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Original Paper

Biological Exposure Indicators for Low Exposure to Toluene

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Abstract

Sixteen male workers were exposed to toluene at sub-occupational exposure limits and examined for the time-weighted average of exposure by diffusive personal sampling. The urine, blood and exhaled air collected during the first and last four hours were analyzed for biological exposure indicators. There was a significant correlation between toluene concentrations in the air on the one hand and the concentrations of toluene in the blood, urine and exhaled air and urinary hippuric acid and o-cresol corrected with creatinine. The levels of five indicators corresponding to threshold limit values of 50 ppm and time-weighted average of toluene (TLV-TWA) were determined. The biological indicators were used to determine discriminative concentrations of toluene (DC_5) which were considered to discriminate exposure from non-exposure within a five percent margin of error. The proper biological indicators were selected from the levels of DC_5, agent specificity, and convenience for sampling and storage procedures. In the present study, the average concentrations of toluene vapors in the breathing zone of workers during the first and last four hours were 14.7 and 25.0 ppm, respectively. Under these conditions, the correlation coefficients between the concentrations of toluene during the whole eight hours and levels of major biological exposure indicators collected during the whole eight hours were higher than the correlation coefficients between concentrations of toluene during the whole eight hours and levels of biological exposure indicators collected during just the last four hours. The shorter biological half life of toluene and the timing of taking specimens for biological monitoring are discussed.

Introduction

Toluene is one of the most popular organic solvents and many workers are exposed to toluene in their work. The five biological indicators for toluene exposure are toluene in the blood (TOL-BL), toluene in exhaled air (TOL-EX), urinary toluene (TOL-U), urinary hippuric acids (HA-U) and urinary o-cresol(CR-U)[1].

In previous papers, the authors reported the use of urinary hippuric acid[2,3] and urinary o-cresol[4] as the biological indicators for urinary biomonitoring for toluene exposure.

The threshold limit value (TLV) of toluene was changed from 100ppm to 50ppm[5], and changes in the biological exposure indicators so that they correspond to 50ppm of toluene have been investigated since then.

A survey was carried out among workers exposed to toluene to investigate the relationship between the amount of exposure to toluene and biological indicator levels. The levels of biological exposure indicators corresponding to 50ppm TLV-TWA of toluene were calculated.

Suitable biological indicators for toluene exposure are also discussed.

Materials and Methods

1. Materials

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

2) Reagents: All reagents used in the present study were reagent grade.

2. Methods

1) Sampling methods:

(1) Sampling procedure: The specimens were collected after the first 4 hours of exposure (0-4h) and at the end of exposure (5-9h). Blood samples were taken at the end of exposure.

(2) Toluene vapors in ambient air: Solvents in the ambient air were absorbed by activated charcoal in a passive sampling tube (M type from Komiyo Kagaku Co, Tokyo) at 1 L/min. Collection was for 60minutes for the A sampling method[6] and 10minutes for the B sampling method[6]. The passive gas tube (Shibata Co, Tokyo) was attached to the collar of each worker and morning and afternoon samples were collected separately. Solvents absorbed on the activated charcoal were desorbed by 2-hydroxypyridine and applied to a headspace-gas chromatograph[7].

(3) Toluene and its metabolites in biological specimens:

a. Toluene in exhaled air: One liter of exhaled air from each individual was collected in a four liter Tedlar bag, and applied to a passive sampling tube (M type).

b. Toluene in urine: The urine samples were immediately transferred and filled into screw-capped glass tubes with a Teflon septum.

c. Toluene in blood: Blood samples (5ml with EDTA-2 Na) were taken from the antecubital vein. Immediately after collection, the blood was transferred and filled into screw-capped vials (4mL) and sealed.

2) Measurement of determinants:

(1) Headspace-gas chromatography: Concentrations of solvent gases were determined by headspace-gas chromatography. The analytical procedure is described in Table 1.

(2) Gas chromatography: For the determination of urinary o-cresol, five mL of urine was put into test tubes with screw caps, one mL of concentrated hydrochloric acid was added and the mixture was heated at 100° for 1 hour in a water bath to hydrolyze the

urine. After cooling, isopropyl ether was added to extract the o-cresol and the isopropyl ether was then injected into a gas chromatograph.

(3) High performance liquid chromatography: Hippuric acid was determined by high performance liquid chromatography in the following manner: Urine was diluted 1:40 with a mixture of methanol and deionized water (1:1) and filtered through a membrane filters with a 0.45 μm pore size. Five μL of the filtrate was applied to a high performance liquid chromatograph (Integra. 4,000, Perkin Elmer Co, USA).

A column (ϕ 4mm x L50mm) packed with octadecyl-silanized silica gel (Tsk gel ODS-80, 5 μm, Tosoh Co, Japan), and a UV detector at 230nm wavelength were used throughout this investigation. A mixed solution of deionized water/methanol/acetic acid (800/250/3 by volume) was used as the mobile phase for the separation of hippuric acid.

Results

The symbols used in the tables for the biological indicators of toluene exposure are listed in Table 2.

1. *Amount of toluene exposure and levels of biological indicators of toluene*

Table 3 summarizes the monitored concentrations of toluene and the corresponding concentrations of biological indicators. Toluene concentrations in the breathing zone of workers during the first four hours were 15.1ppm and concentrations during the last four hours were 21.7ppm, indicating a gradual increase over the eight hour period. The exposure was relatively low.

