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Original Paper

Estimation of Exposure Concentrations of Toluene Using Multiple Regression Analyses of Five Biological Exposure Indicators

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Abstract

In order to estimate exposure concentrations of toluene from its five biological exposure indicators, multiple regression analyses (MRA) were performed. Single correlation analyses showed that the five biological indicator variables were significantly correlated with exposure concentrations of toluene. The multiple correlation coefficients of biological exposure indicator were higher than the single correlation coefficients for each individual indicator. Biological indicators for eight hours were used for the MRA, except for blood toluene (TOL-B) where values for the last four hours of the shift were used.

For the MRA using two variables, the multiple correlation coefficient (M-r) of toluene in exhaled air (TOL-EXH) and hippuric acid in urine (HA-U) with exposure concentration of toluene (TOL-EXP) was 0.914, and the highest among MRAs using two variables followed by the M-r of TOL-EXH and urinary o-cresol (CR-U). Concentrations of TOL-EXH, TOL-U and TOL-B are closely correlated with each other. Therefore, HA-U or CR-U and any one of TOL-EXH, TOL-U and TOL-B are appropriate for use as the variable in MRA. For the MRA using three variables, the M-r of toluene in urine (TOL-U), HA-U and CR-U with TOL-EXP was 0.778. For the MRA using four variables, the M-r of TOL-EXH, TOL-U, HA-U and CR-U with TOL-EXP was 0.945, the highest M-r using four variables. In the case of five variables, the M-r of TOL-EXH, TOL-B, TOL-U, HA-U and CR-U with TOL-EXP were 0.946. For evaluation of the MRA, M-r square adjusted, P value by F test and Akaike's information criterion were applied.

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Introduction

Toluene is widely used as an industrial organic solvent and many workers are exposed to it in their workplace.

The threshold limit value (TLV) of toluene was changed from 100ppm to 50ppm[1], and the suitable biological exposure indicators for 50ppm of toluene have been investigated since the change.

The five biological indicators for toluene exposure [2,3] are toluene in the blood (TOL-BL), toluene in exhaled air (TOL-EXH), urinary toluene (TOL-U), urinary hippuric acid (HA-U) [4-6] and urinary o-cresol (CR-U) [6].

Toluene in blood, exhaled air and urine are determined by measuring toluene itself in biological specimens, which makes these measurements superior in terms of agent specificity. However, the half life of toluene in blood and exhaled air is 30 minutes, similar to the half life of toluene in urine [7]. Therefore, they can be used only if the concentrations of toluene during the working hours are stable[8]. The concentrations of toluene in the blood 16 hours after the end of the shift [1,2] show more personal differences than immediately after. Moreover, a small amount of urinary toluene evaporates during preservation. Urinary o-cresol is excreted in relatively small amounts [6] and is a component excreted in urine due to smoking. Therefore, there are some problems of preservation and non-specificity for toluene exposure.

Urinary hippuric acid is a metabolite of benzoic acid which is found in some foods and soft drinks [4,5]. Therefore, HA is found in the urine of people not exposed to toluene and the level is variable.

These findings suggest that estimation of exposure concentrations of toluene from a single exposure indicator is relatively difficult.

In a previous paper[3], the authors reported the correlation between the personal exposure concentration and each biological indicator for toluene.

In the present report, estimation of the exposure concentration of toluene using multiple regression equations on five biological exposure indicators is described.

Materials and Methods

1. Materials:

   Workers: Sixteen male workers (age 41.1±8.9,m±SD) who were exposed to toluene in factories manufacturing cathode ray tubes (CRT).

2. Methods:

   Sampling and analytical methods for determining toluene vapors in ambient air and toluene and its metabolites in biological specimens have been fully described in a previous report [8].

   Simple correlation analysis was first carried out to investigate the relationship among six

variables. This was followed by multiple regression analyses [9 -11] to evaluate the primary factors associated with personal exposure concentrations of toluene. The TOL-EXH, TOL-B, TOL-U, HA-U and CR-U data for the whole eight hours of working time and those for the last four hours were used as explanatory variables and the personal exposure concentration of toluene for eight hours was used as the criterion variable.

Statistical analyses were carried out with EXCEL (Microsoft, USA) software.

Results

1. Basic statistical data.

   Table 1 shows the basic statistics used in this report and summarizes the monitored concentrations of toluene and the corresponding concentrations of biological indicators. The exposure concentration of toluene during the first four hours was 15.1 ppm, the concentration during the last four hours was 21.7 ppm and the average concentration over the whole eight hours was 18.4 ppm.

