

Urinary Phenylmercapturic Acid as a Marker of Occupational Exposure to Benzene[#]

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Abstract: A hand-saving HPLC method to measure urinary phenylmercapturic acid (PMA) was developed which allows about 35 PMA determinations per day. The method involves conversion of pre-PMA to PMA by the addition of sulfuric acid to a urine sample, extraction into an ether-methanol mixture followed by condensation under a nitrogen stream. The condensate was introduced to a ODS-3 column in a HPLC system, and PMA in the column was eluted into a mobile phase of acetonitrile: methanol: perchloric acid: water. The elution of PMA was monitored at 205 nm. One determination will be completed in 40 min. The method was applied to analysis of end-of-shift urine samples from 152 workers exposed up to 210 ppm benzene, 66 workers exposed to a mixture of benzene (up to 116 ppm) and toluene+xylenes (up to 118 ppm), and 131 non-exposed controls of both sexes. A linear regression was established between time-weighted average intensity of exposure to benzene and urinary PMA. From the regression, it was calculated that urinary PMA level will be about 6.4 mg/l after 8-hour exposure to benzene at 100 ppm, and that PMA in urine accounted for about 0.1% of benzene absorbed. No effects of sex, age, and smoking habit of individuals were detected, and the effect of co-exposure to toluene + xylenes at the levels comparable to that of benzene was essentially nil, which indicates an advantage of PMA as a benzene exposure marker over mono- to tri-phenolic metabolites or t,t-muconic acid.

Key words: Benzene, Biological monitoring, Occupational exposure, Sulfuric acid treatment, Urinary mercapturic acid

Introduction

Whereas carcinogenicity of benzene to humans has been well elucidated through occupational epidemiology^{1,2} e.g. in terms of increased incidence of leukemia among benzene-exposed industrial workers³⁻⁵, benzene is present in automobile gasoline^{6,7}, and is in use in organic synthesis in modern industries. Benzene exposure in cokeries in the iron and steel industry is also known^{8,9}.

This study group has been studying on urinary biomarkers of occupational exposure to benzene such as catechol¹⁰,

quinol¹⁰, 1,2,4-benzenetriol^{11,12} as well as t,t-muconic acid¹³ in addition to a traditional marker of phenol¹⁴. Urinary phenylmercapturic acid (PMA; N-acetyl-S-phenyl-L-cysteine), a N-acetylcysteine conjugate of benzene, has also been evaluated as a marker of exposure to low-level benzene^{8,9,15-20}, but the analytical methods employed are rather complex, e.g., requiring derivatization or clean-up through columns before instrumental analysis²¹⁻²⁵ such as gas chromatography-mass spectrometry¹⁵ or liquid chromatography-mass spectrometry²⁶, and application of the methods to routine occupational health services appear to be not practical.

In the present study, an HPLC method was developed as a hand- and energy-saving and therefore practical method for

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PMA determination in urine, and a linear correlation was established between time-weighted average (TWA) intensity of occupational exposure to benzene and PMA in end-of-shift urine by the application of the established method to urinalysis. Absence of the effects of co-exposure to toluene/xylenes was also observed. The results are presented in this article.

Materials and Methods

Subjects studied

Spot urine samples analyzed were collected near the end of a shift of a work day in the latter half of a work week, from 152 benzene-exposed workers [65 men (exposed up to 92 ppm benzene) and 87 women (up to 210 ppm benzene)], and 66 mixture-exposed workers [49 men (up to 116 ppm benzene and 118 ppm toluene + xylenes) and 17 women (up to 7 ppm benzene and 34 ppm toluene + xylenes)]. In addition, 131 control subjects (43 men and 88 women) in clerical sections of the same factories with no occupational exposure to benzene (B), toluene (T) or xylenes (X) offered spot urine samples (Table 1). Both smokers (1 to 20 cigarettes/day) and nonsmokers were included; about 59% and 1% of men and women, respectively, smoked with the maximum consumption of 20 cigarettes/day. The time-weighted average (TWA) BTX exposure as measured by diffusive sampling, and ages and smoking habits of individuals were cited from previous publications^{14, 27}.