2. *Correlation between toluene concentrations in the air and urinary indicators, with and without correction for urine volume*:

Table 1 Analytical conditions for the determination of solvents in the air by headspace gas chromatography

A. Toluene in exhaled air:
Gas chromatograph: Sigma-2000 from Perkin Elmer Co, USA, was used.
Headspace sampler SH-100, Perkin Elmer Co, H used.

Condition for headspace samplers		Condition for gas chromatography	
Sample temperature	80℃	Column Supercowax-10	
Transfer temperature	150℃	90mx0.32mmI.D. Film	0.5μm
Thermostatting time	30min	Split ratio	30 : 1
Pressurization time	0.5min	Column flow (N_2)	2.0ml
Sampling time	0.02min	Column pressure	26PSI
Withdrawal time	0.2min	Oven temperature	90℃
Vent	−1	Detector temperature	200℃

B. Toluene in urine and blood:

Gas chromatographic conditions were similar to Table 1-A except that Column was SPB-1, 90mx0.32 mmI.D. Film 0.5μm and the split ratio was 15 : 1

C. o-Cresol in urine:

Gas chromatographic conditions were similar to Table 1-A except that column was 5890-II split less, oven temperature 100℃, one min., rate = 60℃/min. and 180℃ for 30 min, injection volume, 1μl., and detector temperature 200℃

The correlation coefficients: The results are listed in Table 4. In order to determine the proper correction for urine volume, uncorrected indicator concentrations, concentrations corrected for creatinine and specific gravity were compared. The correlation coefficients between toluene concentrations in the breathing zone air during the eight hour work period and exposure indicator concentrations in the urine taken during the last four hours of exposure were calculated.

1) Correlation between amount of toluene

Table 2 Explanation of symbols used in the tables.

Tol-Aa	levels of toluene vapor in breathing zone repre. of first 4 H. of exp.
Tol-Ap	levels of toluene vapor in breathing zone repre. of last 4 H. of exp.
Tol-Aa+p	levels of toluene vapor in breathing zone repre. of the whole period of exp.
Tol-EHa	toluene concentrations in exhaled air repre. of first 4 H. of exp.
Tol-EHp	toluene concentrations in exhaled air repre. of last 4 H. of exp.
Tol-EHa+p	toluene concentrations in exhaled air repre. of the whole period of exp.(8 H).
Tol-BLp	toluene concentrations in the blood repre. of last 4 H. of exp.
Tol-Ua	toluene concentrations in the urine repre. of first 4 H. of exp.
Tol-Up	toluene concentrations in the urine repre. of last 4 H. of exp.
Tol-Ua+p	toluene concentrations in the urine repre. of the whole period of exp.
HA-Ua	hippuric acid concentrations in the urine of first 4 H. of exp.
HA-Up	hippuric acid concentrations in the urine of last 4 H. of exp.
HA-Ua+p	hippuric acid concentrations in the urine of the whole period of exp.
o-Cr-Ua	hippuric acid concentrations in the urine of first 4 H. of exp.
o-Cr-Up	hippuric acid concentrations in the urine of last 4 H. of exp.
o-Cr-Ua+p	hippuric acid concentrations in the urine of the whole period of exp.
cor.	correction or corrected.
ob.	observed concentrations of urinary determinants without correction
Cr.	concentrations of urinary determinants corrected with concentration of urinary creatinine
sp.	concentrations of urinary determinants corrected with urinary specific gravity

repre.= representative and exp.=exposure

Table 3 Concentrations of toluene in the air and concentrations of biological exposure indicators for toluene.

period (hr)	parameter	tol in air (ppm)	tol in exh (ppm)	tol in blood (μg/L)	tol in urine (μg/L)	HA in urine (g/L)*	HA in urine (g/gcr)	o-Cr in urine (g/L)*	o-Cr in urine (μg/gcr)
fir.	mean	15.1	2.94	ne	25.8	0.75	0.54	309	220
4hr	S.D.	7.7	1.54		11.0	0.31	0.24	117	88
last	mean	21.7	4.40	319.1	35.1	1.11	0.87	528	404
4hr	S.D.	12.9	3.29	237.7	20.5	0.42	0.42	190	157
tot.	mean	18.4	3.64	ne	30.5	0.93	0.71	419	312
8hr	S.D.	9.5	2.08		12.9	0.31	0.30	138	105

tol = toluene, HA = hippuric acid, o-Cr = o-cresol, exh = exhaled air,
(g/L)*: corrected for the specific gravity of urine (1.024),
cr = creatinine, fir. = first, tot. = total and ne = not examined.

exposure and urinary concentrations of hippuric acid:

Table 4 The correlation coefficients between toluene vapor in the breathing zone and concentrations of urinary determinants indicators in subjects exposed to toluene. Toluene vapors and biological specimens are representative of the first 4 hours, the last 4 hours and whole period of exposure. Urine values were without correction and concentrations corrected with creatinine and specific gravity are presented.

