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# Unmetabolized VOCs in Urine as Biomarkers of Low Level Exposure in Indoor Environments

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**Abstract: Unmetabolized VOCs in Urine as Biomarkers of Low Level Exposure in Indoor Environments: Bing-Ling WANG, *et al*. Department of Public Health, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences**—This study aimed to test the possible use of unmetabolized volatile organic compounds (VOCs) in urine as biomarkers of low-level indoor environmental exposure. Twenty-four subjects in 13 dwellings in a prefecture of Japan participated in this study. Air samples of the breathing zone were collected in the living room and bedroom, along with spot urine samples (before bedtime and first morning voids). Toluene, ethylbenzene, xylene isomers, styrene and *p*-dichlorobenzene in the air and urine samples were measured by gas chromatography/mass spectrometry. For the 21 subjects without solvent exposure at work, there were significant correlations between the time-weighted average air concentrations in the bedroom and morning urinary concentrations for toluene, *o*-xylene, total xylene and *p*-dichlorobenzene (correlation coefficients of 0.54, 0.61, 0.56 and 0.84, respectively). Multiple linear regression analysis showed only air VOCs in the bedroom influenced the morning urinary VOC concentrations. We concluded that unmetabolized VOCs in the urine can provide a reliable biological indicator for air VOC exposures in non-occupational environments.
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**Key words:** Volatile organic compounds (VOCs), Biological monitoring, Indoor environment, Xylene, Toluene, *p*-dichlorobenzene, Urine

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Exposure to high concentrations of volatile organic compounds (VOCs) can lead to adverse health effects, including acute and chronic respiratory effects, plus eye and throat irritation[1–5]. In Japan, toluene, ethylbenzene, xylenes, styrene and *p*-dichlorobenzene are often detected in higher concentrations inside residential buildings than outside. As a consequence, guidelines for these VOCs were established by the Japanese Ministry of Health, Labour, and Welfare (MHLW) in 2000[6, 7]. Some adverse effects such as the symptoms associated with sick building syndrome, however, were found to be related to VOCs at much lower concentrations than their guideline values[8].

Air concentrations and urinary biomarkers have been widely used to evaluate exposure to VOCs in occupational environments[9, 10]. However, there have been few assessments of airborne chemical exposure using urinary biomarkers in indoor non-occupational environments. This is partly because of the poor sensitivity and specificity of the methods. Some urinary biomarkers for occupational VOC exposures are also the metabolites of some daily foods and drinks. Therefore, under exposure to low concentrations of VOCs, the urinary biomarkers of the exposure origin will be less than those of daily food or drink origin. For example, hippuric acid is not advised as a biomarker for toluene when the air concentrations are below 30–50 ppm[11, 12].

Italian investigators have carried out much work on measurements of unmetabolized solvent concentrations in urine, but the approach was limited because of the low excretion. With the improvement of analytical methods, unmetabolized VOCs in urine were again paid much attention from 1990 onwards, mainly by Japanese researchers. Good correlations between air and urinary toluene, xylenes and styrene have been reported in occupational environments with over 0.1 ppm solvent exposure[12–23].

In the case of residential indoor environments, VOC concentrations are much lower than those found in

workplaces. In 1998, the average concentrations for VOCs were 98.3 $\mu g/m^3$ (0.026 ppm) for toluene, 22.5 $\mu g/m^3$ (0.005 ppm) for ethylbenzene, 24.3 $\mu g/m^3$ (0.006 ppm) for *m*- and *p*-xylenes, 10.0 $\mu g/m^3$ (0.002 ppm) for *o*-xylenes, 4.9 $\mu g/m^3$ (0.001 ppm) for styrene, and 123.3 $\mu g/m^3$ (0.021 ppm) for dichlorobenzene in an investigation of indoor air quality in Japan[6]. It was unclear whether at such low levels of exposure, unmetabolized VOCs in urine would still be useful for the assessment of VOC exposure. This study aimed to clarify this issue.