Analysis of urine for phenylmercapturic acid

Under standard assay conditions, each urine sample (0.5 ml) was taken in a 12-ml glass-stoppered glass test tube, and mixed step-wise with 0.5 ml water (or diluted further as necessary), 0.1 ml internal standard solution (20 to 200

mg 3,5-xyleneol dissolved in 30 ml ethanol and diluted to a final volume of 1 l with water), and 0.2 ml 9.9 N sulfuric acid. Within 10 min after the sulfuric acid addition, the strongly acid mixture was made weakly acid by addition of 0.2 ml 7.8 N potassium hydroxide (the final pH being about 0.3). The mixture (about 1.5 ml in volume) was extracted with 4 ml of an ether: methanol mixture (9:1 by volume) by 3-min vigorous mechanical shaking, and the two phases were separated by 10 min centrifugation at 1600 × g. The organic phase (3 ml) was transferred to another test tube, and mixed with 0.5 ml of HPLC mobile phase (for composition, see below). The volume was reduced to about 0.5 ml under nitrogen stream with warming at 25°C. Of the residue, 10 to 50 µl was injected into an HPLC.

The HPLC system (Shimadzu, Kyoto, Japan) employed consisted of an HPLC (Model LC-SPD-10AD), connected with an automated liquid sampler (Model SIL-10AXL), a degasser (DGU-14A), a spectrophotometer (Model SPD-10A), a temperature controller for the detector, and a data-processor (Model C-R7A plus). The column (stainless steel; 150 mm in length and 4.6 mm in inner diameter) was packed with Inertsil ODS-3 (φ 5 µm; GL Science, Tokyo, Japan), and was employed for separation at 55°C. A mixture of acetonitrile (100 ml): methanol (24 ml): 60% perchloric acid (0.5 ml): deionized water (to a total volume of 1 l) was introduced to the system as a mobile phase after thorough degassing by ultrasonication. The mobile phase was allowed to flow at 2.0 ml/min, and the absorption of the effluent was monitored at the wavelength of 205 nm; this wavelength was selected taking low background level, high absorption by PMA and clear separation from other peaks into consideration. Care was taken for complete removal of foams in the flow system. Each determination was completed in

Table 1. Number of subjects by exposure, sex and smoking habits

Group	Men			Women			Men + women		
	S ^a	N ^b	Sum	S	N	Sum	S	N	Sum
Benzene-exposed (Benzene conc.)	46	19	65	0	87	87	46	106	152
	(≤92 ppm)			(≤210 ppm)			(≤210 ppm)		
Mixture-exposed (Benzene conc.) (T + X ^c conc.)	17	32	49	0	17	17	17	49	66
	(≤116 ppm)			(≤7 ppm)			(≤116 ppm)		
	(≤118 ppm)			(≤34 ppm)			(≤118 ppm)		
Non-exposed control	29	14	43	2	86	88	31	100	131
Total	92	65	157	2	190	192	94	255	349

Figures show the numbers of subjects. ^aS: Smokers (Consumption; up to 10 cigarettes/day). ^bN: Nonsmokers. ^cT, X: Toluene and xylenes (three isomers in combination), respectively. The concentration (in ppm) ratio of T to X is approximately 5 to 1.

40 min. A typical chromatogram of urinalysis is shown in Fig. 1 in comparison with that of standard PMA.

In some instances, the PMA concentration in urine was adjusted for creatinine concentration (to be abbreviated as cr^{28}) or a specific gravity of urine of 1.016 (sg^{29}). Creatinine concentration and specific gravity were measured by colorimetry and refractometry, respectively.

Reagents

D,L-phenylmercapturic acid was purchased from Tokyo Kasei Chemicals (Tokyo, Japan). Acetonitrile and methanol (both of HPLC grade) were from Junsei Chemicals (Tokyo, Japan), and perchloric acid (fine analysis grade) was from Wako Pure Chemicals (Osaka, Japan).