cor.	air(x) detor.(y)	Tola	Tolp	Tola+p
	Tol-EHa	0.775**	0.582*	0.707**
	Tol-EHp	0.388	0.829**	0.717**
	Tol-EHa+p	0.587**	0.884**	0.834**
	Tol-Bp	0.304	0.810**	0.669**
	Tol-Ua	0.528*	0.314	0.426
ob	Tol-Up	0.257	0.705**	0.579*
	Tol-Ua+p	0.429	0.693**	0.641**
	HA-Up	0.293	0.450	0.422
	HA-Ua+p	0.222	0.209	0.231
	o-Cr-Up	0.021	0.091	0.052*
	o-Cr-U-a+p	0.075	0.209	0.031
	Tol-Ua	0.619*	0.512*	0.596*
	Tol-Up	0.452	0.818**	0.735**
	Tol-Ua+p	0.610*	0.796**	0.784**
	HA-Ua	0.204	0.359	0.470
cr.	HA-Up	0.590*	0.806**	0.782**
	HA-Ua+p	0.610	0.727**	0.755**
	o-Cr-Ua	0.573*	0.247	0.413
	o-Cr-Up	0.376	0.582*	0.545*
	o-Cr-Ua+p	0.519*	0.544*	0.577*
	Tol-Ua	0.406	0.532*	0.631**
	Tol-Up	0.452	0.844**	0.756**
	Tol-Ua+p	0.640**	0.822**	0.841**
	HA-Ua	0.406	0.198	0.298
sp.	HA-Up	0.574*	0.746**	0.735**
	HA-Ua+p	0.608*	0.618*	0.660**
	o-Cr-Ua	0.449	0.119	0.263
	o-Cr-Up	0.213	0.348	0.320
	o-Cr-Ua+p	0.336	0.289	0.331

*$p<0.05$, **$p<0.01$
cor. = correlation, ob = observed concentration
cr. = corrected with creatinine
sp = corrected with specific gravity

The correlation coefficient between toluene concentrations in the breathing zone air during the eight hour work period and urinary hippuric acid corrected with creatinine taken in the last four hours, 0.782, was the highest.

The correlation coefficient for concentrations corrected with specific gravity was 0.735, while the uncorrected value of 0.422 was the lowest. When hippuric acid concentrations in urine taken during the whole eight hours was used instead of the last four hours, a similar result was obtained.

2) Correlation between amount of toluene exposure and urinary concentration of o-cresol:

The correlation coefficient between toluene concentrations in the breathing zone air during the eight hour work period and concentrations of o-cresol corrected for creatinine in the urine during the last four hours, 0.545, was the highest. The correlation coefficient for urine taken in the last four hours corrected for specific gravity was 0.320 and the correlation coefficient for uncorrected urine taken during the last four hours was the lowest at 0.052. Therefore, urinary hippuric acid and o-cresol values corrected for creatinine were used in the present study.

3) Correlation between amount of toluene exposure and concentration of urinary toluene.

The correlation coefficient between toluene concentrations in the breathing zone air taken during the eight hour work period and concentrations of toluene in the urine taken in the last four hours and corrected for specific gravity was the highest at 0.756. The correlation coefficient for urine taken in the last four hours and corrected for creatinine was also high at 0.735 and the correlation coefficient for uncorrected urine was the lowest at 0.579.

Uncorrected concentrations were used for urinary toluene levels, because toluene diffuses from the blood to the urine and urinary concentrations of toluene are not affected by urine volume. On the other hand, the correlation coefficients between the amount of toluene exposure and urinary toluene concentrations corrected for specific gravity in the urine taken during the last four hours was 0.756 and the whole eight hours was 0.841, which were the highest among the corrected and uncorrected toluene concentrations in the urine. Therefore, the concentration of urinary toluene corrected for specific gravity was used as a supplemental value.

3. *Comparison of correlation coefficients among concentrations of toluene in the breathing zone and concentrations of biological exposure indicators for toluene taken at different times.*

Results are also listed in Table 4. Toluene in exhaled air, toluene in blood, toluene in urine, hippuric acid in urine and o-cresol in urine were used as the biological exposure indicators of toluene.

1) A comparison of the correlation coefficients between intensity of toluene exposure for the whole eight hours and levels of biological indicators of toluene taken during the last four hours and the whole eight hours:

Correlation coefficients between toluene concentrations in the breathing zone air during the whole eight hours and biological indicator concentrations of toluene taken during the last four hours were 0.717 for exhaled air, 0.669 for toluene in blood, 0.579 for toluene in urine, 0.782 for hippuric acid in urine corrected with creatinine and 0.545 for o-cresol in urine corrected with creatinine.

Correlation coefficients between toluene levels in the breathing zone air taken during the whole eight hours and levels of biological indicators of toluene taken during the whole eight hours were 0.834 for exhaled air, 0.641 for toluene in urine, 0.755 for hippuric acid in urine corrected with creatinine and 0.577 for o-cresol in urine corrected with creatinine.

Correlation coefficients between the amount of personal exposure to toluene during the whole eight hours and biological indicator concentrations during the last four hours were lower than those during the whole eight hours, except for a small opposite differences in urinary hippuric acid.

Biological exposure indicators for toluene are excreted in a relatively short period which results in a shorter half life.

These differences in concentrations of indicators in specimens taken at different times arose because the half life of hippuric acid is 1.5 hours and the half life of toluene in the blood and exhaled air is 0.5 hours under the conditions in which toluene concentrations in the morning and afternoon were 15.1 ppm and 21.7 ppm, respectively.

