

Evaluation of biomarkers for occupational exposure to benzene

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Abstract

Objective—To evaluate the relations between environmental benzene concentrations and various biomarkers of exposure to benzene.

Methods—Analyses were carried out on environmental air, unmetabolised benzene in urine, *trans, trans*-muconic acid (*ttMA*), and three major phenolic metabolites of benzene; catechol, hydroquinone, and phenol, in two field studies on 64 workers exposed to benzene concentrations from 0.12 to 68 ppm, the time weighted average (TWA). Forty non-exposed subjects were also investigated.

Results—Among the five urinary biomarkers studied, *ttMA* correlated best with environmental benzene concentration (correlation coefficient, $r = 0.87$). When urinary phenolic metabolites were compared with environmental benzene, hydroquinone correlated best with benzene in air. No correlation was found between unmetabolised benzene in urine and environmental benzene concentrations. The correlation coefficients for environmental benzene and end of shift catechol, hydroquinone, and phenol were 0.30, 0.70, and 0.66, respectively. Detailed analysis, however, suggests that urinary phenol was not a specific biomarker for exposure below 5 ppm. In contrast, *ttMA* and hydroquinone seemed to be specific and sensitive even at concentrations of below 1 ppm. Although unmetabolised benzene in urine showed good correlation with atmospheric benzene ($r = 0.50$, $P < 0.05$), data were insufficient to suggest that it is a useful biomarker for exposure to low concentrations of benzene. The results from the present study also showed that both *ttMA* and hydroquinone were able to differentiate the background level found in subjects not occupationally exposed and those exposed to less than 1 ppm of benzene. This suggests that these two biomarkers are useful indices for monitoring low concentrations of benzene. Furthermore, these two metabolites are known to be involved in bone marrow leukaemogenesis, their applications in biological monitoring could thus be important in risk assessment.

Conclusion—The good correlations between *ttMA*, hydroquinone, and atmospheric benzene, even at concentrations of less than 1 ppm, suggest that they are sensitive and specific biomarkers for benzene exposure.

(*Occup Environ Med* 1995;52:528-533)

Key words: biological exposure indices; biomonitoring; risk assessment

Benzene is an important component in petrol, is a constituent of engine emissions, and is widely used in chemical, paint, and dye industries. The evidence for the association between exposure to benzene and leukemogenic effects has been adequately reviewed.¹ Much controversy still continues on what level of exposure to benzene constitutes an acceptable risk.² The European Community benzene directive calls for an action level of 1 ppm benzene and a limit value of 5 ppm time weighted average (TWA).³ With new evidence on the risk of benzene associated with neoplasia, the American Conference of Government Industrial Hygienists (ACGIH) has recently proposed to lower the threshold limit value (TLV) for eight hours of exposure from 1 to 0.1 ppm.⁴ Thus, studies on biological monitoring must consider the need to identify biomarkers that are sensitive and reliable at these low exposure concentrations.

During the past decade, several new approaches have been developed for the determination of benzene and its metabolites in exhaled air, blood, and urine. Although analysis of exhaled breath has been proposed for biological monitoring of exposure to volatile organic solvents⁵ its use has been limited. By measuring exhaled air at the end of exposure, one theoretically can estimate the amount of exposure to benzene. This technique is, however, complex. A major disadvantage is that the results can vary considerably, depending on the type of sampling technique used and the time of sampling.⁶ On the other hand, collection of blood samples for benzene determination is always unwelcome by the workers.

Traditionally, the most common method used for biological monitoring of benzene exposure is based on measurement of a metabolite of benzene, usually urinary phenol. Nevertheless, recent findings have suggested that measurement of phenol is considered unreliable, especially for low benzene exposure.^{6,7} Analytical methods for the determinations of minor phenolic metabolites such as catechol⁸ and hydroquinone⁹, and unmetabolised urinary benzene^{10,11} have also been developed recently to monitor exposure to benzene. Most of these methods, however, have not been validated, especially those related to low levels of environmental exposure.