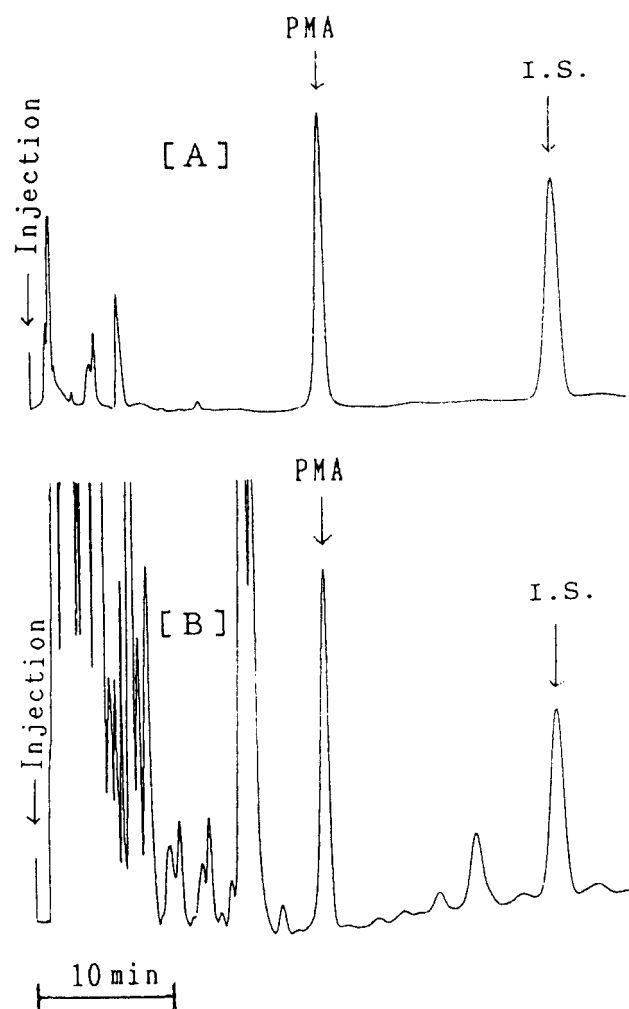


Fig. 1. HPLC chromatograms of [A] authentic phenylmercapturic acid (PMA; 10 mg/l) and [B] a urine sample from a woman exposed to benzene at 67 ppm, in which 12.9 mg/l PMA was detected.

3,5-Xylenol was added as an internal standard (I.S.) at 20 mg/l in both analyses.

Statistical analysis

A PC software STAT-VIEW was employed for multiple and simple regression analyses. AM, ASD, GM and GSD stand for arithmetic mean, arithmetic standard deviation, geometric mean, and geometric standard deviation, respectively.

Results

Analytical conditions for PMA determination

Benzene absorbed is known to be excreted into urine partly as prephenylmercapturic acid or pre-PMA, [N-acetyl-S(1,2-dihydro-2-hydroxyphenyl)-L-cysteine], as a precursor of phenylmercapturic acid³⁰, and it is necessary to pretreat the urine with a mineral acid for removal of a water moiety so that the precursor is measured as PMA in instrumental analysis^{30, 31}. As the authentic precursor was not commercially available, a mixed sample of urine from 5 men and 5 women exposed to about 100 ppm benzene was taken as a precursor source.

When 0.5 ml urine (after dilution with 0.5 ml water to a volume of 1.0 ml) was mixed with 0.2 ml of 12.2 (i.e., 60%), 9.9, 4.9, 2.5, 1.2 or 0.6 N sulfuric acid and kept at room temperature for 10 min before 0.2 ml of 9.8, 7.8, 3.9, 2.0, 1.0 or 0.5 N potassium hydroxide (KOH), respectively, was added to the mixture, the amount of PMA detected by HPLC analysis was largest (22.8 $\mu\text{g/l}$, to be taken as 100% for comparison) after treatment with 9.9 N sulfuric acid and 7.8 N KOH; the recovery when treated with 12.2 N sulfuric acid was slightly lower (i.e., 94%), and treatment with less concentrated sulfuric acid (4.9 to 0.6 N) was accompanied by even lower recovery (i.e., 89 to 69% in relation to sulfuric acid concentration from 4.9 to 0.6 N: Fig. 2).

When the time after addition of 9.9 N sulfuric acid till that of 7.8 N KOH was varied from 0 (i.e., KOH being added immediately after sulfuric acid addition) 1, 2.5, 4, 5, 10, 30, 60 to 120 min, the prolongation to 30 min and over resulted in slight reduction in recovery as a function of time from 96, 92 to 90%, respectively (10 min value being taken as 100%). In contrast, the time interval of less than 10 min (i.e., 0 to 10 min) did not induce a substantial change in the recovery, suggesting that the conversion at the low pH is immediate and complete, whereas exposure to the low pH for more than 10 min may induce further conversion to unidentified compound(s) (Fig. 3).

In a further experiment to examine the effect of pH on the extraction of PMA, 1 ml of 10 mg/l solution of authentic PMA in water was mixed with 0.2 ml of 17.5 (100%), 1.75, 0.175, 0.0175 and 0.00175 N acetic acid (final pH being

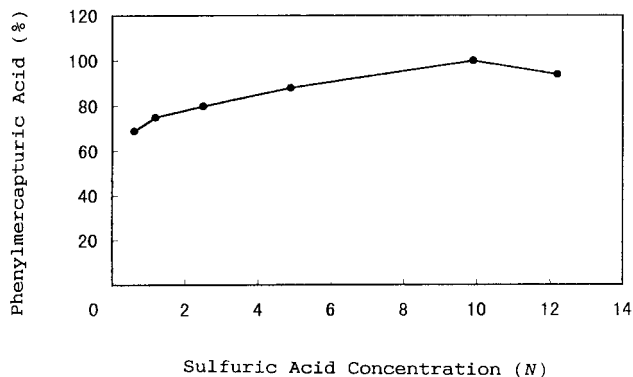


Fig. 2. Effect of sulfuric acid concentration on the detected amount of phenylmercapturic acid.