2) Comparison of correlation coefficients between amount of exposure to toluene during the last four hours and biological indicators concentrations during the last four hours and the whole eight hours:

Correlation coefficients between toluene concentrations in the breathing zone taken during the last four hours and biological exposure indicator concentrations were calculated. In the urine collected during the last four hours, the correlation coefficients were 0.829 for toluene in exhaled air, 0.810 for toluene in the blood, 0.705 for toluene in urine, 0.806 for urinary hippuric acid corrected with creatinine and 0.582 for urinary o-cresol corrected with creatinine.

These coefficients are generally higher than urine collected during the whole eight hours which were 0.884 for toluene in the exhaled air, 0.693 for toluene in the urine, 0.727 for urinary hippuric acid and 0.544 for

urinary o-cresol corrected with creatinine. These differences were also due to the shorter biological half life of toluene.

3) The order of the correlation coefficients between amount of toluene exposure for eight hours and biological exposure indicator concentrations taken during the last four hours:

The correlation coefficients, in descending order, were 0.782 for urinary hippuric acid corrected with creatinine, 0.717 for toluene in exhaled air, 0.669 for toluene in the blood, 0.579 for toluene in the urine and 0.545 for urinary o-cresol corrected with creatinine. The coefficient for urinary toluene corrected for specific gravity was 0.756.

4. *Regression equation:*

Regression equations between toluene concentrations in the breathing zone during the whole eight hours and biological exposure indicator concentrations in specimens taken during the last four hours and during the whole eight hours were calculated.

The regression lines between amount of exposure to workers during the whole eight hours and five biological exposure indicator concentrations collected during the last four hours are shown in Fig. 1. and Fig. 2.

The regression lines between amount of exposure to workers during the whole eight hours and five biological exposure indicators collected during the whole eight hours are shown in Fig. 3. and Fig. 4. The constants for the regression equations between toluene concentrations in the breathing zone air and concentrations of biological exposure indicator concentrations in specimens taken at different times are listed in Table 5.

5. *Values of the biological exposure indicators of toluene corresponding to 50 ppm of toluene.*

The mean values, the 5 % confidence range for the mean and the 5 % predictive range for each specimen, corresponding to 50 ppm toluene of TLV-TWA, were calculated from regression equations and are shown in Table 6.

The mean values of the biological exposure indicators corresponding to 50 ppm TLV-TWA toluene were calculated to be 13.1 ppm for toluene in exhaled air, 846 μg/L for toluene in blood, 74.6 μg/L for urinary toluene, 92.7 μg/L for urinary toluene corrected for specific gravity of 1.024, 1.98 g/gcr.for urinary hippuric acid and 688 μg/gcr. urinary o-cresol. These values are for specimens taken during the last four hours.

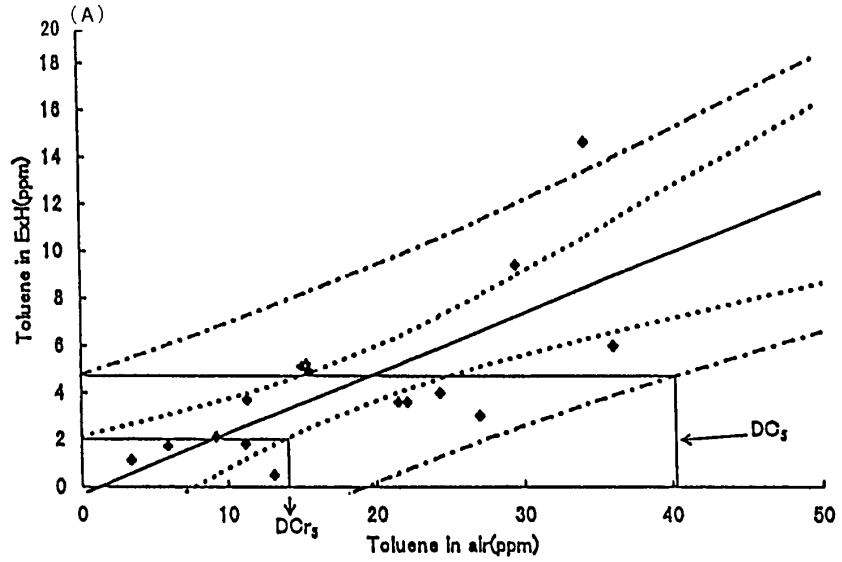
The corresponding mean values for specimens during the whole eight hours were 9.44 ppm for toluene in exhaled air, 58.0 μg/L for urinary toluene, 80.8 μg/L for urinary toluene corrected for a specific gravity of 1.024, 1.44 g/gcr. for urinary hippuric acid and 514 μg/gcr. for urinary o-cresol.