S-phenylmercapturic acid (SPMA) has

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Accepted 11 April 1995

been shown recently to be more specific and sensitive than phenol for the estimation of exposure.¹² For SPMA determination, however, sophisticated gas chromatography-mass spectrophotometry (GC-MS) is required for adequate sensitivity; this is some way from being an efficient technique for use as a biomarker for routine monitoring. *Trans, trans*-muconic acid (*tt*MA), a metabolite of *trans, trans*-mucoaldehyde has also been proposed as a biomarker for exposure to benzene.^{13,14} Nevertheless, its use for low level benzene exposure has not been studied extensively.

If an exposure limit has to be considered, the most appropriate biological exposure index has first to be validated before it can be widely used. Our study was thus carried out to fulfill this objective. In this paper we report the results of two comprehensive studies designed to evaluate various biomarkers of benzene exposure under field conditions. Unmetabolised benzene, *tt*MA, and three phenolic metabolites—catechol, hydroquinone, and phenol—were analysed on 64 workers exposed to a relatively wide range of benzene concentrations.

Materials and methods

SUBJECTS

The first field study was conducted in Kuching, east Malaysia in 1992. Urine samples were collected from nine car mechanics and 13 pump attendants from three busy petrol kiosks. Preliminary environmental monitoring indicated that the exposure was generally below 1 ppm. The second field study was carried out in Qunshan, China, in 1994. Forty two workers in a shoe manufacturing plant participated in this investigation. Workers in this factory used benzene as a diluent for adhesive. The exposure level is generally above 1 ppm, occasionally above 100 ppm. The other solvents used were monochlorobenzene and toluene. The atmospheric concentrations of these two compounds were low, normally below 1 ppm.

None of the workers in the shoe factory were smokers, whereas five of the workers in the first field study were light smokers (< five cigarettes daily). These workers were advised not to smoke on the day of sample collection. Workers with known heart, lung, liver, kidney, or any other chronic diseases were excluded. None of the subjects drank alcohol on a regular basis. It was also noted that no respirators were used by any of the workers in both studies.

Both environmental and biological monitoring were carried out in the middle of the week. Forty non-smokers who had no history of solvent exposure were used for evaluation of the analytical methods. Most of the non-exposed subjects were hospital staff or graduate students in the medical school.

ENVIRONMENTAL AIR SAMPLES

Individual exposure to benzene at the workplace was monitored with a 3M organic vapour monitor (model 3500) throughout the

whole work shift of eight hours. The diffusive samplers were attached to the collar or shirt pocket of the workers before they entered the plant. The samplers were detached at the end of the shift and stored at -4°C until analysis. Determination of the benzene in the dosimeter was carried out within one week. An autosampler was used together with an integrator for the gas chromatographic determination of benzene. The injection volume was $2\ \mu\text{l}$.

DETERMINATION OF CATECHOL, HYDROQUINONE, AND PHENOL IN URINE

Urine samples were collected in polycarbonate bottles at the end of the workshift and stored at -4°C . Determination of phenolic compounds in urine was carried out according to the method of Lee *et al*⁹ with high performance liquid chromatography (HPLC) and variable wavelength fluorometric detection. Hydroquinone, catechol, and phenol were detected at 2.9, 6.8, and 13.6 minutes, respectively.

The results are presented after correction for creatinine concentration (mg/g creatinine).¹⁵ The creatinine was measured by an Abbott autoanalyser based on Jaffe's method.

DETERMINATION OF *TRANS, TRANS*-MUCONIC ACID

Measurement of *tt*MA was carried out according to an HPLC method recently developed in our laboratory.¹³ The *tt*MA was detected at 10.2 minutes with an ultra violet/visible spectrophotometer at wavelength 265 nm.

DETERMINATION OF BENZENE IN URINE

The analysis of unmetabolised benzene in urine was carried out according to the method of Kok and Ong.¹¹ For the analysis, 1 ml of urine in a headspace vial containing chlorobenzene as an internal standard was incubated at 60°C for 30 minutes and 0.5 ml headspace gas was used for gas chromatographic analysis. Benzene in urine was detected at 2.5 minutes with a silicone gum capillary column and a photoionisation detector.

STATISTICAL ANALYSIS

Statistical analysis was performed with the statistical package for sociological studies (SPSS) package (window version) on an IBM compatible personal computer. Earlier studies have indicated that there is no sex difference in the biotransformation of benzene.^{8,13} The data from male and female subjects in the study were thus analysed together.