To a mixture of 0.5 ml urine and 0.5 ml water in a tube, 0.2 ml of sulfuric acid (at the concentration shown in the figure) was added. The tube was allowed to stand for 10 min before 0.2 ml of potassium hydroxide (0.5 to 9.8 N; for details, see text) was added. The amount of phenylmercapturic acid in the tube, analyzed by the standard method as described in the Materials and Methods section, was expressed in percentage taking 22.8 $\mu\text{g/l}$ (the maximum amount detected when 9.9 N sulfuric acid was added) as 100%.

1.2, 1.6, 2.0, 2.4 and 2.7, respectively) and the mixture was subjected to solvent extraction and then HPLC analysis. The recovery taking the amount at pH 1.2 as 100% was 99.7%, 85.9%, 60.4%, and 55.9%, in the order, suggesting that the pH 1.2 or lower appeared to be necessary. When the solvent extraction was repeated at this pH, about 92%, 8% and none (0%) of authentic PMA was recovered in the first, second and third extraction, respectively.

In a subsequent experiment, 0.5 ml each of the urine samples from 5 subjects (exposed to 68 to 153 ppm benzene) was diluted with 0.5 ml water, and then mixed with 0.2 ml 17.5 N acetic acid (the final pH about 1.2). The mixtures (with no KOH addition) was subjected to the solvent extraction and subsequent analysis. In parallel, the same urine-water mixture samples were treated as usual with 9.9 N sulfuric acid and then 7.8 N KOH followed by the HPLC analysis. The comparison in the amount of PMA after acetic acid (followed by no alkali addition) or sulfuric acid - KOH treatment showed that the amount of PMA under the former condition was 14 to 31% (mean 22% with no correlation with intensity of benzene exposure) of what was detected after the latter treatment. The findings suggested that a majority of PMA (78% on an average in a range of 69 to 86%) was excreted in urine as a precursor (possibly as pre-PMA).

When 10 samples each of authentic PMA dissolved in water at final concentrations of 10 and 20 mg/l were analyzed after the standard procedures (for details of the procedures,

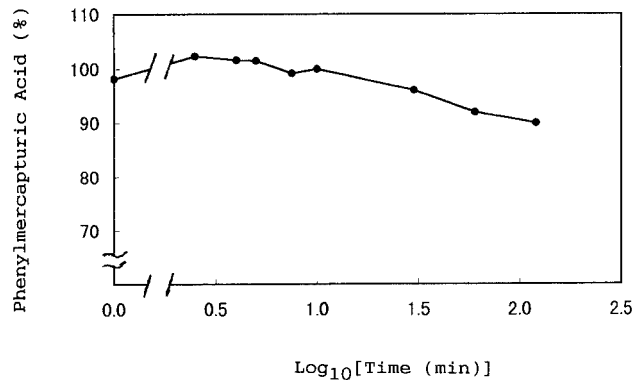


Fig. 3. Effect of time between addition of sulfuric acid and that of potassium hydroxide.

To a mixture of 0.5 ml urine and 0.5 ml water, 0.2 ml 9.9 N sulfuric acid was added. O (i.e., immediately) to 120 min (see the figure for time) after sulfuric acid addition, 0.2 N 7.8 N potassium hydroxide was added to the tube, and the mixture was analyzed for phenylmercapturic acid by the standard method as described in the Materials and Methods section. The amount of phenylmercapturic acid detected was expressed taking the amount detected with 10 min lapse of time (22.8 $\mu\text{g/l}$) as 100%.

see the Materials and Methods section), the accuracy and precision of the method was such that the recovery was 97.5% and 98.5%, respectively (98.0% in total) with standard deviation of 4.7% and 4.5% (4.6% in total); the coefficient of variation was thus 4.8% and 4.5%, or 4.7% in total. The detection limit was 0.02 mg/l when a P/N ratio of 2 was taken.

No detection of PMA in the urine from non-exposed subjects

The standard procedures thus established was applied to urine samples from 131 non-exposed subjects [43 men (including 29 smokers) and 88 women (including 2 smokers)]. None of the urine samples contained PMA above the detection limit of 0.02 mg/l, irrespective of the smoking habits of the individuals.

Possible effects of sex, age and smoking on urinary PMA excretion

In order to have an over-all view on the effects of solvent exposure as well as personal factors such as age, sex and smoking, two exposure groups (i.e., the benzene-exposed and the mixture-exposed) of both sexes were combined (218 men and women), and the combination was subjected to multiple regression analysis, in which sex, age (years), smoking (number of cigarettes consumed per day), and intensities of exposure (ppm) to benzene, toluene and xylenes were taken as independent variables. One of PMAob, PMAcr or PMAsg was taken as a dependent variable.