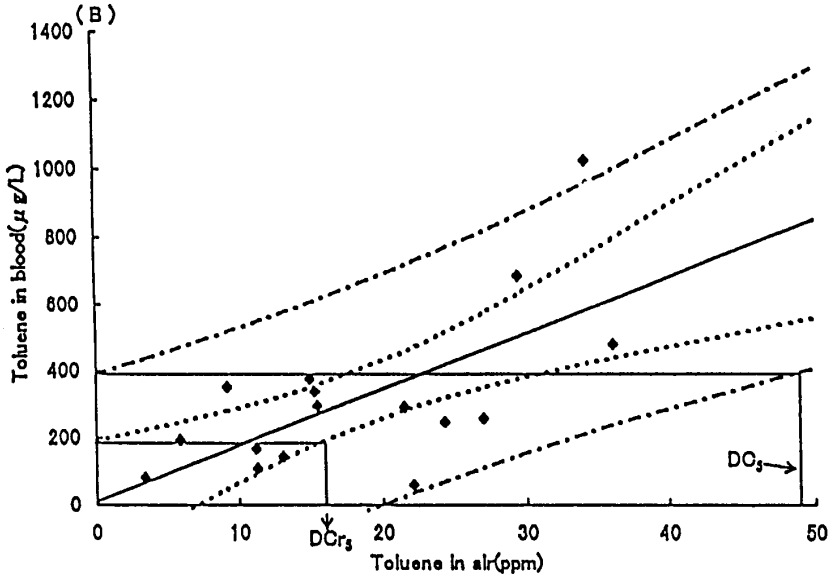
There are some differences in the regression equations between amount of personal exposure in working days and five biological indicators of toluene collected during the first and last four hours and the whole eight hours. This is due to the fact that the half lives of biological exposure indicators are relatively short, as described in the discussion.

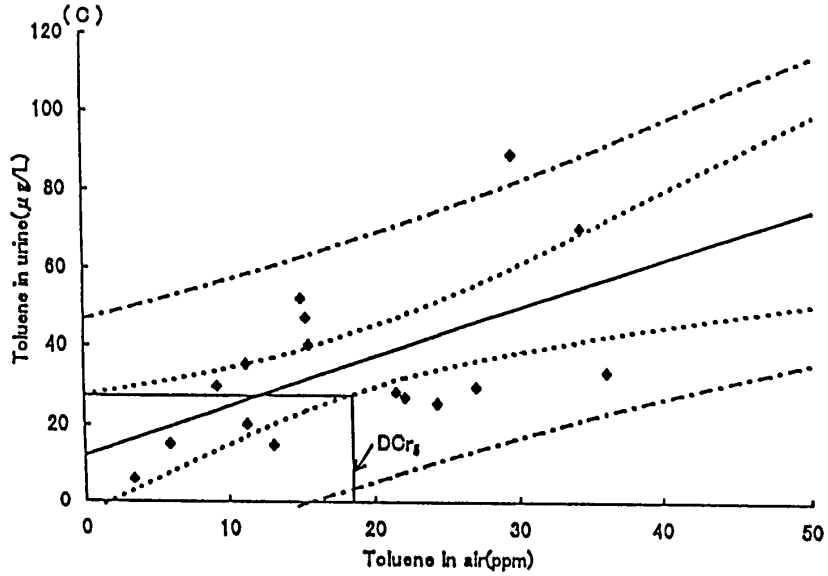
6. *The matrix of correlation coefficients among determinants derived from ambient toluene in biological specimens*

The matrix of correlation coefficients among toluene in exhaled air and toluene and hippuric acid and o-cresol in urine are listed in Table 7.

There was a significant correlation between toluene levels in blood and toluene levels in exhaled air, ($r = 0.93$, $p < 0.01$), toluene levels in blood and toluene levels in urine, ($r = 0.83$, $p < 0.01$), and toluene levels in blood and hippuric acid levels in urine, ($r = 0.54$, $p < 0.05$), when specimens taken during the last four hours of exposure were used.