## Materials and Methods

*Subject recruitment*

The subjects were recruited from people who had participated in an earlier investigation of the prevalence of sick building syndrome[24]. To be included in the investigation, the subjects' houses had to be built no more than 5 yr before the year of the study (2003). With the inclusion criteria, 906 dwellings were randomly selected as possible subjects. Responses were obtained for 519 households who were all invited to participate in this study. Ultimately, the occupants of thirteen dwellings, 24 people, agreed to participate in the study (Table 1). All subjects received careful training on how to collect the air and urine samples.

This study was conducted with all the subjects' informed consent and the approval of the Institutional Ethical Board for Epidemiological Studies at Okayama University Graduate School of Medicine and Dentistry.

*Airborne VOC monitoring*

VOC monitoring was carried out both in the bedroom where the subjects were sleeping and in the living room with passive air samplers (VOC-SD diffusion sampler, Sigma-Aldrich, Japan). The monitoring time was the sleeping period of the subjects for the bedroom (Table 1) and 24 h for the living room. Samplers were put at the same height as the subjects' breathing zones, i.e. near the pillow, 20 cm above the bed in the bedroom, and 150 cm above the floor in the living room. Simultaneously, field blank samples were collected, and temperature and relative humidity were measured.

VOCs were desorbed by carbon disulfide mixed with an internal standard (toluene-d8), and analyzed by an Agilent 6890N/Agilent 5973 inert gas chromatography-mass spectrometer (GC/MS). The column was DB-5 MS (30 m in length, 0.25 mm in inner diameter and 0.5 $\mu$m in film thickness).

The oven temperature was first kept at 40°C for 5 min, and then increased to 200°C at 10°C/min. The ion source temperature was 230°C. The carrier gas was helium with 10 ml/min flow rate. The injection temperature was 200°C with a split ratio of 20:1. The detection mode was EI SIM (m/z=(99, 100) for toluene-d8, (91, 92) for toluene, (91, 106) for ethylbenzene, (91, 106) for xylenes, (78, 104) for styrene, and (146, 148) for *p*-dichlorobenzene). The limit of quantitation (LOQ) was 0.1 $\mu g/m^3$ for six chemicals (*m*- and *p*-xylene appeared with the same peak).

**Table 1.** Characteristics of the subjects

| | |
|---|---|
| Gender | |
| Male (%) | 14 (58.3) |
| Female (%) | 10 (41.7) |
| Age | |
| Mean ± SD (yr) | 32 ± 15 |
| Range | 5 – 51 |
| Alcohol drinkers (%) | 6 (25) |
| Smokers (%) | 4 (16.7) |
| Beverage drinkers (%) | 4 (16.7) |
| Occupational solvent exposure (%) | 3 (12.5) |
| Time spent in the living room | |
| Mean ± SD (h) | 8.5 ± 6.3 |
| Range | 0.2 – 18.9 |
| Time spent in the bedroom | |
| Mean ± SD (h) | 7.8 ± 1.4 |
| Range | 5.1 – 10.8 |

SD, standard deviation.

*Urine sampling and VOC analysis*

Spot urine samples were collected with paper cups before bedtime and first morning voids. Ten milliliters of urine samples were transferred to 22-m*l* glass vials capped with silicone-free plugs, and refrigerated at 4°C. The participants were asked to finish all procedures within 2 min after voiding. The analysis was performed within a few days after collection. VOCs in the urine were detected by a GC/MS connected to an automatic headspace sampler (Agilent G1888). Other conditions were the same as those used in the analysis of the air samples. The LOQ was 0.01 ng/m*l* for six chemicals (*m*- and *p*-xylene appeared with the same peak).

