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Method for Analyzing Urinary Toluene and Xylene by Solid-Phase Microextraction (SPME), and Its Application to Workers Using Organic Solvents

F. Asakawa,[1,2] F. Jitsunari,[2] J. Choi,[2] S. Suna,[2] N. Takeda,[2] T. Kitamado[2]

[1] Human-Environmental Science Research Laboratory, College of Liberal Arts and Science, Kurashiki University of Science and the Arts, Kurashiki-shi, Okayama 712-8505, Japan
[2] Department of Hygiene and Public Health, School of Medicine, Kagawa Medical University, Miki-cho, Kagawa 761-0793, Japan

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In Japan, workers using organic solvents (8 types) are required by law to undergo examination of urinary metabolites. This biological monitoring using urinary metabolites as indices of biological exposure aims to prevent health disorders due to organic solvents. For toluene (TOL), urinary hippuric acid (HA-U) is examined. However, food-derived HA is present in urine even in the general population (Ikeda et al. 1969, Kawai et al. 1981) and the upper normal limit of HA-U that can be statistically considered to indicate TOL exposure is approximately 0.6 g/g creatinine (Ogata et al. 1986). This corresponds to approximately 40 ppm TOL in the air (Ogata et al. 1986). On the other hand, the occupational exposure level-time weighted average (OEL-TWA) of TOL recommended by the Japan Society for Occupational Health (JSOH) is now 50 ppm instead of the conventional 100 ppm (JSOH 1996). Thus, TOL exposure by examination of HA-U is expected to be a difficult evaluation.

As a biological index to replace HA-U, we noted urinary toluene (TOL-U) reported by Imbriani et al. (1986) partly because TOL-U is not detected in people unexposed to TOL. However, compared with HA-U in the order of g/L, the amount of TOL-U is very small (in the order of μg/L). Therefore, we have undertaken to examine a more sensitive method for the biological monitoring of TOL, and have evaluated a method to measure TOL-U using solid-phase microextraction (SPME) reported by Arthur et al. (1992a). SPME is a type of solid phase extraction based on the principles of distribution in which volatile organic compounds (VOCs) are directly extracted from liquid specimens or from the head space (HS) of liquid or solid specimens, and measured by gas chromatography (GC). Although this method has been applied to the analysis of environmental contamination (Arthur et al. 1992b, Potter et al. 1992), there have been no studies on its application to urinary samples.

Correspondence to: F. Asakawa

We developed a sensitive method in which TOL is directly extracted by SPME from urinary samples, and quantitatively analyzed by GC. At one industrial facility, parameters such as TOL-U were measured by this SPME method in workers using organic solvents in addition to air monitoring, and good results were obtained.

MATERIALS AND METHODS

For the application of SPME to measure TOL-U, we evaluated a method by which TOL is extracted by fused silica fibers with an absorbent (liquid phase) from head space (HS) of urinary samples, and measured by GC. The extraction conditions (extraction time, addition of salt, heating, etc.), reproducibility, and linearity of the calibration curve of HS-SPME were evaluated. In addition to TOL, xylenes (*o*-, *m*-, and *p*-XYL), which are frequently used in work places, were examined. As the standard solution, a standard reagent for water quality analysis (Wako Pure Chemical Osaka, Japan.) was diluted with methanol to prepare mixed standard solutions at each concentration. The other reagents used were those for water quality analysis or equivalent ones. For SPME, fibers with polydimethylsiloxane with a film thickness of 100 μm (SUPELCO No.5-7300 Pennsylvania, USA) were used.

Urine samples (5 mL) were placed in 10 mL volume vials, mixed with 50 μL methanol (because of standard solution dissolved in methanol) and 1 g sodium chloride (NaCl). The vials were sealed with a septum with a Teflon liner. The contents were thoroughly mixed and left for 1 hour at room temperature (25 °C). SPME fiber was inserted into HS of the vial, and extraction was performed for 5 minutes while the sample solution was stirred using a stirrer. The SPME fiber was immediately injected into the GC and held for 2 minutes.

GC was performed using a Shimadzu GC-8A (Kyoto, Japan) with a flame ionization detector (FID) under the following conditions: column, DB-WAX (J & W California, USA), 30 m x 0.53 mm I.D., 1.5 μm (film thickness); carrier, He 10 mL/min; make up, N_2 40 mL/min; oven, 55 °C; injection / detector, 150 °C.