Results

Table 1 shows the geometric mean (GM), SD, and ranges of the various biological measurements for 64 exposed and 40 non-exposed subjects. The exposed groups are categorised into low exposure (< 1 ppm); intermediate exposure (1–5 ppm) and high exposure (>5 ppm). The data show that for

Table 1 Geometric means (GSDs) and ranges of various biomarkers in urine from 40 non-exposed people and 64 exposed workers.

Exposure	Catechol (mg/g creatinine)	Hydroquinone (mg/g creatinine)	Phenol (mg/g creatinine)	<i>tt</i> MA (mg/g creatinine)	Benzene (nmol/l)
Non-exposed (n=40)	1.52 (1.2) (0.25-4.78)	0.32 (0.4) (0.04-1.90)	4.8 (9.8) (3.9-26.3)	0.11 (0.07) (0.01-0.29)	1.49 (2.69) (0.64-10.42)
Low-exposure (n=26) <1ppm benzene in air	1.71 (2.02) (0.47-10.86)	0.64 (0.34) (0.16-1.58)	9.3 (11.6) (4.21-21.25)	0.36 (0.22) (0.09-1.56)	— —
Intermediate exposure (n=27) 1-5 ppm benzene in air	1.86 (1.88) (0.15-7.74)	0.89 (1.01) (0.06-4.14)	21.4 (15.1) (2.64-66.3)	4.59 (5.9) (1.49-24.87)	61.6 (68) (6.9-696)
High-exposure (n=11) >5 ppm benzene in air	3.98 (2.03) (1.50-9.74)	2.95 (1.90) (1.23-6.90)	46.7 (24.7) (22.8-101.5)	20.89 (11.3) (4.73-47.6)	138.0 (280) (6.7-757)

most of the biomarkers studied there is an increase in urine metabolite with an increase in benzene exposure. Significant differences were noted between non-exposed subjects and workers exposed to low concentrations of benzene for *tt*MA and hydroquinone (table 1). This suggests that these two biomarkers were able to differentiate the background level of exposure and those exposed to concentrations of around 1 ppm.

The relations between benzene concentration in breathing zone air and various biomarkers studied were examined by pooling the data from both field investigations. Statistical analyses were performed with consideration of the highly skewed distribution of variables and environmental benzene concentrations, correlations were therefore carried out by logarithmic transformation of the data. Correction for urinary creatinine seemed to improve the correlations with breathing zone air for all urinary metabolites. Data were thus analysed with values corrected for urinary creatinine.

Figures 1 to 5 show the scatter diagrams between benzene concentrations in air and various biomarkers of exposure. It was noted that there were significant associations between all of the urinary biomarkers and benzene in breathing zone air, except urinary catechol (fig 2). The best correlation with

breathing zone air was *tt*MA (fig 4, $r = 0.87$) followed by urinary hydroquinone (fig 3, $r = 0.70$). Figure 5 shows the correlation coefficient between benzene concentrations in the breathing zone air and unmetabolised benzene in urine collected at the end of the shift. Although data were not available for low concentrations of exposure to benzene, it is noteworthy that there was a significant correlation ($r = 0.50$, $P < 0.01$) between these two exposure variables.

When the data were analysed according to the concentrations of benzene exposure the correlations were higher for those workers who were more intensively exposed (table 2). The results indicate that the phenolic metabolites did not seem to show significant correlations with atmospheric benzene at low levels of exposure, except hydroquinone. Significant correlation for urinary phenol was found ($r = 0.67$, $P < 0.05$) only when the environmental benzene concentration exceeded 5 ppm. No correlation could be found between catechol in urine and benzene exposure at any level of exposure. In contrast, *tt*MA was the only biomarker that showed significant correlation ($P < 0.001$) with environmental benzene for all categories of exposure (table 2).

There seems to be a good correlation ($r = 0.70$) between unmetabolised benzene in

Figure 1 Relation between urinary phenol and breathing zone air. Points are individual values.