*Personal information collection*

The subjects were asked to record their times of leaving and returning to the dwelling and their times of going to bed and getting up. They were also asked to provide information on age, gender, smoker or non-smoker, number of cigarettes smoked, alcohol drinker or teetotaller, volume and type of alcohol drunk (beer, distilled spirit, sake, others), beverage drinker or not, volume of beverage drunk, use of drugs or similar things and urination times. If a child was too young to write, his or her parent was asked to record the information. The volume of alcohol consumed was calculated

**Table 2.** Differences and correlations of VOC air concentrations in living rooms and bedrooms

| VOCs | Guideline value[a] | Living room | | | Bedroom | | | r |
|---|---|---|---|---|---|---|---|---|
| | | GM | GSD | Range | GM | GSD | Range | |
| Toluene | 260 | 8.28 | 1.55 | 3.8 – 15.2 | 9.92* | 1.78 | 3.2 – 24.5 | 0.83 |
| Ethylbenzene | 3800 | 2.21 | 1.49 | 1.2 – 3.7 | 2.85** | 1.75 | 1.3 – 6.2 | 0.9 |
| *m*- and *p*-Xylene | – | 2.67 | 1.4 | 1.6 – 4.7 | 1.41** | 1.84 | 0.5 – 3.6 | 0.94 |
| *o*-Xylene | – | 1.12 | 1.41 | 0.7 – 1.9 | 1.2 | 1.93 | 0.4 – 3.2 | 0.9 |
| Total xylene | 870 | 3.79 | 1.39 | 2.6 – 6.6 | 2.62** | 1.85 | 1.0 – 6.8 | 0.95 |
| *p*-Dichlorobenzene | 240 | 4.57 | 3.27 | 0.7 – 58.9 | 6.23** | 3.34 | 1.0 – 60.7 | 0.97 |
| Styrene | 220 | ND | | | ND | | | |

GM, geometric mean; GSD, geometric standard deviation; ND, not detectable; r, Correlation coefficient; [a]Recommended by the Ministry of Health, Labour and Welfare of Japan; *$p$<0.05; **$p$<0.01. The limit of quantitation for all six chemicals was 0.1 $\mu$g/m$^3$. n=24, unit: $\mu$g/m$^3$

**Table 3.** Differences and correlations between night and morning urine VOC concentrations

| VOC | Night urine | | | Morning urine | | | r[a] | $p$[b] |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Range | Mean | SD | Range | | |
| Toluene | 0.091 | 0.065 | 0.037–0.306 | 0.079 | 0.054 | 0.025–0.230 | 0.830** | 0.134 |
| Ethylbenzene | 0.017 | 0.014 | 0.005–0.06 | 0.015 | 0.016 | 0.005–0.072 | 0.730** | 0.442 |
| *m*- and *p*-Xylene | 0.008 | 0.006 | 0.005–0.025 | 0.008 | 0.007 | 0.005–0.032 | 0.395 | 0.929 |
| *o*-Xylene | 0.006 | 0.002 | 0.005–0.015 | 0.008 | 0.005 | 0.005–0.019 | 0.509* | 0.008 |
| Total xylene | 0.015 | 0.008 | 0.005–0.038 | 0.017 | 0.012 | 0.005–0.051 | 0.611** | 0.342 |
| *p*-Dichlorobenzene | 0.023 | 0.022 | 0.005–0.082 | 0.027 | 0.030 | 0.005–0.107 | 0.971** | 0.094 |
| Styrene | 0.290 | 0.261 | 0.014–1.179 | 0.156 | 0.205 | 0.016–0.796 | 0.939** | <0.001 |

SD, standard deviation; [a]correlation coefficient; [b]paired sample t test. *$p$<0.05; **$p$<0.01, n=24, unit: ng/m*l*.

according to the alcohol percentages of different types.

*Statistic analysis*

The data below LOQ for urinary VOC concentrations were set to half the LOQ, 0.005 ng/m*l*. A paired t-test was used to test the difference between night and morning urinary VOC concentrations. Multiple linear regression analysis was adopted to identify the main influencing factors on the morning urinary VOC concentrations. Independent variables included age, gender, smoker or non-smoker, number of cigarettes smoked, alcohol drinker or teetotaller, volume of alcohol drunk, beverage drinker or not, volume of beverage drunk, organic solvent exposure or not, urination times, time spent in the living room, sleep time (consistent with sampling time), airborne VOCs concentration in the bedroom. Airborne VOC concentrations in the living room were not entered because of the high correlation between airborne VOC concentrations in the living room and in the bedroom. The variables were entered in the regression model stepwise. For all statistical analyses, the two-tailed test and a 5% level of significance were applied. $p$<0.10 was used as the criterion to select influential independent variables.

