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Masana Ogata[*] Shohei Kira[†] Yoshihiro Shimada[‡]

Hirokazu Ohsaki[**] Reiko Sugihara[††] Toshiko Fujii[‡‡]

[*]Okayama University,
[†]Okayama University,
[‡]Okayama University,
[**]Okayama University,
[††]Okayama University,
[‡‡]Okayama University,

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Abstract

The levels of hippuric acid in the urine of people exposed to toluene vapour were measured by paper chromatography, direct colorimetry, high performance liquid chromatography and gas chromatography. The control was a similar group not exposed to toluene vapour. The values were analyzed statistically, conversion equations calculated, and the propriety of these equation discussed. Since the three chromatographic methods gave similar values, the measurement of urinary hippuric acid by these methods can be used as an index of toluene exposure. The colorimetric method gave higher levels the chromatographic methods, especially for the urine of people not exposed to toluene. This may have been due to glycine conjugates (other than hippuric acid) developing a similar color, resulting in elevated values for hippuric acid. This colorimetric method should be used with caution for biological evaluation of workers with low toluene exposure.

KEYWORDS: measurement of hippuric acid, toluene, conversion equation

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COMPARISON OF SEVERAL METHODS FOR THE MEASUREMENT OF URINARY HIPPURIC ACID AS AN INDEX OF TOLUENE EXPOSURE

Masana OGATA, Shohei KIRA, Yoshihiro SHIMADA, Hirokazu OHSAKI*, Reiko SUGIHARA** and Toshiko FUJII

*Department of Public Health, Okayama University Medical School, Okayama 700, Japan ; *Department of Industrial Science, School of Engineering, Okayama University, Okayama 700, Japan and **Department of Home Economics, School of Education, Okayama University, Okayama 700, Japan*

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Abstract. The levels of hippuric acid in the urine of people exposed to toluene vapour were measured by paper chromatography, direct colorimetry, high performance liquid chromatography and gas chromatography. The control was a similar group not exposed to toluene vapour. The values were analyzed statistically, conversion equations calculated, and the propriety of these equations discussed. Since the three chromatographic methods gave similar values, the measurement of urinary hippuric acid by these methods can be used as an index of toluene exposure. The colorimetric method gave higher levels the chromatographic methods, especially for the urine of people not exposed to toluene. This may have been due to glycine conjugates (other than hippuric acid) developing a similar color, resulting in elevated values for hippuric acid. This colorimetric method should be used with caution for biological evaluation of workers with low toluene exposure.

Key words : measurement of hippuric acid, toluene, conversion equation.

Toluene has been widely used as an industrial organic solvent. Estimation of the exposure levels or inhaled amount of toluene is important in monitoring the health of solvent workers. It is difficult or inconvenient to measure the atmospheric concentrations of solvent in the work yard alongside workers, so the level of urinary hippuric acid (HA), a major metabolite of toluene excreted in urine (1), has been used as an index for the estimation of toluene exposure. To determine the levels of HA, the authors have developed several methods: paper chromatography (PC) (2, 3), colorimetry using benzene sulfonyl chloride (BSC) (4, 5), and dimethyl amino benzaldehyde (DAB) (6, 7). Gas chromatography (GC) (8) and high performance liquid chromatography (HPLC) (9, 10) have also been used to determine urinary HA. Some of these methods have

been used to monitor levels in solvent workers and in experimental studies. Comparison of HA levels in volunteers with those in workers was difficult because of differences in the methods used. This was also difficult when the reverse comparison, workers to volunteers, was made. A conversion equation for data from different methods was needed. The present experiment was designed to assess four methods which are now popularly used in industrial health administration.

MATERIALS AND METHODS

Urine samples were collected from healthy persons who had not been exposed to toluene and xylene.

In an exposure chamber, volunteers were exposed to toluene vapour for 3 h in the morning and 4 h in the afternoon with a break of an hour in between. The vapour concentration was determined by GC. From each volunteer, all the urine was collected from 4 to 8 h after entering the exposure chamber as described in the previous report (3). The urine was kept frozen at $-20°C$ until analysis.

Urine samples were analyzed by the following methods: a) PC; a modification described by Ogata and others (2), b) direct colorimetry (7), c) GC (8) and d) HPLC (9). All reagents used were analytical grade.