(A)
Toluene in ExH(ppm)
Toluene in air(ppm)
DCr5
DC5


(B)
Toluene in blood(μg/L)
Toluene in air(ppm)
DCr5
DC5


(C)
Toluene in urine(μg/L)
Toluene in air(ppm)
DCr5

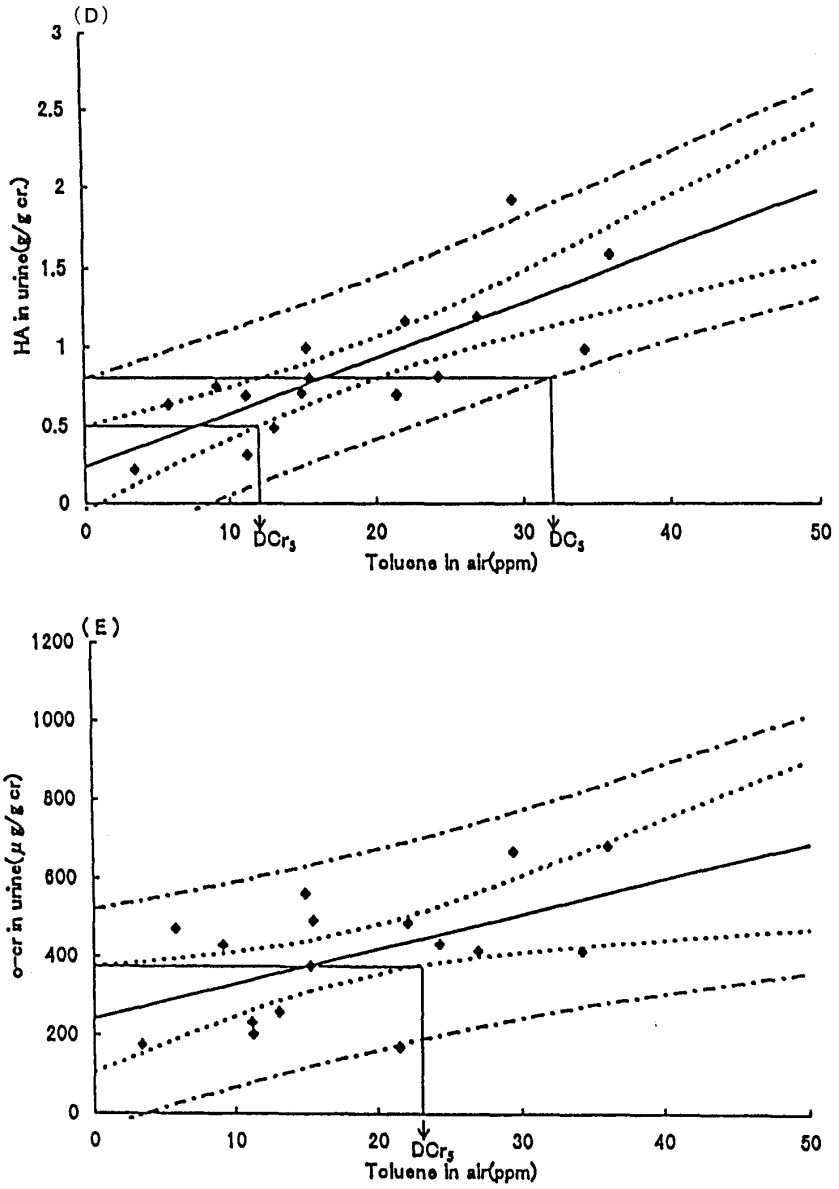


Fig. 1 Regression lines with a 90 percent confidence and predictive intervals between toluene concentrations in the breathing zone air taken during the whole eight hours and the concentrations of biological exposure indicators of toluene collected during the last four hours.
Dotted lines indicate the 90 % confidence intervals of the regression line and chain lines indicate the 90 % predictive intervals of the individual samples.
Fig. A = toluene in air versus toluene in the exhaled air. Fig. B = toluene in air versus toluene in the blood. Fig. C = toluene in air versus toluene in the urine. Fig. D = toluene in air versus hippuric acid in the urine. Fig. E = toluene in air versus o-cresol in the urine. Toluene in air is on the x-axis on all figures. DC_5 = The discriminant concentration of toluene at a 5 percent level of error from individual samples. DCr_5 = The discriminant concentration of toluene at a 5 percent margin of error from the 90 percent confidence interval of regression lines.

Discussion

A comparison of correlation coefficients between amount of exposure and biological exposure indicators taken at different times was carried out. The data indicate that the correlation coefficients between amount of exposure to toluene during the whole eight