Next, at an industrial facility using organic solvents (TOL and XYL etc.), urine samples were collected in 10 mL volume vials sealed with a septum with a Teflon liner after work from 27 male workers who mainly engage in

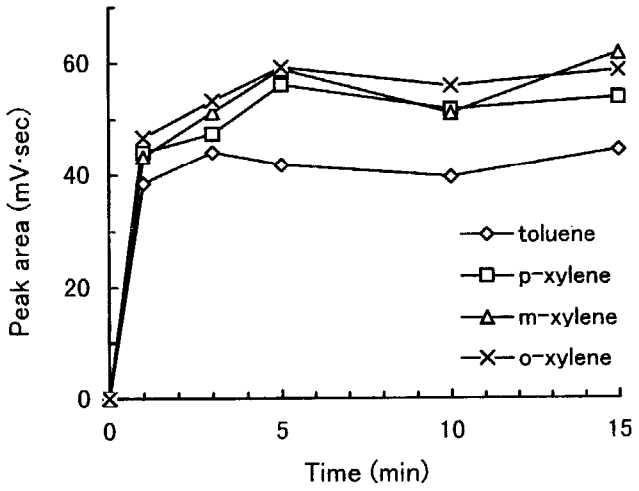

Figure 1 Absorption-time profile for toluene and xylenes absorbed by SPME from urine containing 50 µg/L of each compound (left at 25 °C for 1 h without addition of NaCl, and then stirred for extraction).

printing on plastic films. Urine samples were kept in a cooler until morning, and then TOL-U and XYL-U were measured by HS-SPME-GC. Simultaneously, the amount of exposure of each worker to organic solvents in the breathing zone during work was investigated using diffusive samplers (3M #3500 Organic Vapor Monitor Minnesota, USA), and metabolites such as HA-U and methyl HA-U were measured. All subjects gave their informed consent prior to their inclusion in the study.

Analysis of diffusive samplers was performed according to the manufacturer's analysis guide (3M 1992); after elution with 1.5 mL of carbon disulfide, GC analysis was performed under the same conditions as above. Metabolites such as HA-U were measured by high performance liquid chromatography (HPLC) using a Shimadzu LC-4A under the following conditions: column, STR-ODS (Shimadzu), 150 mm x 4.6 mm I.D.; mobile phase, pH 3.3 phosphate buffer (20 mmol/L) : acetonitrile = 87 : 13; flow rate, 0.7 mL/min; oven, 40 °C; detection wavelength, 225 nm.

RESULTS AND DISCUSSION

Fig. 1 shows the relationship between the HS-SPME extraction time and the

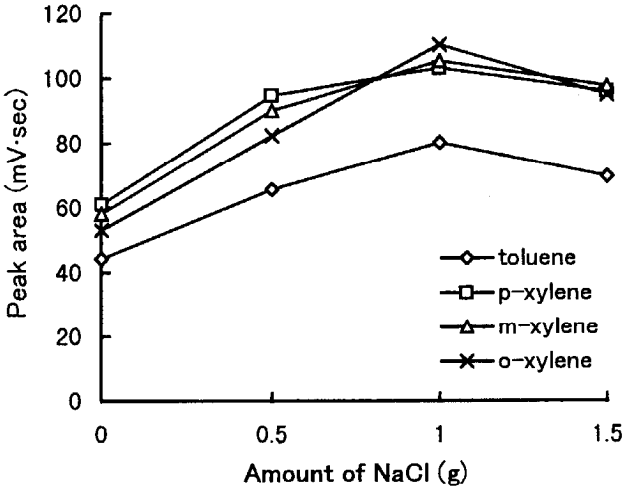

Figure 2 Effect of NaCl addition on SPME of toluene and xylenes in urine containing 50 μg/L of each compound (left at 25 °C for 1 h, and then stirred for 5 min for extraction).