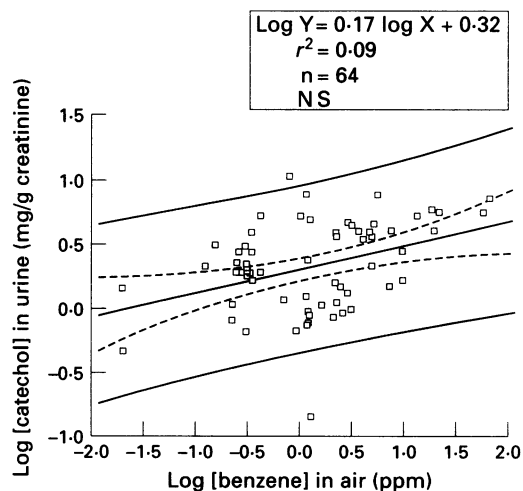
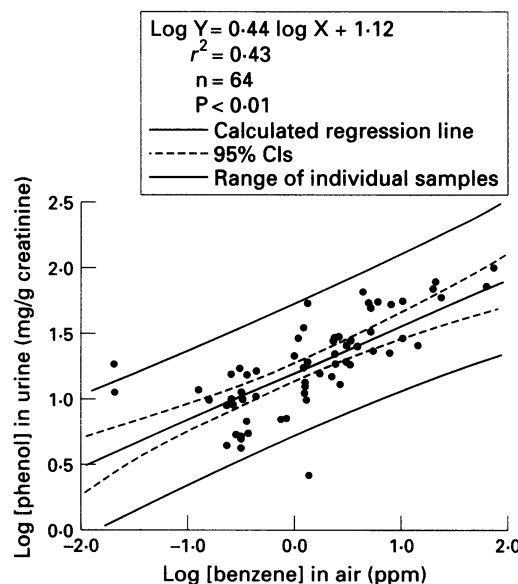


Figure 2 Relation between urinary catechol and breathing zone air.

Figure 3 Relation between urinary hydroquinone and breathing zone air.

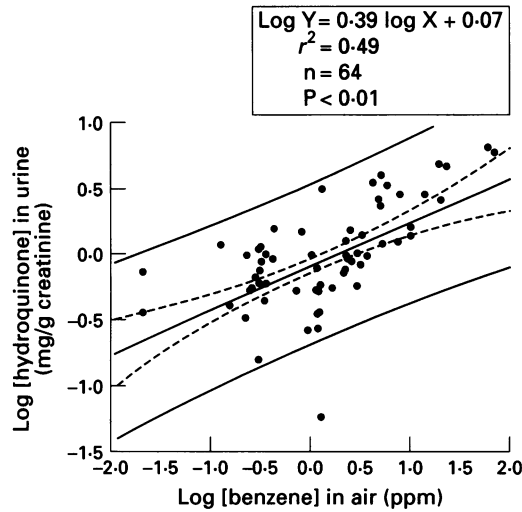
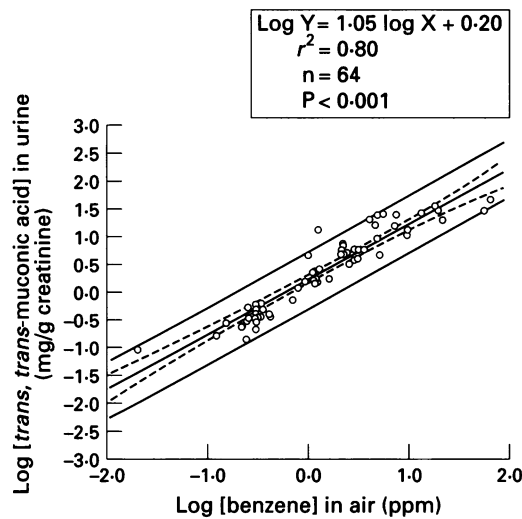


Figure 4 Relation between urinary trans, trans-muconic acid and breathing zone air.



urine and benzene in air for exposure concentrations from 1–5 ppm (table 2). No such correlation was found for higher exposures. Correction for urinary creatinine concentration lessened the correlations with the determinants (data not shown). This finding is in agreement with several earlier investigations. It is thought that adjustment for creatinine

Table 2 Correlations between various biomarkers in urine and environmental benzene for different levels of exposure.

