops in two shoe factories and two small printing plants. Control subjects were 43 men (39·3 ± 11·9 years) and 88 women (26·9 ± 8·4 years) who worked in the same factories but had not been exposed to solvents. Those under medical treatment with drugs were excluded.

COLLECTION AND ANALYSES OF URINE SAMPLES

Each subject was asked to pass urine at about 1300. The urine sample for analysis was collected at about 1500 when the seven hour shift was over and the urinary phenol concentration was expected to reach a maximum; the urine was kept at −80°C before analy-

sis. Phenol concentration was determined gas chromatographically by the method previously described for o-cresol[4]: 1 ml of urine was mixed with 0·5 ml 15% HCl and heated for one hour for acid hydrolysis. Then, 3,5-xylenol (either 1·25 or 5 μg) was added as an internal standard and the mixture was extracted with 2 ml of carbon disulphide. The organic phase was treated with sodium sulphate for desiccation. An aliquot of 1 ml was transferred to a test tube and the volume was reduced to about one twentieth or less under a stream of nitrogen. An aliquot of 1 to 5 μl was injected into a Hitachi FID gas chromatograph (model 163) equipped with 60–80 mesh KG-02 on Uniport HP glass columns (3 mm in inner diameter and 5 m in length). The injection port and the oven were heated at 210°C and 180°C, respectively. The carrier gas (nitrogen) was allowed to flow at a rate of 30 ml/min and the supplies of hydrogen and air to the detectors were at 1·0 kg/cm^2 and 1·5 kg/cm^2, respectively. Under such conditions phenol was clearly separated from o-, m-, and p-cresols on the chromatogram. The phenol concentration was expressed as a measured value, as the ratio to the creatinine concentration,[6] or after correcting to the specific gravity of the urine of 1·016.[7] The creatinine concentration was measured colorimetrically[8] and the specific gravity by refractometry.

DETERMINATION OF BENZENE CONCENTRATION IN BREATHZONE AIR

Badge type passive samplers with K-filter 1600[9] were used for personal breathzone air sampling to measure the time weighted average exposure (from about 0800 to about 1500). The precise duration of sampling was recorded for each individual or workshop. After exposure, the activated carbon filters were brought to an analytical laboratory distant from the survey sites within two days and kept refrigerated before analysis (within two weeks).[10]

An additional study disclosed that, when kept at 4°C, there was no significant ($p < 0·10$) loss in benzene on the carbon over a six month period. The gas chromatrographic analysis of the carbon disulphide extract showed that the major component of the solvent vapour in the air was benzene; other contaminants such as toluene, n-hexane, or ethyl acetate were detected at trace level (less than 5% of that of benzene at most) only occasionally. When the benzene concentrations were expressed on a group basis, a log normal distribution was assumed.[11]

Results

PHENOL CONCENTRATIONS IN THE URINE OF THE NON-EXPOSED SUBJECTS

Phenol concentrations in the control urine samples accumulated in the range of 0–10 mg/l in the cases of observed values and after correction for a specific gravity (1·016) or 1–10 mg/g when corrected for creatinine. A skew towards higher levels was observed suggesting a log normal rather than a normal distribution. Accordingly, geometric means (GMs) and geometric standard deviations (GSDs) were calculated with an assumption of a log normal distribution; the results are summarised in table 1. The difference in the GMs between the two sexes was small even though statistically significant in some cases. When the results for the two sexes were combined, the GMs were either below 10 mg/l (the observed value and the value corrected for a specific gravity) or 10 mg/g (the value corrected for creatinine).

CORRELATION BETWEEN BENZENE CONCENTRATIONS IN BREATHZONE AIR AND PHENOL CONCENTRATIONS IN URINE

When the exposed workers were grouped by workshop and by sex, and benzene GM and phenol GM compared (table 2), the range of both breathzone benzene concentrations and urinary phenol concentrations was wide but the former were generally paralleled by the latter even though the number of workers in some workshops was small (workshops A and D, for example). It should also be noted that GSDs were greater than 2 in several cases, indicating that the exposures, even in a single workshop, might vary

Table 1 *Phenol concentration in urine samples from non-exposed subjects*

Non-exposed subject	*No of examinees*	*Value† corrected for*		
		Observed value† (mg/l)	*Creatinine‡ (mg/g)*	*Specific gravity§ (mg/l)*
Men + women	131	6·91 (2·622)	8·63 (1·921)	7·10 (1·983)
Men	43	7·65 (2·861)	10·27 (1·845)*	9·09 (1·892)**
Women	88	6·57 (2·511)	7·93 (1·932)	6·29 (1·968)

* and ** indicate that the difference between the two sexes is statistically significant (*$p < 0·05$, **$p < 0·01$).
†Geometric mean (geometric standard deviation).
‡Phenol concentration divided by creatinine concentration.
§Phenol concentration adjusted to a specific gravity of 1·016.