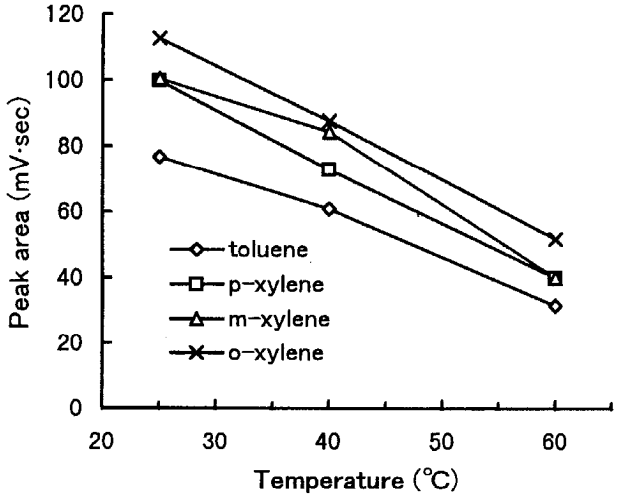

Figure 3 Effect of temperature on SPME of toluene, xylene in urine containing 50 μg/L of each compound (left at each temperature for 1 h with 1g NaCl, and then stirred for 5 min for extraction).

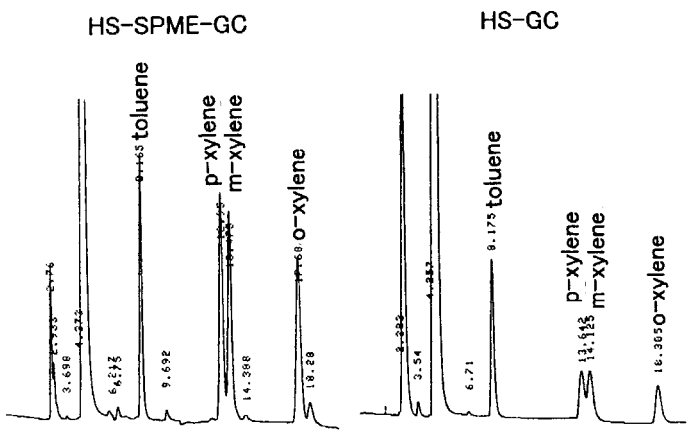

Figure 4 Chromatograms of urine sample containing toluene and *p*-, *m*-, and *o*-xylenes at 50 μg/L. Comparison between HS-SPME-GC and HS-GC methods.

amount of extraction. This relationship was obtained after each urine sample (5 mL) was mixed with 50 μL of 5 mg/L mixed standard solution (abbreviated as 50 μg/L urine below) left at room temperature (25 °C) without addition of NaCl, and then stirred for extraction. Both TOL and XYL reached equilibrium after approximately 5 minutes. Next, the effects of the addition of NaCl, heating, and stirring of samples on the amount of extraction were evaluated. Concerning the effect of salt, Fig. 2 shows the results of measurements after 50 μg/L urine samples were mixed with NaCl, left at room temperature, and stirred for 5 minutes for extraction. The amounts of TOL and XYL increased 1.5 - 2 times after addition of the salt. On the other hand, as shown in Fig. 3, heating (40 - 60 °C) decreased the amounts of TOL and XYL by 20 - 60 %, showing that heating was not useful. Without stirring during extraction, the amounts of TOL and XYL decreased by approximately 40 % (data not shown).

Therefore, the conditions for HS-SPME were established to be the addition of 1 g NaCl, standing at room temperature, and stirring for 5 minutes. Under these conditions, reproducibility was evaluated (50 μg/L urine, n = 5). The coefficient of variance was 1.7 % for TOL, 4.5 % for *p*-XYL, 3.0 % for *m*-XYL, and 3.5 % for *o*-XYL. The calibration curves for both TOL and XYL showed linearity up to a urinary concentration of 100 μg/L. Fig. 4 shows a gas chromatogram of 50 μg/L urine by this HS-SPME-GC method

Table 1 Toluene exposure concentration (TOL-TWA) and urinary toluene (TOL-U), urinary hippuric acid (HA-U) concentration of workers using organic solvents.

	TOL-TWA (ppm)	TOL-U (μg/L)	HA-U (g/L)
Mean (n = 27)	10.6	11.9	0.62
Range	0.8 – 33.6	ND – 44.5	0.07 – 1.58

ND; Not detected at the detection limit of 0.5 µg/L

and that by HS-GC (injection of 0.5 mL HS). The sensitivity (area ratio) of HS-SPME-GC was 1.2, 2.5, 2.5 and 4.7 times higher than that of HS-GC for TOL, *p*-XYL, *m*-XYL and *o*-XYL, respectively. The detection limit was estimated to be at least 0.5 µg/L with a signal-to-noise of 6 : 1. TOL-U and XYL-U at a concentration of approximately 1 µg/L could be measured by HS-SPME-GC without problems. This HS-SPME-GC method showed good separation of peaks and high sensitivity and reproducibility, and is appropriate for the biological monitoring of workers using organic solvents.