## Results

*Indoor air concentration of VOCs*

With the exception of styrene, all analyzed chemicals were detected in the air of subjects' living rooms and bedrooms (Table 2). The concentrations in the bedroom were significantly higher than those in the living room except for xylenes. However, their correlations between living rooms and bedrooms were very high with correlation coefficients all over 0.8.

*Urinary VOC concentrations*

All of the analyzed VOCs were detected in the night and morning urine samples (Table 3). No significant differences were found except for *o*-xylene and styrene: for *o*-xylene the average concentration in the morning urine was higher than that in the night urine, while for styrene the opposite trend was observed. Concentrations in the night urine samples showed a high correlation with those in the morning (r>0.50), except for *m*- and *p*-xylene.

**Table 4.** Correlations between morning urine VOCs and bedroom air VOCs concentrations[a]

| VOCs | All subjects (n = 24) | Subjects without occupational solvent exposure (n=21) |
|---|---|---|
| Toluene | 0.26 | 0.54* |
| Ethylbenzene | 0.04 | 0.28 |
| *m*-and *p*-Xylene | 0.19 | 0.42 |
| *o*-Xylene | 0.56** | 0.61** |
| Total xylene | 0.38 | 0.56** |
| *p*-Dichlorobenzene | 0.84** | 0.84** |

[a]Correlations by Pearson Correlation Coefficient. *$p$<0.05; **$p$<0.01.

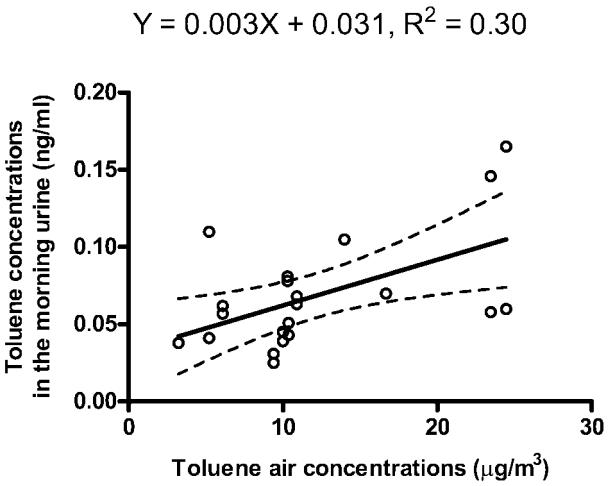


**Fig. 1.** Correlation between urine and air toluene concentrations. The interrupted lines show the 95% confidence interval of the means.

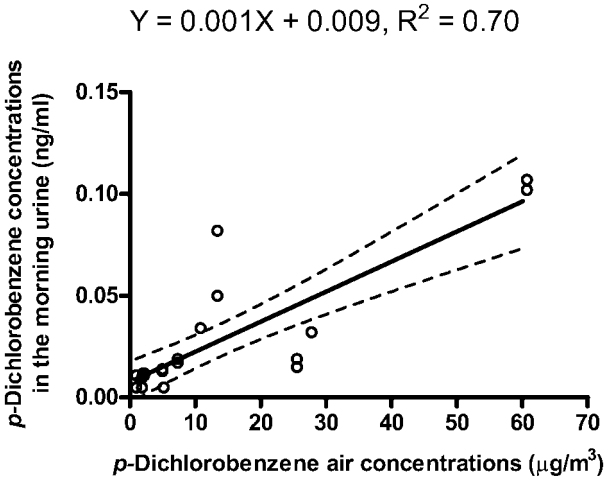


**Fig. 2.** Correlation between urine and air *p*-dichlorobenzene concentrations. The interrrupted lines show the 95% confidence interval of the means.

*Correlations between air and urine VOC concentrations*

Bedroom air and morning urine concentrations for *o*-xylene and *p*-dichlorobenzene showed relatively high correlations (r=0.56, 0.84, respectively) (Table 4). No significant correlations were found among other VOCs.