Parameters correlated	Correlation coefficient (r)	Slope	Intercept	P value
Low exposure, <1 ppm (n = 26):				
Log CAT in urine v log BZ in air	0.10	0.23	0.44	NS
Log HQ in urine v log BZ in air	0.25	0.07	-0.15	<0.05
Log PH in urine v log BZ in air	0.14	-0.9	0.90	NS
Log tMA in urine v log BZ in air	0.82	0.71	-0.40	< 0.001
Log BZ in urine v log BZ in air	—	—	—	—
Intermediate exposure, 1–5 ppm (n = 27):				
Log CAT in urine v log BZ in air	0.24	0.48	0.10	NS
Log HQ in urine v log BZ in air	0.64	1.08	-0.40	< 0.05
Log PH in urine v log BZ in air	0.50	0.63	1.12	NS
Log tMA in urine v log BZ in air	0.75	1.14	-0.29	< 0.001
Log BZ in urine v log BZ in air	0.70	1.79	1.12	<0.01
High exposure, >5 ppm (n = 11):				
Log CAT in urine v log BZ in air	0.46	0.31	0.26	NS
Log HQ in urine v log BZ in air	0.67	0.47	-0.6	=0.01
Log PH in urine v log BZ in air	0.70	0.42	1.19	< 0.05
Log tMA in urine v log BZ in air	0.76	0.43	-0.86	< 0.001
Log BZ in urine v log BZ in air	0.22	0.35	1.74	NS

BZ = benzene; CAT = catechol; HQ = hydroquinone; PH = phenol; tMA = trans, trans-muconic acid.

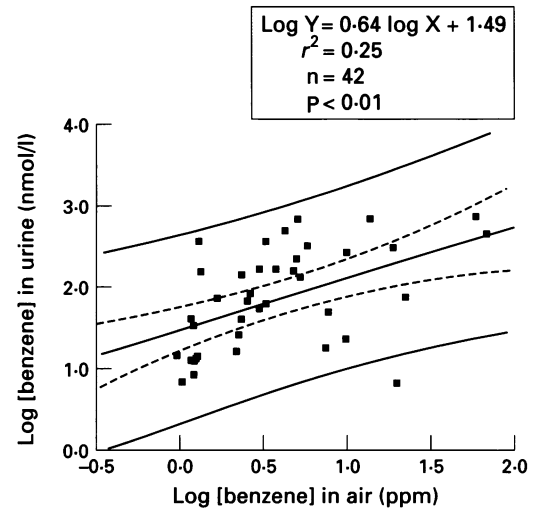


Figure 5 Relation between unmetabolised urinary benzene and breathing zone air. Study was not carried out on workers in east Malaysia.

excretion is not relevant for unmetabolised volatile compounds, as they are eliminated by tubular diffusion rather than glomerular filtration.¹⁶

Discussion

The growing concern about benzene exposure and its effect on health has led to a call for more studies to be conducted to identify appropriate biomarkers for low level exposure. The present study aimed to evaluate the relations between exposure and various recently developed biomarkers so that a biological exposure index corresponding to exposure can be estimated. Evaluations were performed on unmetabolised benzene in urine, tMA, and three major phenolic metabolites of benzene.

PHENOLIC METABOLITES AS BIOMARKERS

The metabolic fate of benzene has been well characterised. Over 90% of inhaled benzene is absorbed by the lungs and undergoes biotransformation to benzene oxide. This active metabolite is subsequently metabolised to several phenolic products—phenol (50%), catechol (up to 25%) and hydroquinone (5%). The main metabolite, phenol is by far the most widely investigated and well documented as a biological monitor of benzene. The determination of phenol has evolved from the classic spectrophotometric method to the more sensitive capillary gas chromatography and reversed phase high performance liquid chromatography (RP-HPLC). The early methods failed to distinguish between phenol, catechol, hydroquinone, and various isomers of cresol, and hence lacked specificity.⁶ Better correlations have been found in recent studies with the chromatographic method of determination.⁷ In the present study HPLC was used for determination of phenol in urine, the results showed that although there was a good correlation between phenol and environmental benzene, this variable was not specific

for concentrations below 5 ppm (tables 1 and 2). For non-occupationally exposed people, the concentration of phenol usually does not exceed 20 mg/g creatinine (table 1), however, in some cases the values may reach 30 mg/g creatinine.⁶ Monitoring for an occupational exposure limit of benzene in air for a TWA of 1 ppm as stipulated by ACGIH¹⁷ is thus limited by the inadequate specificity.