The data was analysed by a microcomputer. Tests for significance were done according to Hald's method (11).

RESULTS

Table 1 shows the correlations of the values obtained from the four different methods. The correlation coefficient (r), the slope (a) and the y-intercept (b) for pairs of methods were calculated separately from the data of the non-exposed group ($n=26$) and the exposed group ($n=12$). Although correlation coefficients were satisfactory, data from the exposed group showed a higher correlation ($0.926 \leq r \leq 0.998$) than from the non-exposed group ($0.818 \leq r \leq 0.948$).

Next data analysis shown in Table 2 was tested for the significance of regression coefficients (a) shown in Table 1 between the data from non-exposed and exposed groups for each pair of methods. Three slopes of combinations, the direct method against GC, HPLC and PC, showed significant differences of $p<0.05$ between the regression coefficients of the non-exposed group and exposed groups. On the other hand, regression of PC versus (vs) GC, and GC vs PC: PC vs HPLC, and HPLC vs PC: PC vs D, and D vs PC: GC vs HPLC, and HPLC vs GC: GC vs D, and D vs GC: and HPLC vs D, and D vs HPLC: were acceptably similar as far as slope. The result was that, for the four combinations of methods listed in Table 1, the values obtained from one method could be converted to the partner method using a regression equation derived from the total data. The regression equations of combinations marked with

TABLE 1. CORRELATION AND THE REGRESSION EQUATIONS AMONG VARIOUS METHODS FOR THE CONVERSION OF URINARY HIPPURIC ACID

Method		Non-exposed person (n=26)			Exposed person (n=12)			Total of them (n=38)		
x	y	r	a	b	r	a	b	r	a	b
PC	GC	0.948	0.969	−0.017	0.995	0.994	−0.030	0.996	0.992	−0.023
GC	PC		0.927	0.042		0.995	0.051		1.001	0.030
PC	HPLC	0.936	1.116	−0.006	0.994	1.105	0.058	0.996	1.122	0.002
HPLC	PC		0.785	0.036		0.895	−0.028		0.884	0.005
PC	D	0.913	1.319	0.111	0.989	1.172	0.244	0.991	1.202	0.154
D	PC		0.631	−0.027		0.834	−0.159		0.818[a]	−0.113
GC	HPLC	0.883	1.027	0.042	0.997	1.108	0.097	0.996	1.127	0.032
HPLC	GC		0.759	0.018		0.897	−0.075		0.880	−0.021
GC	D	0.818	1.159	0.182[b]	0.926	1.182	0.108	0.961	1.161	0.172
D	GC		0.579	−0.029		0.726	0.219		0.796[a]	−0.074
HPLC	D	0.906	1.101	0.141[b]	0.998	1.065	0.174[b]	0.994	1.071	0.153
D	HPLC		0.745	−0.056		0.935	−0.155		0.927[a]	−0.135

a the rightness of fit of the slope of these equations is discussed in the test of result.
b significant difference ($p<0.01$) between y intercept and zero.
PC=paper chromatography, GC=gas chromatography, HPLC=high performance liquid chromatography, D=direct colorimetric method with DAB at pyridine, r=correlation coefficient, regression equations are $y=ax+b$, a=the slope, b=y; the intercept.

TABLE 2. TEST FOR THE SIGNIFICANCE OF REGRESSION COEFFICIENTS BETWEEN THE DATA FROM NON-EXPOSED AND EXPOSED GROUPS BY EACH COMBINATION OF METHODS

Method	t (observed)	Degree of freedom calculated	Difference
PC ⟶ GC	0.335	33	ns
PC ⟶ HPLC	0.097	31	ns
PC ⟶ D	1.115	32	ns
GC ⟶ PC	0.943	33	ns
HPLC ⟶ PC	1.595	33	ns
D ⟶ PC	2.876	34	$p<0.05$
GC ⟶ HPLC	0.699	27	ns
GC ⟶ D	0.118	27	ns
HPLC ⟶ GC	1.616	27	ns
D ⟶ GC	2.976	29	$p<0.01$
HPLC ⟶ D	0.336	26	ns
D ⟶ HPLC	2.615	27	$p<0.05$

ns: not significant; Other abbreviations are the same as shown in Table 1.

(a) in Table 1 should be calculated separately from the data for the non-exposed and exposed groups, and those with (b) should be used carefully.