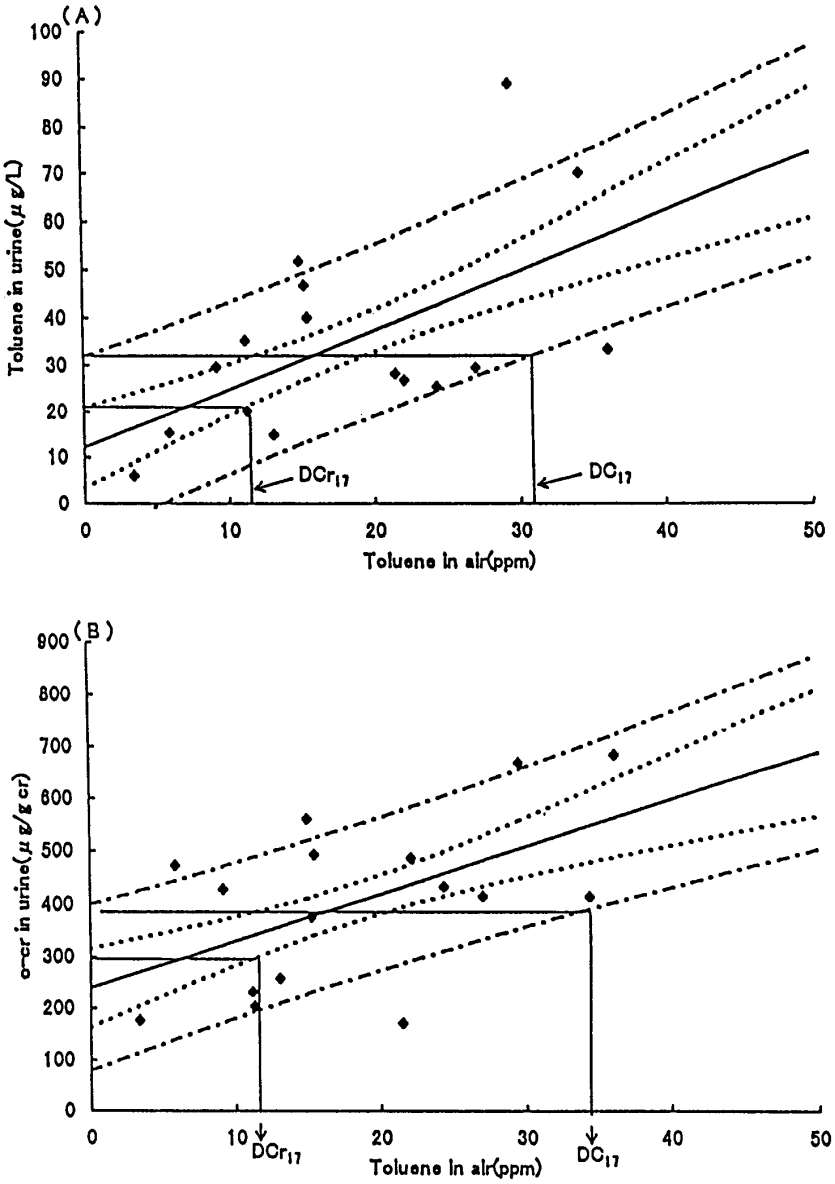
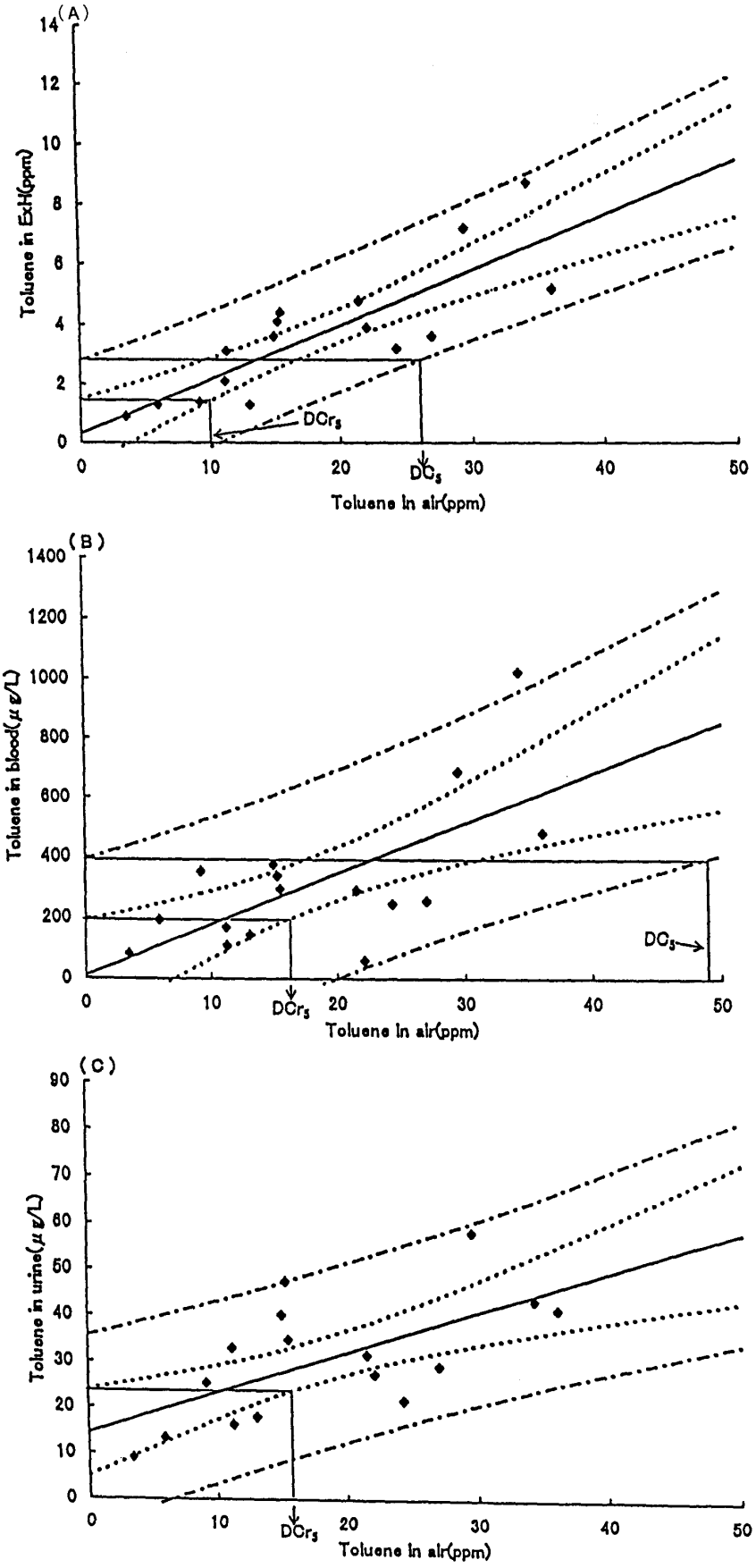


Fig. 2 Regression lines with a 66 percent confidence and predictive intervals between toluene concentrations in the breathing zone air taken during the whole eight hours and the concentration of biological exposure indicators of toluene collected during the last four hours.
Dotted lines indicate the 66 % confidence intervals of the regression line and chain lines indicate the 66 % predictive intervals of the individual samples.
Fig. A = toluene in air versus toluene in the urine. Fig.B = toluene in air versus o-cresol in the urine. DC_{17} = The discriminant concentration of toluene at a 17 percent level of error from individual samples. DCr_{17} = The discriminant concentration of toluene at a 17 percent margin of error from the 66 percent confidence interval of regression lines.

hours and biological exposure indicator concentrations during the last four hours were lower than the coefficients during the whole eight hours.