Determination of catechol in urine may be considered as complementary to phenol in urine as catechol is a metabolite derived from phenol through further oxidation. In a recent study with HPLC measurement of catechols, a significant correlation was found between urinary catechol and environmental benzene exposure ($r = 0.85$).⁸ Most of the subjects in the study were exposed to high concentrations of benzene (up to 200 ppm) and the correlation below 10 ppm of exposure was not assessed. In the present study, we were unable to show any significant correlation between urinary catechol and breathing zone air for all concentrations of benzene exposure (fig 2 and table 2). Furthermore, this biomarker was unable to distinguish exposed from unexposed subjects (table 1). Its use as a biomarker for low concentrations of benzene exposure is thus limited by its sensitivity.

Very few studies have been conducted to examine the relation between urinary hydroquinone and exposure to benzene. An earlier study showed that there was a good correlation ($r = 0.81$) between exposure to benzene and excretion of urinary hydroquinone in a group of workers exposed to high concentrations of benzene.³ The present study shows that even at low levels of exposure there was still a close correlation between benzene exposure and urinary hydroquinone ($P < 0.05$, table 2). Data in table 1 also show that this biomarker was able to differentiate those exposed to less than 1 ppm of benzene from those without exposure ($P < 0.01$).

TRANS, TRANS-MUCONIC ACID AS A BIOMARKER

It has been estimated that less than 2% of the absorbed benzene is eliminated as *tt*MA in urine. It is well established, however, that the only significant formation of *tt*MA in the body is through the metabolism of benzene; thus it is specific to benzene exposure.¹⁸ In our earlier study, *tt*MA was also shown to be very reliable and has the ability to detect background levels of benzene exposure in the general population.¹⁹ The results here further show that among all the metabolites studied, *tt*MA correlated best with atmospheric benzene. Tables

1 and 2 show that *tt*MA is sensitive and specific for low benzene exposures, it is still feasible even if the benzene concentration is below the current exposure limit of 1 ppm. These findings suggest that it has a promising role in the biological monitoring of benzene for environmental as well as occupational exposure.

Based on the present data, with a calculated exposure of 1 ppm, about 1.7 mg of *tt*MA/g of creatinine was expected at the end of the workshift. This value is in good agreement with the preliminary finding of 1.4 mg/g creatinine reported by Lauwery *et al* recently from 38 garage and coke oven workers.²⁰

UNMETABOLISED BENZENE IN URINE AS A BIOMARKER

As benzene is not usually present in the urine, the measurement of this compound tends to be specific. Although not many studies have been conducted on measurement of unmetabolised benzene in urine, good correlations between urinary benzene concentrations and environmental exposure have been reported.^{10,11} Furthermore, laboratory analysis of unmetabolised benzene in urine is relatively easy and sensitive with a photoionisation detector, making the determinations of benzene in urine a suitable method for biological monitoring. The data here also showed that there was a significant correlation ($r = 0.50$, $P < 0.01$, fig 5) between unmetabolised benzene in urine collected at the end of the workshift and environmental benzene. Unfortunately, data were insufficient for evaluating benzene exposure of less than 1 ppm. It is important to point out the fact that just like other volatile organic solvents, only a very small proportion of absorbed benzene will not be metabolised and excreted in the urine. For low concentrations of exposure, the sensitivities tend to depend much on the method of detection. The use of unmetabolised benzene in the urine for routine monitoring of low exposures requires further study.

EVALUATION OF VARIOUS BIOMARKERS

The ideal biomarker for benzene exposure should be specific, available for analysis with non-invasive techniques, detectable in trace concentration and most importantly quantitatively related to the degree of exposure. Measurement of benzene or its metabolites in urine is usually preferred as the procedure is non-invasive and is thus suitable for routine monitoring. In the present study the data collected for various urinary biomarkers covered a relatively wide range of exposure, it is thus possible to make a quantitative estimation between benzene exposure and urinary concentration of each biomarker. Table 3 summarises these results. For urinary phenol, a urinary concentration of 25.9 mg/g creatinine would correspond to 5 ppm of benzene in air. This value is in line with the BEI recommended value of 50 mg/g creatinine for exposure of 10 ppm.¹⁷

The overall results from the present investigations indicated that among the five

Table 3 Estimated concentrations of various biomarkers in urine in subjects exposed to 5 ppm benzene for eight hours