Table 2 *Phenol concentration in the urine of workers exposed to benzene in various workshops*

Workshop	No of workers	Benzene concentration in breathzone air* (ppm)	Phenol concentration*		
			Observed value (mg/l)	Value corrected for Creatinine† (mg/g)	Value corrected for Specific gravity‡ (mg/l)
Men:					
A	3	1·0 (1·000)	12 (2·798)	7 (1·812)	8 (1·869)
B	24	11·1 (2·398)	32 (2·145)	42 (2·125)	34 (2·321)
C	20	32·6 (1·421)	106 (1·784)	147 (1·498)	119 (1·456)
D	2§	7, 76	16, 85	15, 160	16, 136
E	15	60·2 (1·282)	277 (1·657)	313 (1·320)	206 (2·259)
Women:					
A	1§	1	3	5	4
B	19	18·1 (2·792)	76 (3·945)	102 (3·041)	77 (2·818)
C	37	42·4 (2·485)	134 (2·235)	169 (2·143)	124 (2·116)
D	2§	5, 37	5, 100	6, 165	4, 114
E	29	76·4 (1·576)	319 (2·178)	427 (1·783)	315 (1·662)

*Geometric mean (geometric standard deviation).
†Phenol concentration divided by creatinine concentration.
‡Phenol concentration adjusted to a specific gravity of 1·016.
§Individual values shown.

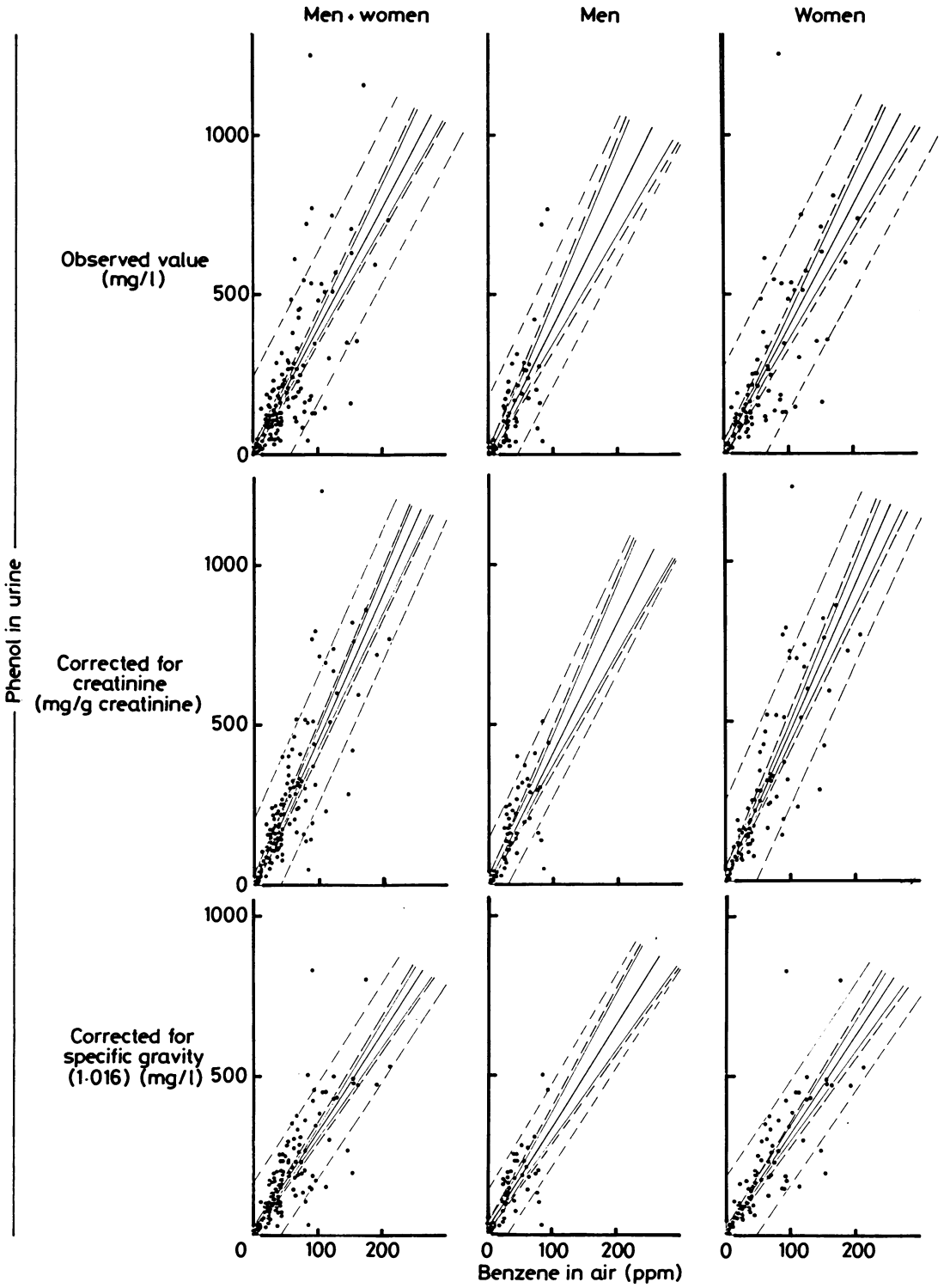
depending on the individual worker. Accordingly, further comparisons between the two measurements were made on an individual basis using scatter diagrams as shown in the figure. Despite the difference in the maximum exposure between the sexes (some of the female workers were exposed well above 100 ppm whereas exposure of male workers never exceeded this concentration), the correlation appeared to be similar for the two sexes. It should also be noted that the variation around the regression was smaller in every case when the phenol concentrations were corrected for creatinine concentration than when observed phenol concentrations or specific gravity corrected phenol concentrations were used.