A preliminary simple regression analysis among the independent variables showed that toluene and xylenes correlated significantly ($r=0.310$, $P<0.01$) with each other whereas benzene was independent to other two aromatics (the absolute value of $r<0.15$, $P>0.10$). Accordingly, the combination of concentrations of toluene and xylenes were introduced as one independent variable, and benzene was taken separately.

The results (Table 2) indicated that benzene was the only independent variable with significant ($P<0.05$) influence; the partial correlation coefficient (PCC) was 0.76 to 0.79 (depending on which one of PMAob, PMAc or PMAsg was taken) so that about 60% of total variation in PMA could be explained by benzene exposure alone. Other variables such as age, sex and exposure to other two aromatics did not contribute significantly ($P>0.10$). It should be added that the PCC for smoking was negative (i.e., below zero) although statistically insignificant.

Quantitative correlation between urinary PMA and benzene exposure

Benzene exposure-dependent increase in urinary PMA was examined to compare the quantitative relationship among benzene-exposed workers with that among mixture-exposed workers. Because no effects of age and smoking were detected in the multiple regression analysis, both smokers and non-smokers were combined, and subjects of various ages were treated together. Cases of men and women were calculated separately and also in combination. The calculation was carried out also for the combination of the exposed and the controls, i.e., the benzene + control group, and the mixture + control group.

In the benzene-exposed group, the regression was significant ($P<0.01$) in men, in women and also in men+women (the top half in Table 3); no significant ($P>0.10$) difference was observed between the two regression lines for men and women. It should be noted that the correction for urine density did not give substantial increase in the correlation coefficients. Table 3 further shows that the inclusion of the non-exposed controls did not induce changes in the regression lines. The regression of PMAob, PMAc or PMAsg with benzene exposure among the benzene + control group is depicted in Fig. 4 for visual understanding of the correlation.

In the mixture group (the bottom half in Table 3), only 17 women were available and the intensity of the exposure to benzene was also low (up to 7 ppm; Table 1). These limitations most probably were responsible for small and insignificant correlation coefficients (cases marked with #

Table 2. Influential independent variable in multiple regression analysis

Dependent variable ^a	Independent variable		
	Item	(PCC ^b , P)	R ²
PMAob	Benzene	(0.762, <0.01)	0.581
PMAc	Benzene	(0.793, <0.01)	0.629
PMAsg	Benzene	(0.769, <0.01)	0.591

Only the independent variable with significant ($P<0.10$) influence is shown among the independent variables of sex, age, cigarette consumption and concentrations of benzene and toluene+xylenes. ^aPMAob, PMAc and PMAsg: Phenylmercapturic acid as observed (mg/l), corrected for creatinine (mg/g creatinine) or corrected for a specific gravity of 1.016 (mg/l) respectively.

^bPCC: Partial correlation coefficient.

in Table 3). In men, in contrast, the number of subjects and exposure concentrations were comparable between the benzene group (65 men exposed up to 92 ppm benzene; Table 1) and in the mixture group (49 men exposed up to 116 ppm benzene). Accordingly, men in the benzene group and in the mixture group were considered appropriate to examine possible effect of co-exposure to toluene and xylenes on metabolism of benzene to PMA. Comparison of three (i.e., with PMAob, PMAc and PMAsg) pairs of regression lines for the men showed that the line for the mixture group did not differ significantly ($P>0.05$) from that for the benzene group when PMAob was taken as the exposure marker, whereas the slopes tended to be greater ($0.01<P<0.05$) in the mixture group than in the benzene group when PMAc or PMAsg was taken (Fig. 5).

Discussion

The present trial to develop a simple HPLC method for determination of PMA in urine has succeeded to establish a protocol which enables to complete one assay in 40 min without complicated pretreatment such as derivatization or column-based clean-up procedure. Experiences show that one analytical chemist can complete the measurement of about 35 samples per day, which is productive enough for the application to occupational health practice on a routine basis. The fact that the analytical system can be run overnight unattended is an advantage of HPLC analysis over FID-GC.