   The concentration of TOL-EX and three urinary exposure indicators (TOL-U, HA-U and CR-U) for the whole eight hours and the concentrations of TOL-EXH, TOL-B and three urinary indicators for the last four hours were used in the following multiple regression analyses.

2. Comparison between single and multiple correlation coefficients.

   The single correlation coefficients (single r) of all variables are listed in Table 2. The multiple correlation coefficients (multiple r) for various combinations of two to five explanatory variables with personal exposure concentration as the criterion variable are listed in Table 3. The results were as follows:

   1) In the case of two factors:
      - ① The multiple r of HA-U and CR-U concentrations for eight hours (am+pm) was 0.762 (Table 3. f.2-③), which was a little higher than the single r of 0.755 (f.1) for HA-U alone.
      - ② The multiple r of TOL-EXH and HA-U for eight hours was 0.914 (f.2-⑤), which was higher than the single r (0.834) for HA-U alone. The multiple r of 0.914 was the highest among the multiple r using two factors.
      - ③ The multiple r of TOL-B and HA-U for the last four hours was 0.835 (f.2-⑧), which was higher than single r (0.782) for HA-U alone.

   2) In the case of three factors:

      The multiple r of TOL-U, HA-U and CR-U for eight hours was 0.793, which was a little higher than the single r (0.755) for HA-U alone.

Table 1. Toluene exposure concentrations to workers as the criterion variable and concentrations of biological exposure indicators for toluene as explanatory variables used in the multiple regression analyses

Period (hr)	Ind.	Tol-EXP (ppm)	Tol-EXH (ppm)	Tol-B (μg/L)	Tol-U (μg/L)	HA-U (g/g cr)	CR-U (μg/g cr)
Last	Mean	21.7	4.40	319.1	35.1	0.87	404
4hr	S.D.	12.9	3.29	237.7	20.5	0.42	157
Tot.	Mean	18.4	3.64	ne	30.5	0.71	312
8hr	S.D.	9.5	2.08	ne	12.9	0.30	105

Ind.= indicator, Tol=toluene, EXP=exposed air, EXH=exhalation, B=blood, U=urine, HA=hippuric acid, CR=o-cresol, cr=creatinine, tot.=total, ne=not examined. Number of workers in Tables 1 $\sim$ 5 is sixteen.

Table 2. Correlation matrix of variables used in the analyses

A. Variables from data for whole eight hours except the values of toluene in blood which were for the last four hours.

	TOL-U	HA-U	CR-U	TOL-EXH	TOL-B	TOL-EXP
TOL-U	1.000					
HA-U	0.682*	1.000				
CR-U	0.581*	0.661*	1.000			
TOL-EXH	0.789**	0.548*	0.500	1.000		
TOL-B	0.711*	0.407	0.416	0.871**	1.000	
TOL-EXP	0.642*	0.755**	0.577*	0.845**	0.670	1.000

TOL-EXP=exposed concentration of toluene, *=$p<0.05$, **=$p<0.01$

B. Variables of biological exposure indicators from data for the last four hours and personal exposure data for the whole eight hours

EXP	TOL-U	HA-U	CR-U	TOL-EXH	TOL-B	TOL-EXP
TOL-U	1.000					
HA-U	0.686*	1.000				
CR-U	0.555*	0.790**	1.000			
TOL-EXH	0.832**	0.536*	0.443	1.000		
TOL-B	0.826**	0.541*	0.464	0.928**	1.000	
TOL-EXP	0.580*	0.781**	0.545*	0.717*	0.670*	1.000

3) In the case of four factors:

The multiple r of TOL-EXH, TOL-U, HA-U and CR-U for eight hours was 0.945, which was higher than the single r (0.834) of HA-U alone and was the highest among multiple r tested using four explanatory variables.The multiple r for TOL-B for the last four hours and TOL-U, HA-U and CR-U for eight hours was 0.923, which was higher than the single r (0.755) for eight hours of HA-U alone.

4) In the case of five factors:

The multiple r for TOL-EXH, TOL-B (pm), TOL-U, HA-U and CR-U for the whole eight hours was 0.946, which was the highest among multiple r tested. The multiple r for TOL-EXH, TOL-B, TOL-U, HA-U and CR-U for the last four hours was 0.924.