The correlation coefficients are summarised in table 3. The correlation was significant ($p < 0·01$) and the value of the coefficient was about 0·8 or higher in all cases; the largest coefficient was obtained when phenol concentrations were corrected for creatinine concentration. The intercepts on the phenol axis (table 3) were essentially the same as the GMs for the non-exposed subjects (table 1), and rather small by comparison with the corresponding slopes (equivalent to 1 to 3 ppm benzene exposure). As both the

Table 3 *Correlation between breathzone benzene concentration and urinary phenol concentration*

Measurement group	No of examinees*	A†	B†	C‡
Observed value (mg/l):				
Men + women	283	3·816	9·4	0·822
Men§	108	4·110	4·3	0·789
Women‖	175	3·763	10·6	0·828
Women¶	*159*	*4·003*	*6·9*	*0·746*
Value corrected for creatinine (mg/g):**				
Men + women	283	4·451	13·4	0·891
Men§	108	4·097	15·6	0·858
Women‖	175	4·503	14·7	0·894
Women¶	*159*	*4·784*	*8·0*	*0·893*
Value corrected for specific gravity (mg/l):††				
Men + women	283	3·129	15·3	0·881
Men§	108	3·351	13·3	0·840
Women‖	175	3·099	14·5	0·890
Women¶	*159*	*3·639*	*7·0*	*0·861*

*Including non-exposed subjects (43 men and 88 women).
†Slope (A) and the intercept on the Y axis (B) in the figure as: $Y = AX + B$, where Y is phenol concentration (unit; as described in the table) in urine and X is time weighted average benzene exposure concentration (ppm).
‡Correlation coefficient. $p < 0·01$ for all correlation coefficients.
§Exposed up to 92 ppm.
‖Exposed up to 210 ppm.
¶Women exposed to less than 100 ppm.
**Phenol concentration divided by creatinine concentration.
††Phenol concentration adjusted for a specific gravity of 1·016.



Relation between benzene in breathzone air and phenol in urine. Points indicate individual values. Lines and curves are calculated regression line (solid line in centre), 95% confidence ranges of sample means (thin curves), 95% confidence ranges of the regression line (the broken lines), and 95% confidence ranges of the individual samples (the outmost broken lines).

intercept and the slope were similar in men and women, it was clear that the regression lines did not vary between the two sexes. This was the case even when women exposed to less than 100 ppm were selected (taking the difference in maximum exposure levels between the sexes into consideration).