The present analysis of end-of-shift urine samples from benzene-exposed workers (up to 210 ppm) and non-exposed control subjects for PMA shows that urinary PMA correlates linearly to TWA exposure to benzene (Table 3). The effect of co-exposure to toluene and xylenes on urinary PMA level

Table 3. Correlation of PMA in end-of-shift urine samples with TWA benzene exposure

Group Correction for Sex	No. ^a	Regression parameter ^b		
		α	β	r^c
Benzene				
None				
Men	65 (108)	- 201 (- 72)	67.6 (65.0)	0.677 (0.752)
Women	87 (175)	- 82 (- 23)	65.5 (64.9)	0.699 (0.793)
Men + women	152 (283)	- 130 (- 43)	66.1 (65.0)	0.717 (0.789)
Creatinine concentration				
Men	65 (108)	184 (66)	63.0 (65.3)	0.676 (0.776)
Women	87 (175)	59 (16)	79.7 (80.1)	0.742 (0.831)
Men + women	152 (283)	- 72 (- 24)	78.8 (78.2)	0.758 (0.826)
Specific gravity ^d				
Men	65 (108)	179 (64)	50.6 (52.8)	0.672 (0.774)
Women	87 (175)	303 (84)	53.1 (55.4)	0.696 (0.804)
Men + women	152 (283)	195 (65)	53.6 (55.1)	0.718 (0.803)
Mixture				
None				
Men	49 (92)	- 31 (- 14)	74.3 (74.0)	0.928 (0.937)
Women	17 (105)	186 (9)	11.6 (54.3)	0.111 [#] (0.652)
Men + women	66 (197)	- 22 (- 6)	74.1 (73.8)	0.930 (0.940)
Creatinine concentration				
Men	49 (92)	- 129 (- 59)	97.6 (96.5)	0.868 (0.882)
Women	17 (105)	281 (14)	8.8 (73.2)	0.056 [#] (0.608)
Men + women	66 (197)	- 84 (- 23)	96.9 (95.8)	0.871 (0.887)
Specific gravity				
Men	49 (92)	- 46 (- 21)	71.7 (71.3)	0.908 (0.920)
Women	17 (105)	160 (8)	12.2 (48.8)	0.136 [#] (0.670)
Men + women	66 (197)	- 37 (- 10)	71.5 (71.0)	0.912 (0.924)

Numbers are for the benzene or mixture group. Those in parentheses are for benzene + control or mixture + control groups. ^aNumber of subjects. ^b α and β are parameters of a regression line, $Y = \alpha + \beta X$, where X is TWA benzene (ppm) in breathing zone air and Y is PMA ($\mu\text{g/l}$ or $\mu\text{g/g}$ creatinine) in end-of-shift urine samples. ^c r are correlation coefficients. ^eAll correlation coefficients are statistically significant ($P < 0.01$), except the coefficients for women in the mixture group (those marked with #) for which $P > 0.10$. ^dA specific gravity of 1.016.

was absent when examined by multiple regression analysis (Table 2), and of marginal significance when the regression lines were compared between the benzene exposure group and the mixture exposure group. It is not possible to explain why the effect of co-exposure was of marginal significance when urinary PMA was corrected for urine density although it was absent when PMA was evaluated without any correction for urine density. Nevertheless, the observation as a whole suggests that the effect should be small if present.

Thus, the present survey not only supports the opinion that PMA is a good biomarker of benzene exposure^{8, 9, 15-19}, but has demonstrated that PMA is useful even under the condition of co-exposure to other aromatics and most probably to other solvents in general. The latter observation

suggests that, as a benzene exposure marker, PMA is superior to mono- to tri-hydroxylated benzenes [phenol²⁷], quinol²⁷) and catechol²⁷], 1,2,4-benzenetriol¹¹] and t,t-muconic acid¹³, because the excretion of these urinary benzene metabolites other than PMA are all known to be suppressed by co-exposure to other aromatics. For example, urinary excretion of phenolic metabolites were suppressed by 33 to 60%¹¹, and it was as large as 74% in case of t,t-muconic acid¹³, when metabolite excretion of by workers exposed to benzene and toluene together was compared with the excretion of those exposed to benzene only.

Despite the observation that one cigarette will yields 6 to 73³²) or 50 μg benzene³³) when smoked, no effect of smoking was detected in the present study. This lack of effect may