Three subjects were exposed to organic solvents at work, while the other subjects were office clerks or with no work. Given that the residential air exposure level is usually low, the influence of organic solvent exposure at work should conceal this kind of low level exposure; therefore we analyzed the data for the subjects without occupational organic solvents exposure further. For these subjects' data, bedroom air and morning urine concentrations for toluene, *o*-xylene, total xylene and *p*-dichlorobenzene all showed significant correlations (r=0.54, 0.61, 0.56 and 0.84, respectively) (Table 4, Figs. 1 and 2).

*Multiple linear regression analysis*

Multiple linear regression analysis showed that occupational solvent exposure was the main influencing factor for all six VOCs except for *p*-dichlorobenzene which only had airborne concentration in the bedroom as an influencing factor (Table 5). Alcohol drinking showed an influence on ethylbenzene, and the number of cigarettes smoked had a significant influence on ethylbenzene and *o*-xylene. Sleep time in the bedroom as a factor related to exposure was only found to be significant for *o*-xylene, and gender for only toluene. For styrene, no influence factor was found.

When the subjects exposed to solvents at work were excluded, airborne concentrations in bedrooms were found to be the main influencing factor for toluene, *o*-xylene, total xylenes and *p*-dichlorobenzene. However, no significant influence factor was found for ethylbenzene or *m*- and *p*-xylene.

**Table 5.** Differences of significant influence factors for the morning urine VOCs concentrations with and without occupational solvent exposure involvement

| DV ($C_{mu}$) | All subjects (n=24) | | | | | Subjects without solvents exposure (n=21) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | IV | $CR^2$ | PCC | *p* | $R^2$ | IV | $CR^2$ | PCC | *p* | $R^2$ |
| Toluene | Solvent | 0.282 | 0.682 | <0.001 | 0.514 | $C_{ba}$ | 0.289 | 0.587 | 0.007 | 0.406 |
| | $T_l$ | 0.182 | –0.507 | 0.016 | | Sex | 0.176 | –0.498 | 0.025 | |
| | $C_{ba}$ | 0.113 | 0.460 | 0.031 | | | | | | |
| ethylbenzene | $N_{smo}$ | 0.237 | 0.385 | 0.077 | 0.417 | – | – | – | – | – |
| | Alc | 0.142 | 0.460 | 0.031 | | | | | | |
| | Solvent | 0.114 | 0.428 | 0.047 | | | | | | |
| *m*- and *p*-Xylene | Solvent | 0.381 | 0.617 | 0.001 | 0.353 | – | – | – | – | – |
| *o*-Xylene | $C_{ba}$ | 0.244 | 0.778 | <0.001 | 0.659 | $C_{ba}$ | 0.319 | 0.706 | 0.001 | 0.473 |
| | Solvent | 0.200 | 0.726 | <0.001 | | $T_s$ | 0.206 | 0.551 | 0.012 | |
| | $T_s$ | 0.188 | 0.689 | 0.001 | | | | | | |
| | $N_{smo}$ | 0.086 | 0.484 | 0.026 | | | | | | |
| Total xylene | Solvent | 0.296 | 0.617 | 0.002 | 0.424 | $C_{ba}$ | 0.314 | 0.56 | 0.008 | 0.278 |
| | $C_{ba}$ | 0.178 | 0.502 | 0.015 | | | | | | |
| *p*-Dichlorobenzene | $C_{ba}$ | 0.697 | 0.835 | <0.001 | 0.683 | $C_{ba}$ | 0.689 | 0.836 | <0.001 | 0.683 |
| Styrene | – | – | – | – | – | – | – | – | – | – |

DV, dependent variable; IV, independent variable; $CR^2$, contribution for $R^2$; PCC, partial correlation coeffecient; $R^2$, adjusted $R^2$; $C_{mu}$, VOC concentrations in the morning urine; $C_{ba}$, air VOC concentrations in the bedroom; Alc, alcohol; $N_{smo}$, Number of cigarette smoked; $T_l$, time spent in the living room; $T_s$, sampling time.