Biomarker	Estimation at 5 ppm (95% CIs)	r ² (adjusted)
Catechol (mg/g creatinine)	2.79 (0.62-12.5)	0.09
Hydroquinone (mg/g creatinine)	1.55 (0.38-6.2)	0.49
Phenol (mg/g creatinine)	29.51 (9.4-91.2)	0.43
Trans, trans-muconic acid (mg/g creatinine)	8.51 (2.71-25.7)	0.80
Benzene (μmol/l)	89.1 (6.95-1122.0)	0.25

biomarkers *tt*MA correlated best with environmental benzene. Hydroquinone also showed significant correlation with benzene exposure even at concentrations of below 1 ppm. They can thus be used as biomarkers for low concentrations of exposure to benzene. It is worthwhile to point out that the application of these two biomarkers for biological monitoring could be of relevance to health, particularly in predicting the effect induced by benzene. Recent studies have shown that *trans*, *trans*-mucoaldehyde, a precursor of *tt*MA, and hydroquinone are highly myelotoxic.^{21,22} In particular, hydroquinone is known to be DNA reactive and is a potential inducer of aneuploidy in cultured human lymphocytes.²³ This compound was found to be seven to nine times more effective than other benzene metabolites at inducing DNA adducts.²⁴ Recent findings from our laboratory also indicated that hydroquinone was the most toxic among the benzene metabolites in killing cells as well as in inducing DNA strand breakage.²⁵ On the other hand, it has also become increasingly clear that the effect of benzene on bone marrow is conferred by a combination of active metabolites. Snyder *et al* reported that when hydroquinone and *trans*, *trans*-mucoaldehyde were given together they were most effective in inhibiting the production of erythrocytes.²⁶ These findings need to be recognised when biomarkers are used in risk assessment. The use of *tt*MA and hydroquinone as biomarkers will thus be of significance as they would reflect better the biologically active components of benzene metabolites.

The conditions and concentrations of exposure to benzene found in the present study are considered to be representative of other chemical industries that use benzene; nevertheless, possible interference by co-exposure to other organic solvents or due to skin exposure has not been investigated, further studies are thus needed in this area. Our earlier studies have shown that cigarette smoking was associated with higher concentrations of benzene metabolites in the urine.^{13,19} In depth studies will also be required to evaluate the potential influence of cigarette smoking on these biomarkers.

Conclusion

In this paper we report our findings on the evaluation of five biomarkers that have been suggested for biological monitoring of benzene exposure. The results suggest that measurement of *tt*MA in urine is the most reliable biomarker for monitoring of benzene exposure from 0.12 to over 68 ppm. Among the three phenolic metabolites of benzene, hydroquinone seems to be more reliable than catechol and phenol. With the decrease in environmental exposure and the low specificity of urinary phenol and catechol, it is expected that determination of these two metabolites would become less relevant.

We are grateful for the participation of the workers and the cooperation of the local health authorities in the People's Republic of China and the management in both factories. This work was in part supported by the National University of Singapore Grant No 920387. We thank Professors Shouzhun Xue and Bingen Tao of Shanghai Medical University and Nanjing University for their assistance.