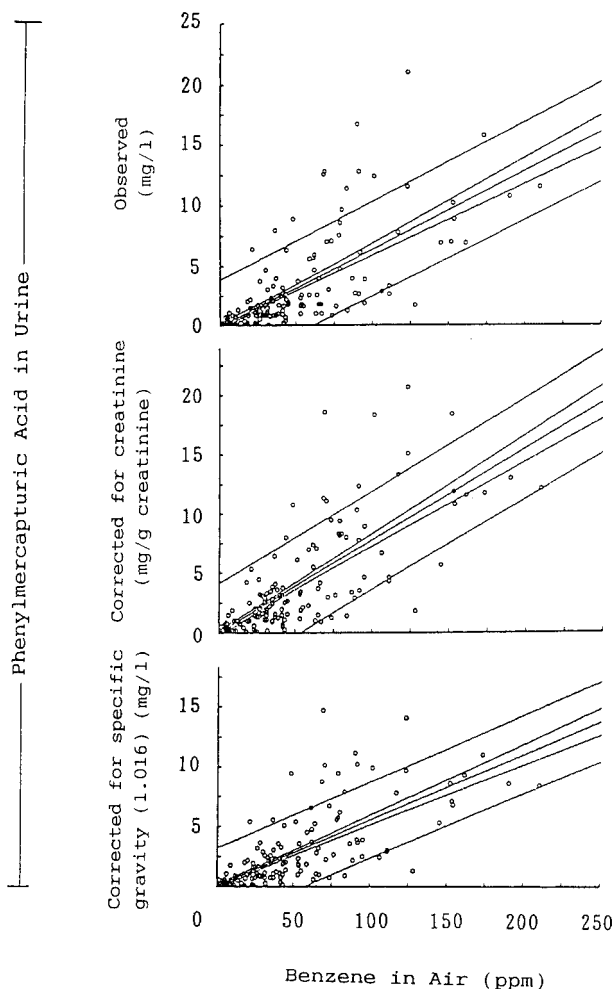


Fig. 4. Scatter diagrams of phenylmercapturic acid levels in end-of-shift urine samples ([A] as observed, [B] after correction for creatinine concentrations, and [C] after correction for creatinine concentrations) against time-weighted average benzene concentrations in breathing zone air.

Each dot shows a case. Lines in the middle are calculated regression lines, and the curves on both sides show the 95% confidence ranges of the means. The outer-most curves show the 95% confidence ranges of the individual values.

probably be due to the fact that the occupational benzene exposure was much higher (up to 210 ppm; Table 1) in the present study than the levels in the cigarette smoke and that there was no real heavy smokers among the subjects studied (i.e., with consumption of up to 20 cigarettes/day). Simple comparison suggests that 8-hour occupational exposure to benzene at 100 ppm (or 320 mg/m^3) will be accompanied by 2.3 g benzene intake via respiration when a respiration rate of 15 l/min [i.e., $100 \text{ ppm (or } 320 \text{ mg/m}^3) \times 15 \text{ l/min} \times 60 \text{ min} \times 8 \text{ hours}$]¹¹ is assumed. By smoking 20 cigarettes/day, the benzene intake will be 1.46 mg ($73 \text{ } \mu\text{g} \times 20$) even

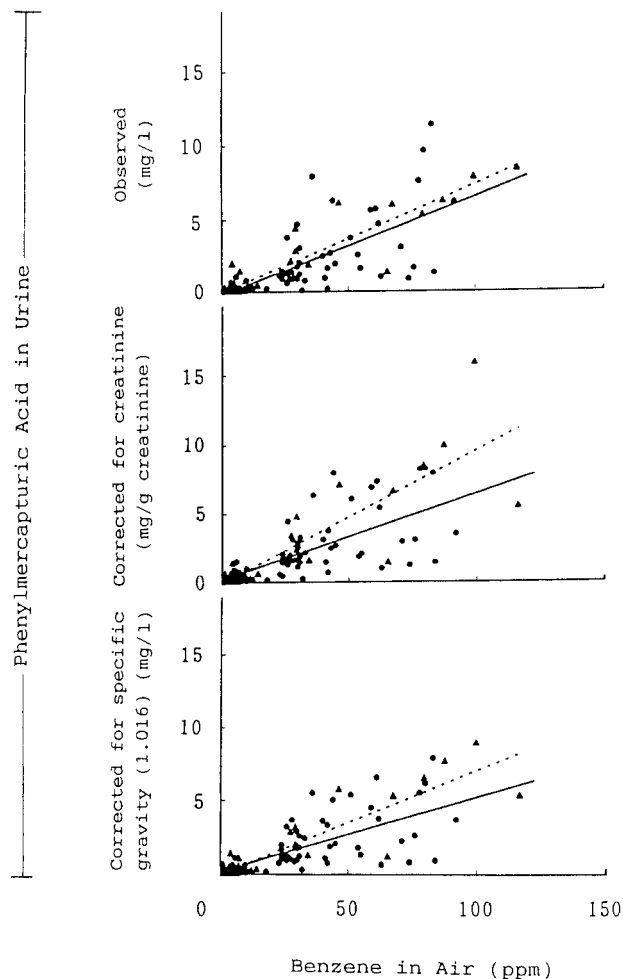


Fig. 5. Comparison of regression between men in the benzene group and men in the mixture group.