Discussion

The present study clearly demonstrates that the phenol concentration in urine (collected at around 1500 in the latter half of the week) is proportional to the benzene concentration in breathzone air (expressed as a time weighted average) at least up to 200 ppm. The slope of the regression line obtained in the present study—3 to 4 mg phenol/l urine (or g creatinine)/ppm benzene—was larger than the values reported in some previous studies. For example, a slope of 0·33 (unit: mg phenol/litre urine/ppm × hour benzene) was calculated by Lauwerys[2] primarily from the data of Rainsford and Lloyd Davies,[7] Berlin *et al*,[12] and Sherwood,[13] which is equivalent to 2·3 mg/l/ppm when a seven hour exposure is assumed. Most of the high values cited in the calculations of Lauwerys were, however, derived from workers who spent substantial periods in uncontaminated air,[7] whereas spot workroom air samples were used for benzene determination. Thus the intensity of exposure might have been overestimated. In another study in which phenol concentrations (measured colorimetrically by the Theis-Benedict method) were adjusted to a specific gravity of 1·024 a regression line with a slope estimated to be 7·0 mg/l/ppm was found.[14] Adjusting the specific gravity to 1·016 gives a slope of 4·7 mg/l/ppm, a value slightly higher than the present results. The data from Pagnotto and Bethlehem Steel as cited by the National Institute for Occupational Health and Safety gave a slope of 7·2 and 3·9 mg/l/ppm, respectively, assuming a linear regression.[15] Assuming that urinary phenol concentrations were adjusted to a specific gravity of 1·024 by Pagnotto, readjustment to 1·016 results in a slope of 4·8 mg/l/ppm. If cases with "normal" phenol concentrations are excluded from Bethlehem Steel data the slope becomes 3·8 mg/l/ppm.

One of the critical points in evaluating urinary phenol as a biological estimator of benzene exposure is to determine whether the estimator can separate those exposed to benzene at a given occupational exposure limit such as 10 ppm from those not exposed. Comparison of the lower 95% confidence range of the mean phenol concentrations at 10 ppm benzene (figure) and the upper 95% confidence range of the phenol concentrations in the non-exposed subjects as calculated by GM × $(GSD)^2$ (table 1) shows that the former was larger than the latter in men, women, and men + women when phenol concentrations were corrected for either creatinine or a specific gravity, although this was not the case with observed (uncorrected) phenol concentrations. For example, the lower 95% confidence value (using phenol concentrations corrected for creatinine) for men + women was 45·1 mg/g creatinine: the corresponding upper 95% confidence limit for the non-exposed men + women was 31·9 mg/g creatinine. Thus it is reasonable to conclude that the separation of those exposed to 10 ppm benzene from those not exposed is possible at least on a group basis, so far as the effects of medication[16-18] are excluded. It is clearly impossible to make the separation on an individual basis as the wide 95% confidence ranges indicate (figure).

No follow up of phenol excretion was made in the present study to cover the entire period of phenol excretion. It is possible, however, to make a cross sectional balance study between the amount of benzene absorbed and the excretion of phenol in urine at the end of work with exposure to benzene at, say, 10 ppm, using three assumptions; that about 50% of inhaled benzene is absorbed through the lungs (by analogy with toluene[19]) and that the rates of respiration and urinary excretion under the conditions studied are 15 l/min and 1 ml/min, respectively. The input will be 10 ppm ($=31·9$ mg/m^3) $\times 15 \times 10^{-3}$ (m^3/min) $\times 0·5 = 0·23925$ mg/m^3 whereas the output into urine in the form of phenol is 38·16 mg/l $\times 1 \times 10^{-3}$ l/min $= 0·003816$ mg/min or 0·003167 mg/min (0·03816 mg/min × 78·11/94·11) as benzene, where 78·11 and 94·11 are molecular weight of benzene and phenol, respectively. Thus at the end of the workshift about 13% ($=0·04755/0·23925 \times 100$) of benzene absorbed through the lungs will be converted to phenol and excreted into the urine.

Further analyses of the urine samples are currently in progress for polyhydroxylated benzene metabolites such as catechol and hydroquinone, possible contributors to the development of benzene toxicity[20-22] to correlate the urinary concentrations with the intensity of benzene exposure.

We thank the Health Bureau of Hefei City and Dr S-L Fu, Ms R-G Zhang, Mr W-G Wu, Ms G-F Cui, Mr L-H Zai, Ms J-F Wan, and Ms L-S Hong for their supportive cooperation in the field investigation.

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