Table 3. The multiple correlation coefficient of concentrations of biological exposure indicators in workers associated with toluene exposure concentrations to workers. Biological specimens are from the whole period of exposure (a+p) and the last 4 hours (p).

f.	pe.	TOL-B	TOL-EXH	TOL-U	HA-U	CR-U
1.	am+pm	ne	0.834**	0.641**	0.755**	0.577*
	pm	0.669**	0.717**	0.579*	0.782**	0.545*
2.			① TOL-U,HA-U	② TOL-U,CR-U		③ HA-U,CR-U
	am+pm		0.775	0.690		0.762
	pm		0.784	0.639		0.790
			④ TOL-EXH,TOL-U	⑤ TOL-EXH,HA-U		⑥ TOL-EXH,CR-U
	am+pm		0.846	0.914		0.863
	pm		0.718	0.858		0.761
			⑦ TOL-B,TOL-U	⑧ TOL-B,HA-U		⑨ TOL-B,CR-U
	pm		0.672	0.835		0.720
3.				TOL-U,HA-U,CR-U		
	am+pm			0.778		
	pm			0.792		
4.			① TOL-EXH,TOL-U,HA-U,CR-U			② TOL-B,TOL-U,HA-U,CR-U
	am+pm		0.945			0.923
	pm		0.861			0.880
5.				TOL-EXH,TOL-B,TOL-U,HA-U,CR-U		
	am+pm			0.946		
	pm			0.924		

f. = number of factors. pe. = period of exposure. unit of TOL-U= observed concentration. unit of HA-U and CR-U = corrected concentration with creatinine. * = $p<0.05$, ** = $p<0.01$ in $f = 1$.

3. Multiple regression analysis and multiple regression coefficients squared

Table 4 shows the results of multiple regression analyses using two explanatory variables.

According to the determinant coefficient, the following results were obtained: Two factors, TOL-EXH and HA-U (No.2-5-m), explained 81.0% of the total variance in the observed exposure concentrations of toluene while TOL-EX and CR-U (No.2-6-m) explained 70.6%. Smaller values of Akaike's information criterion were obtained in the above combinations compared to other combinations of two factors. The P values calculated by the F test in analysis of variance were less than 5% and 12 of 15 combinations of explanatory variables were less than 1%.

Table 5 also presents the results of three, four and five variables in multiple regression analyses. Three urinary specimen factors explained only 53.5% of the total variances in the observed exposure concentrations. The four factors of TOL-EXH plus and three urinary specimens explained 85.5% and yielded the lowest value of Akaike's information criteria of 91.7. The four factors of TOL-B (pm) plus three urinary specimens for eight hours (am + pm) explained 64.8%. The five factors of TOL-EXH plus three urinary specimens (am + pm) and TOL-B (pm) explained 84.1% of the total variance in the observed exposure concentrations of toluene. All the P values listed in Table 5 were less than 1%.

Table 4. Results of multiple regression analyses of two factors associated with toluene exposure concentrations to workers.

No. fac.	pe.	MCC	MCC-squ. adjust.*	α_i	β_1	β_2	P %	AIC
2-①	ft.				TOL-U	HA-U		
	am+pm	0.775	0.539	-0.394	0.175	19.13	0.26	108.8
	pm.	0.784	0.555	2.92	0.038	16.25	0.21	108.3
2-②	ft.				TOL-U	CR-U		
	am+pm	0.690	0.395	-0.686	0.341	0.028	1.51	113.2
	pm.	0.639	0.317	3.939	0.186	0.020	3.30	115.1
2-③	ft.				HA-U	CR-U		
	am+pm	0.762	0.519	-0.559	21.41	0.012	0.35	109.6
	pm.	0.790	0.567	4.91	20.92	-0.012	0.17	107.8
2-④	ft.				TOL-EX	TOL-U		
	am+pm	0.846	0.671	4.84	4.09	-0.048	0.03	103.4
	pm.	0.718	0.441	9.88	2.14	-0.026	0.90	111.9
2-⑤	ft.				TOL-EX	HA-U		
	am+pm	0.914	0.810	-1.40	2.81	13.47	0.01>	94.6
	pm.	0.858	0.695	2.37	1.17	12.48	0.02	102.2
2-⑥	ft.				TOL-EX	CR-U		
	am+pm	0.863	0.706	0.157	3.39	0.019	0.01	101.6
	pm.	0.761	0.514	4.15	1.66	0.017	0.36	109.7
2-⑦	ft.				TOL-B	TOL-U		
	pm	0.672	0.367	9.36	0.024	0.039	2.00	113.9
2-⑧	ft.				TOL-B	HA-U		
	pm	0.835	0.650	2.38	13.3	0.014	0.04	104.4
2-⑨	ft.				TOL-B	CR-U		
	pm	0.720	0.445	4.27	0.021	0.018	0.86	111.8

pe.=period of exposure. MCC= multiple correlation coefficient. *= MCC-square adjusted for degree of freedom. P % is obtained by F test in analysis of variance. AIC = Akaike's information criterion. ft.=factors. $Y_2 = \alpha_2 + \beta_1 x_1 + \beta_2 x_2$