Each symbol shows one case, and lines show regression lines. Circles and solid lines are for men in the benzene group, whereas triangles and broken lines are for men in the mixture group. For statistical comparison of the lines, see text.

when the highest benzene yield of $73 \text{ } \mu\text{g/piece}$ is assumed. The latter value is less than 10^{-3} of the former.

It is of interest to make quantitative estimation of relative amount of PMA in the total amount of absorbed benzene. At the end of a hypothetical 8-hour exposure to benzene at 100 ppm, a worker will absorb $2392.5 \text{ } \mu\text{g}$ benzene/min, assuming that the absorption rate in the lungs is 50%¹¹ in addition to the respiration rate of 15 l/min. With an assumption that the rate of urine excretion is 1 ml/min^{11,12}, the observation in the present study (Table 3; observed value for men+women) suggests that the benzene-equivalent amount of PMA to be excreted in urine at the end of a shift with 100 ppm benzene exposure will be $[(64.5 \times 100) - 41.0] \text{ } \mu\text{g/l} \times 10^{-3} \text{ l/min} \times 78.11/239.29 = 2.09 \text{ } \mu\text{g/min}$, where

Table 4. Quantitative correlation of PMA in end-of-shift urine samples with TWA benzene exposure; a literature survey

Ref. ^a	Type of workshops surveyed	Benzene exposure (ppm)	Regression parameter ^b			PMA at 1 ppm benzene
			α	β	r	
8)	Cokery, Garage, etc.	<7.3				12 $\mu\text{g/g cr}^c$
9)	Cokery	<15	- 8.2	66.3	0.99	58 $\mu\text{g/g cr}$
15)	Chemical plants, natural gas refinery, etc.	<2				46 $\mu\text{g/g cr}^c$
16)	Car repair shop	<2.5	4.1	35.1	0.81	39 $\mu\text{g/l}$
17)	Gas prod., Chem. mfg., Oil refinery, etc.	<3.2				47 $\mu\text{g/g cr}$
18)	Gas prod., Chem. mfg., Oil refinery, etc.	<6				44 $\mu\text{g/g cr}$
19)	Chemical plants	<20	1.59 ^d	0.66 ^d	0.63 ^d	39 $\mu\text{g/g cr}$
Present study	Shoe-making (men + women, observed)	<210	- 41	64.5	0.78	24 $\mu\text{g/l}$
	Ibid. (men + women, cr. corr.)		- 19	77.7	0.82	59 $\mu\text{g/g cr}$

^aReferences: ^b α and β are parameters of a regression line, $Y = \alpha + \beta X$, where X is TWA benzene (ppm) in breathing zone air and Y is PMA ($\mu\text{g/l}$) in end-of-shift urine samples; when benzene or PMA concentration was expressed in mg/m^3 or mmol , it was converted to ppm or $\mu\text{g/l}$. r are correlation coefficients; all coefficients are statistically significant ($P < 0.01$).

^cCorrected for creatinine concentration. ^dDouble logarithmic regression. ^eOriginally 6 $\mu\text{g/g cr}$ for 0.5 ppm benzene.

78.11 and 239.29 are the molecular weight of benzene and PMA, respectively. Thus, 0.09% [i.e., $(2.09/2392.5) \times 100\%$] of benzene absorbed will be excreted in urine as PMA. When compared with other urinary metabolites i.e., phenol (13.2%), catechol (1.6%), quinol (10.2%), 1,2,4-benzenetriol (0.5%) and t,t-muconic acid (1.9%)¹¹⁾, the share of PMA is apparently smallest.

Major findings available in literature on quantitative relationship of PMA in end-of-shift urine with TWA benzene exposure^{8, 9, 15–19)} are summarized in Table 4. The reported values for PMA corresponding to 1 ppm benzene cluster around 40 $\mu\text{g/g cr}$ with two extremes of 12 and 58 $\mu\text{g/g cr}$. In making comparison with the present observation (Table 4), it should be noted that the benzene exposure in the present study (up to 210 ppm) is much higher than the levels reported for the populations in literature (up to 20 ppm), and that possibility exists that the metabolism of benzene may vary depending on the exposure intensity³⁴⁾. Nevertheless, it appears likely that there is a general agreement between the present observation and findings available in literature, when urinary PMA level corresponding to 1 ppm benzene exposure is estimated from individual regression equations (the right-most column in Table 4).

Studies are currently in progress in this study group to improve the analytical method for a better sensitivity with a lower detection limit. The goal is to develop a method which is sensitive enough to be applicable to biomonitor workers with low occupational exposure such as less than 1 ppm benzene³⁵⁾.

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