- 1 Yardley-Jones A, Anderson D, Parke D. The toxicity of benzene and its metabolism and molecular pathology in human risk assessment. *Brit J Ind Med* 1991; 48:437-44.
- 2 Rinsky RA. Benzene and leukemia: An epidemiologic risk assessment. *Environ Health Perspect* 1989;82:189-92.
- 3 Environmental Health Executive. *Occupational exposure limits*. London: HMSO, 1994. (EH40/94).
- 4 American Conference of Governmental Industrial Hygienists. Notice of intended changes—Benzene. *Applied Occupational and Environmental Hygiene* 1990; 5:453-9.
- 5 Perbellini L, Faccini GB, Pasini F, Cazzoli F, Pistoia S, Rosellini R, Valsecchi M, Brugnone F. Environmental and occupational exposure to benzene by analysis of breath and blood. *Br J Ind Med* 1988;45:345-52.
- 6 Ong CN, Lee BL. Determination of benzene and its metabolites: Application in biological monitoring of environmental and occupational exposure to benzene. *J Chromatogr* 1994;660:1-22.
- 7 Ducos P, Gaudin R, Bel J, Maire C, Francin JM, Robert A, Wild P. *Trans*, *trans*-muconic acid, a reliable biological indicator for the detection of individual benzene exposure down to the ppm level. *Int Arch Occup Environ Health* 1992;64:309-13.
- 8 Inoue O, Seiji K, Kasahara M, Nakatsuka H, Watanabe T, Yin SG, *et al*. Determination of catechol and quinol in the urine of workers exposed to benzene. *Br J Ind Med* 1988;45:487-92.
- 9 Lee BL, Ong HY, Shi CY, Ong CN. Simultaneous determination of hydroquinone, catechol and phenol in urine using high-performance liquid chromatography with fluorimetric detection. *J Chromatogr* 1993;619:259-66.
- 10 Ghittori S, Fiorentino ML, Maestri L, Cordioli G, Imbriani M, Urinary excretion of unmetabolized benzene as an indicator of benzene exposure. *J Toxicol Environ Health* 1993;38: 233-43.
- 11 Kok PW, Ong CN. Blood and urinary benzene determined by headspace gas chromatography with photoionization detection. *Int Arch Occup Environ Health* 1994, 66:195-201.
- 12 van Sittert NJ, Boogaard PJ, Beulink GDJ. Application of the urinary S-phenylmercapturic acid test as a biomarker for low levels of exposure to benzene in industry. *Br J Ind Med* 1993;50:460-9.
- 13 Lee BL, New AL, Kok PW, Ong HY, Shi CY, Ong CN. Urinary *trans*, *trans*-muconic acid determined by liquid chromatography: application in biological monitoring of benzene exposure. *Clin Chem* 1993;39:1788-92.
- 14 Bechtold WE, Henderson RF. Biomarkers of human exposure to benzene. *J Toxicol Environ Health* 1993; 40:377-386.
- 15 Ong CN, Lee BL, Foo SC. Specific gravity adjustment for urinary analysis. *Am Ind Hyg Assoc J* 1985;46:10-11.
- 16 Boeniger MF, Lowry LK, Rosenberg J. Interpretation of urine results used to assess chemical exposure with emphasis on creatinine adjustment: a review. *Am Ind Hyg Assoc J* 1993;54:615-27.
- 17 American Conference of Governmental Industrial Hygienists. *Threshold limit values and biological exposure indices for 1994*. Cincinnati, Ohio: 1993.
- 18 Johnson ES, Lucier G. Perspectives on risk assessment impact of recent reports on benzene. *Am J Ind Med* 1992;21:749-57.
- 19 Ong CN, Lee BL, Shi CY, Ong HY, Lee HP. Elevated levels of benzene-related compounds in the urine of cigarette smokers. *Int J Cancer* 1994;59:177-80.
- 20 Lauwerys RR, Buchet JP, Andrien F. Muconic acid in urine: a reliable indicator of occupational exposure to benzene. *Am J Ind Med* 1994;15:297-300.
- 21 Leavey G, Bodell WJ. Potentiation of DNA adduct formation in HL-60 cells by combination of benzene metabolites. *Proc Natl Acad Sci USA* 1992;89:7105-09.
- 22 Witz G, Gad SC, Tice RR, Oshiro Y, Piper CE, Goldstein BD. Genetic toxicity of the benzene metabolites *trans*, *trans*-mucoaldehyde in mammalian and bacterial cells. *Mutat Res* 1990;240:295-306.
- 23 Irons RD, Stillman WS, Colagiovanni DB, Henry VA. Synergistic action of the benzene metabolite hydroquinone on myelopoietic stimulating activity of granulocyte/macrophage colony-stimulating factor *in vitro*. *Proc Natl Acad Sci USA* 1992;89:3691-5.
- 24 Kochalana P, Subrahmanyam VV, Meyer KB, Zhang L, Smith MT. Benzene and its phenolic metabolites produce oxidative DNA damage in HL60 cells *in vitro* and in bone marrow *in vivo*. *Cancer Res* 1993;53:1023-6.
- 25 Sze CC, Shi CY, Ong CN. Cytotoxicity and DNA strand breaks induced by benzene and its metabolites in chinese hamster ovary cells. *J Appl Toxicol* 1995 (in press).
- 26 Snyder R, Dimitriadis E, Guy R, Hu P, Cooper K, Bauer H, *et al*. Studies on the mechanism of benzene toxicity. *Environ Health Perspect* 1989;82:31-5.