Table 3 shows the mean values and the sample standard deviations of urinary HA levels determined by the four methods. These four methods had different means; the direct method showed the highest (357 μg/ml) and GC the lowest (147 μg/ml) for urine samples from the non-exposed group. The three chromatographic methods had similar means (147–182 μg/ml).

TABLE 3. LEVELS OF URINARY HIPPURIC ACID IN NON-EXPOSED PERSON AS CALCULATED BY VARIOUS METHODS (μg/ml)

	Observed concentration				Corrected concentration			
	Logarithm[a]		Real quantity		Logarithm[a]		Real quantity	
	Mean	SD[b]	Mean	Deviation[c]	Mean	SD[b]	Mean	Deviation[c]
PC	2.240	0.404	174	27-1117	2.217	0.355	165	32- 845
GC	2.166	0.432	147	20-1072	2.142	0.304	139	23- 851
HPLC	2.259	0.420	182	26-1256	2.336	0.370	172	31- 955
D	2.552	0.303	357	88-1439	2.529	0.240	338	112-1007

a calculated with an assumed log normal distribution (12); *b* SD: standard deviation, *c* m$\pm$2SD, corrected concentration=corrected concentration for urinary specific gravity 1.024, real quantity=the values were converted from logarithmic value into real quantity. Other abbreviations are the same as shown in Tables 1 and 2.

Table 4 shows data converted using the equations listed in Table 1. The original data is from our report (13) determined by PC. The calculated values were close to each and seemed to allow conversion of the data obtained by the conversion equations. This suggests the rightness of fit of the data obtained from solvent workers by these chromatographic methods.

TABLE 4. EXAMPLE ON CONVERTED VALUES OF CORRECTED HIPPURIC ACID CONCENTRATION IN AFTERNOON-URINE OF EXPERIMENTAL EXPOSURE TO TOLUENE, FROM PAPER CHROMATOGRAPHY TO OTHER METHODS (m$\pm$SD, mg/ml)

Method	Equations from exposed group		Equations from total group	
	100 ppm toluene	200 ppm toluene	100 ppm toluene	200 ppm toluene
PC	2.81$\pm$0.66	5.85$\pm$1.24	2.81$\pm$0.66	5.85$\pm$1.24
GC	2.76$\pm$0.66	5.78$\pm$1.23	2.76$\pm$0.65	5.78$\pm$1.23
HPLC	3.16$\pm$0.73	6.52$\pm$1.36	3.15$\pm$0.73	6.57$\pm$1.38
D	3.54$\pm$0.77	7.10$\pm$1.59	3.53$\pm$0.88	7.19$\pm$1.66

Urine sampls were collected immediately after the experimental exposure simulated to ordinal work-time, for 3 h in the morning, 1 h break, and for 4 h in the afternoon. The number of male was 10, female was 26, and there was not significant difference between them. Abbreviations are the same as those in Tables 1, 2 and 3.

DISCUSSION

Referring to a comparative study between colorimetry and other chromatographic methods for HA, Hasegawa and others (14) reported a trend confirmed by our present results although they used BSC as a reagent for color development instead of the DAB used by us. One reason why the direct method showed higher values might be the presence of other glycine conjugates such as hydroxyhippuric acid, methylhippuric acid and salicyluric acid in the urine of those not exposed to toluene. These are derived from foods or food conservatives, react with BSC and DAB, and develop a similar color. In urine from non-exposed workers DAB seemed to have a higher specificity for HA than BSC because of the difference of the slopes given: 0.444 for BSC and 0.579 for DAB by GC.

On the other hand, when the direct method with DAB was used for urine from the exposed group, the slope increased from 0.579 to 0.796 with GC and the correlation coefficient rose from 0.818 (non-exposed) to 0.961 (Table 1). The results suggest that two conversion equations, one for the non-exposed group and one for the exposed group, might be needed for combination of the direct method and the PC, GC or HPLC methods.

The following characteristics should be considered when these methods are used to monitor toluene workers. The direct method is available as a mass-screening test for the determination of HA in urine, because it only needs the widely available spectrophotometer. Chromatographic methods such as GC and HPLC, however, require expensive instruments and have high running costs, but, show high specificity for HA and are especially good for detailed analysis of urine samples from workers exposed to low levels of toluene.

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