Therefore, at an industrial facility using organic solvents, in addition to the investigation of worker exposure to organic solvents and the measurement of metabolites such as HA-U and methyl-HA-U, TOL-U and XYL-U were measured by HS-SPME-GC. Results are shown in Table 1. However, since measurement using diffusive samplers in the breathing zone of workers revealed TOL as the primary organic solvent, only the TOL exposure concentration (TOL-TWA) and the amounts of HA-U and TOL-U are shown in this table. As Fig. 5 shows, a high positive correlation observed between TOL-TWA and TOL-U (r = 0.820). Concerning this correlation, a value of r = 0.87 was reported by Imbriani et al. (1986) and Ghittori et al. (1987) and a value of r = 0.84 by Kawai et al. (1996). Our value was similar to these values. On the other hand, there was a correlation between TOL-TWA and HA-U (r = 0.614). Kawai et al. (1996) reported a value of r = 0.522.

Thus, TOL-TWA values were better correlated with TOL-U than with HA-U. TOL-U at an OEL-TWA of 50 ppm obtained from the regression line between TOL-TWA and TOL-U was 47.8 µg/L. We established a simple and rapid HS-SPME-GC method in which TOL-U and XYL-U were directly extracted from urine samples, and quantified by GC. This method showed good separation of peaks and high sensitivity and reproducibility,

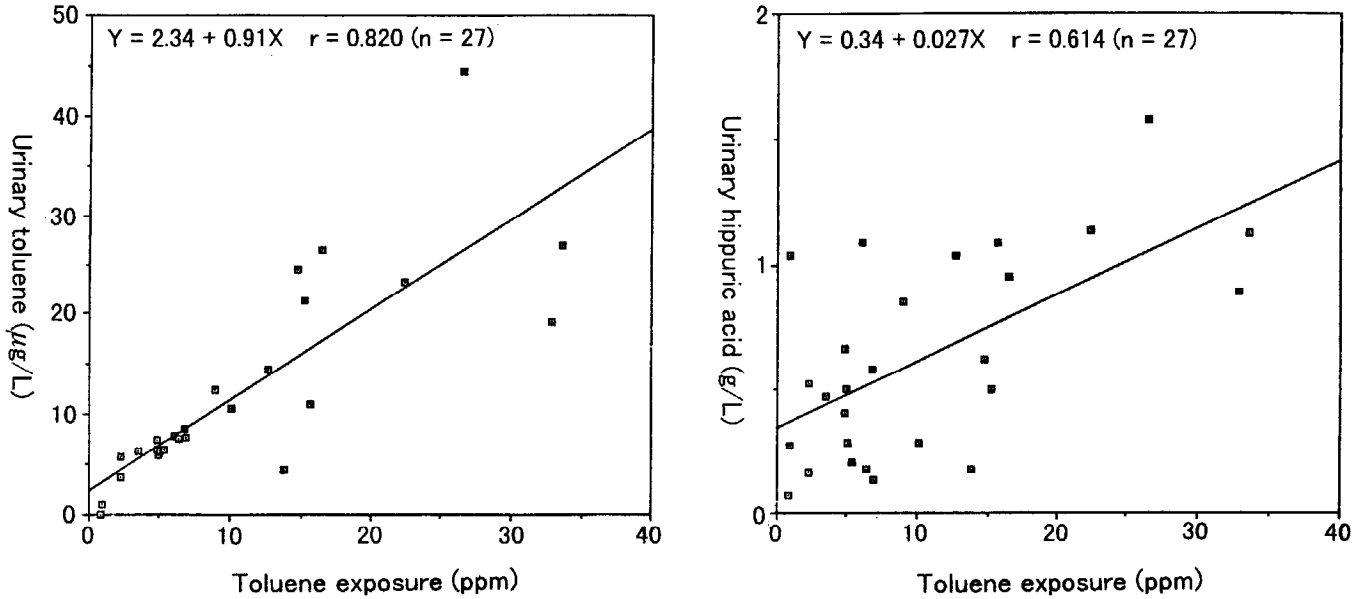

Figure 5 Correlation of toluene exposure (TOL-TWA) and urinary toluene (TOL-U) or urinary hippuric acid (HA-U) in workers using organic solvents.

and may be applicable to the biological monitoring of workers using organic solvents.

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