## Discussion

This study verified the usefulness of using unmetabolized toluene, xylenes and *p*-dichlorobenzene in urine as biomarkers that complement evaluation of the indoor air exposure to VOCs. By using the regression equations (Figs. 1 and 2) obtained from the subjects without solvent exposure, we were able to get theoretical urine VOC concentrations with monitored air VOC concentrations. For example, the subject with occupational solvent exposure had observed urinary VOC concentrations of 0.194 ng/m*l* toluene and 0.009 ng/m*l* *p*-dichlorobenzene. However, the theoretical concentrations were 0.041 ng/m*l* toluene and 0.011 ng/m*l* *p*-dichlorobenzene. The significantly higher observed urinary toluene concentration suggests possible high exposure outside the bedroom, although at the same time the possible effects of individual difference in metabolic rate, contamination during sampling and analysis should be considered. Increasing the sample size in a future study might be needed to confirm the present results.

In order to decrease the possible volatile loss of VOCs, we asked the participants to finish all urine collections within 2 min from urine voiding to vial capping, which was even faster than the 3 min recommended by other researchers[25].

Adjustment of either urinary density or creatinine has been recommended for VOCs metabolites significantly influenced by kidney functions, drinking habits, sweating, etc. However, excretion of unmetabolized VOCs seems to be related to the urine/blood partition coefficient and to urinary volume[26]. Therefore, our urinary data are presented without correction.

Confounding factors such as cigarette smoking and alcohol drinking did not show significant influences on urinary toluene, xylenes and *p*-dichlorobenzene excretion. We found that gender was a confounding factor for urinary toluene levels. This can be explained by adipose tissues which average 36.5% in females and 21.1% in males[27]. Fat tissue has the ability to efficiently extract highly lipophilic solvents during exposure, leading to a delay in VOC excretion. Following a decrease in exposure level this confounding effect will become more observable. Takeuchi *et al.*[18] did not find a confounding influence of gender on urinary VOCs levels, perhaps partly because the relatively high exposure range in their study (0.4–54.3 ppm for toluene and 0.4–27.3 ppm for xylenes) made the influence from exposure much higher than that from gender.

In our study, in order to get personal exposure concentrations the measures were carried out during the hours of sleep. This neglected the influences of normal physical activity and assured a stable condition. As a result of enhanced physical activity, the amount of xylene retained and subsequently absorbed by the body is increased due to enhanced pulmonary ventilation and cardiac output[28, 29]. The uptake rates of chlorinated solvents are approximately doubled even at light physical

exercise (50 W) compared with the value at rest[20]. In addition, it has been reported that blood and brain toluene levels in rats exposed to 2,000 or 4,000 ppm for 4 h in the light were significantly higher at the end of exposure and 40 min after the cessation of exposure than in animals exposed in the dark[30]. This suggests that circadian rhythms may have an influence on toluene absorption, distribution and excretion. Accordingly, the urinary VOC concentrations interpolated from our regression equations will be different from those in non-sleeping conditions.

Our 21 subjects without occupational solvent exposure included 5 children aged less than 18 years old. Although age was not a significant factor for urinary VOCs levels in multivariate analysis, the power of the analysis will be limited because of this selection bias.

Although direct measurement of VOCs in spot urine samples has been used as stable biomarkers in occupational environments in some countries, there are still few studies for non-occupational low-level VOCs exposures. To our knowledge the exposure ranges in our study are the lowest of all field studies that have been reported in the literature. Their reliability should be examined in similar exposure ranges in other field investigations. However, we encountered some difficulty in quantifying all of the levels that we measured. In present study, half of the xylene concentrations in urine were around the level of limit of quantification; therefore, the results of the statistical analyses are likely to have been biased. We suggest that new methods need to be established to lower the limits of quantification, alternatively, subjects with relative higher exposures could be selected.

From the results of the present study, we conclude that toluene, *o*-xylene, total xylene and *p*-dichlorobenzene in urine can be regarded not only as biomarkers of industrial exposure, but also as indicators of low level non-occupational indoor exposures.

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