Table 5. Results of multiple regression analyses of three, four and five factors associated with toluene exposure concentrations to workers

No. fac.	pe.	MCC	MCC-squ. adjust.*	α_i	β_1	β_2	β_3	β_4	β_5	P %	AIC
3	ft.				TOL-U	HA-U	CR-U				
	m.	0.778	0.506	-1.41	0.159	17.7	0.01			0.91	
	pm.	0.792	0.535	4.69	0.343	35.63	-0.01			0.64	109.7
4	ft.				TOL-EXH	TOL-U	HA-U	CR-U			
	m.	0.945	0.855	0.256	4.01	-0.33	18.2	0.002		0.01>	91.7
	pm.	0.861	0.800	5.44	2.41	-0.31	21.26	0.1		0.01	96.8
4	ft.				TOL-B	TOL-U	HA-U	CR-U			
	m.	0.932	0.648	0.204	0.021	-0.153	20.92	0.004		0.30	105.8
	pm.	0.880	0.693	5.296	0.027	-0.231	21.19	-0.015		0.14	103.6
5	ft.				TOL-U	HA-U	CR-U	TOL-EXH	TOL-B		
	m.	0.946	0.841	0.330	-0.34	18.4	0.0024	3.86	0.002	0.01	93.6
	pm.	0.924	0.781	5.45	-0.32	21.3	0.013	2.23	0.003	0.06	98.7

pe.=period of exposure. ft.= factors. m.= am+pm. MCC= multiple correlation coefficient. *= MCC-square adjusted for degree of freedom. AIC = Akaike's information criterion. $Y_3 = \alpha_3 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3$, $Y_4 = \alpha_4 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4$. $Y_5 = \alpha_5 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_5 x_5$

Discussion

In a previous paper [3], the single correlation coefficient between amount of personal exposure and each biological exposure indicator for the whole eight hours or the last four hours was reported. The data indicated that the correlation coefficient of exposure concentration was in the descending order of TOL-EXH, HA-U, TOL-U and CR-U for eight hours and HA-U, TOL-EXH, TOL-B, TOL-U and CR-U for the last four hours.

In the present report, multiple correlation coefficients of exposure concentrations of toluene with concentrations of five biological determinants for eight hours and for four hours, except that only TOL-BL p.m. was used, were calculated and compared with single correlation coefficients.

With regard to the collection of biological specimens, the biological half lives of toluene and its metabolites in biological samples are relatively short. The half life of TOL-EX and TOL-BL are 0.5 hour while that of HA-U is 1.5 hours [7]. Therefore, in the survey of workers exposed to 15.1 ppm in the a.m. and 21.7 ppm in the p.m., the correlation coefficients for exposure concentrations of TOL-EX and TOL-BL for the whole eight hours are higher than the coefficients for the last four hours [3]. In addition, levels of biological exposure indicators for the last four hours were used for the biological exposure indices of the ACGIH in the USA [1].

In the present study, considering the results described above, the levels of biological exposure indicators for the last four hours and whole eight hours were used as explanatory variables, except that levels of toluene in the blood collected during the last four hours were used.

There are many combinations for explanatory variables, and the ones useful in a practical way were selected in this study as follows:

1) In the case of two factors, combinations of ① two of the three urinary indicators and ② toluene in exhaled air or toluene in blood plus one urinary indicator were selected. The correlation coefficient of one pair among TOL-EXH, TOL-B and TOL-U showed higher values, because TOL-EXH is diffused from the blood to alveolar air according to the air / blood partition coefficient and TOL-U is toluene diffused from the blood to the glomerular filtrate in the kidneys according to the glomerular filtrate / blood partition coefficient. The half lives of TOL-EXH ,TOL-B and TOL-U are shorter than that of HA-U. Therefore, TOL-EXH ,TOL-B and TOL-U are useful only if the toluene densities in the air are stable during the work period. As a result, adding HA-U and/or CR-U to TOL-EXH, TOL-B and TOL-U as factors in the multiple regression equations resulted in improved correlation coefficients even though HA-U levels are affected by benzoic acid and CR-U levels are affected by smoking [3].
2) In the case of three factors, three urinary variables were used.
3) In the case of four factors, toluene in exhaled air or toluene in blood in combination with the three urinary variables were selected.

In a previous paper [12], amounts of toluene inhaled by workers wearing protective masks were estimated by using single regression equations on determinants derived from toluene in biological specimens. Percentages of toluene removed by wearing a mask are estimated to be 34 percent by multiple regression equations. These findings will be reported in detail in a forthcoming paper.

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