The difference is due to the fact that the half lives of the biological exposure indicators are relatively short. The half life of hippuric acid is 1.5 hours and half life of toluene in the blood and exhaled air is 0.5 hour[8]. Therefore, biological exposure indicator concentrations can be useful only in the case that toluene concentrations are relatively stable


(A)
Toluene in ExH(ppm)
Toluene in air(ppm)
DCr$_s$
DC$_s$
(B)
Toluene in blood(μg/L)
Toluene in air(ppm)
DCr$_s$
DC$_s$
(C)
Toluene in urine(μg/L)
Toluene in air(ppm)
DCr$_s$

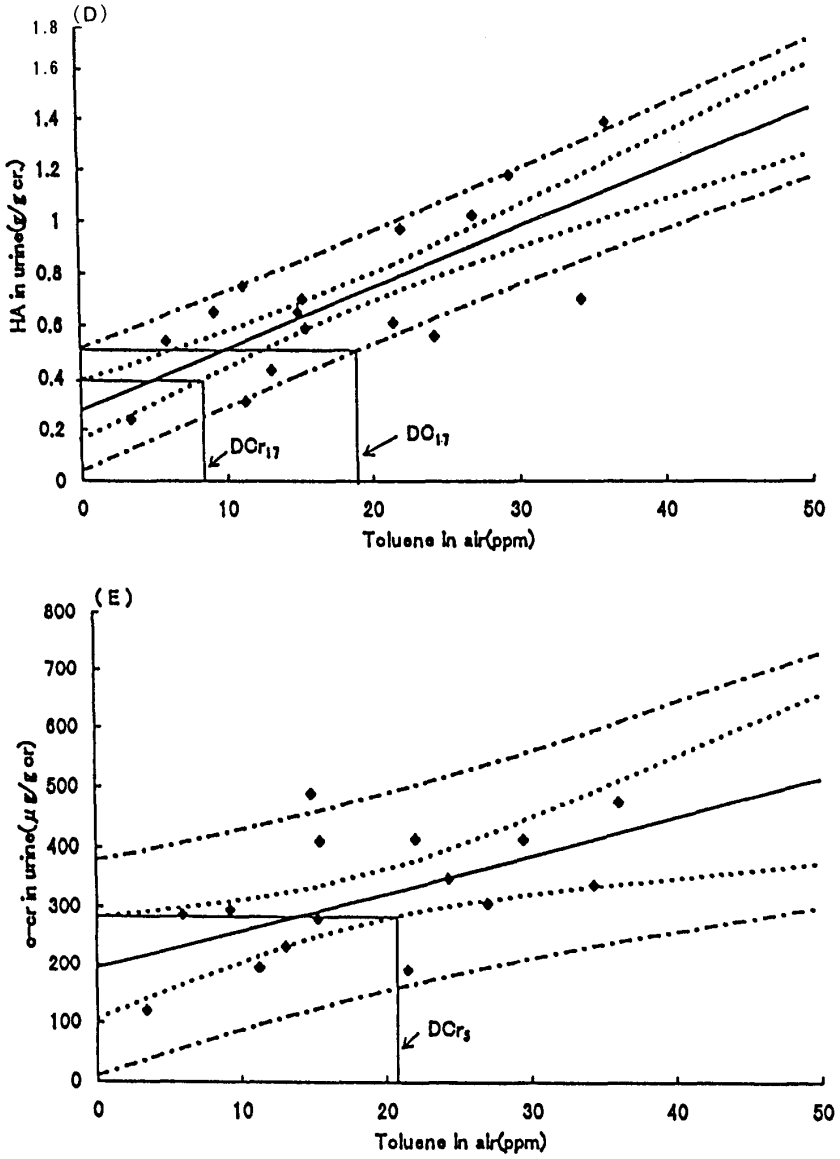


Fig. 3 Regression lines with a 90 percent confidence and predictive intervals between toluene concentrations in the breathing zone air taken during the whole eight hours and the concentration of biological exposure indicators of toluene collected during the whole eight hours.
Dotted lines indicate the 90 % confidence intervals of the regression line and chain lines indicate the 90 % predictive intervals of the individual samples.
Fig. A = toluene in air versus toluene in the exhaled air. Fig. B = toluene in air versus toluene in the blood (an exceptional case: the blood was taken at the end of the last four hours). Fig. C = toluene in air versus toluene in the urine. Fig. D = toluene in air versus hippuric acid in the urine. Fig. E = toluene in air versus o-cresol in the urine. DC_5 = Discriminant concentration of toluene at a 5 percent level of error from individual samples. DCr_5 = The discriminant concentration of toluene at a 5 percent margin of error from the 90 percent confidence intervals of regression lines. note: in Fig. D, 66 percent confidence and predictive intervals were depicted.

during the working day. The results suggest that when worker exposure to toluene is different in the morning and afternoon, biological exposure indicator concentrations for the whole working day are a better indicator of exposure than the concentrations at the

end of a shift.

Determinant concentrations in the present study were compared with the results of other scientists. In the present study, concentrations of toluene in blood, urinary toluene, urinary hippuric acid and urinary o-cresol in specimens taken during the last four hours were 0.85mg/L, 74.6μg/L, 1.98g/gcr. and 0.69mg/gcr., respectively. Urinary toluene, urinary hippuric acid, and urinary o-cresol in specimens taken during the whole eight hours were 58.0μg/L., 1.44g/gcr and 0.51mg/gcr.,

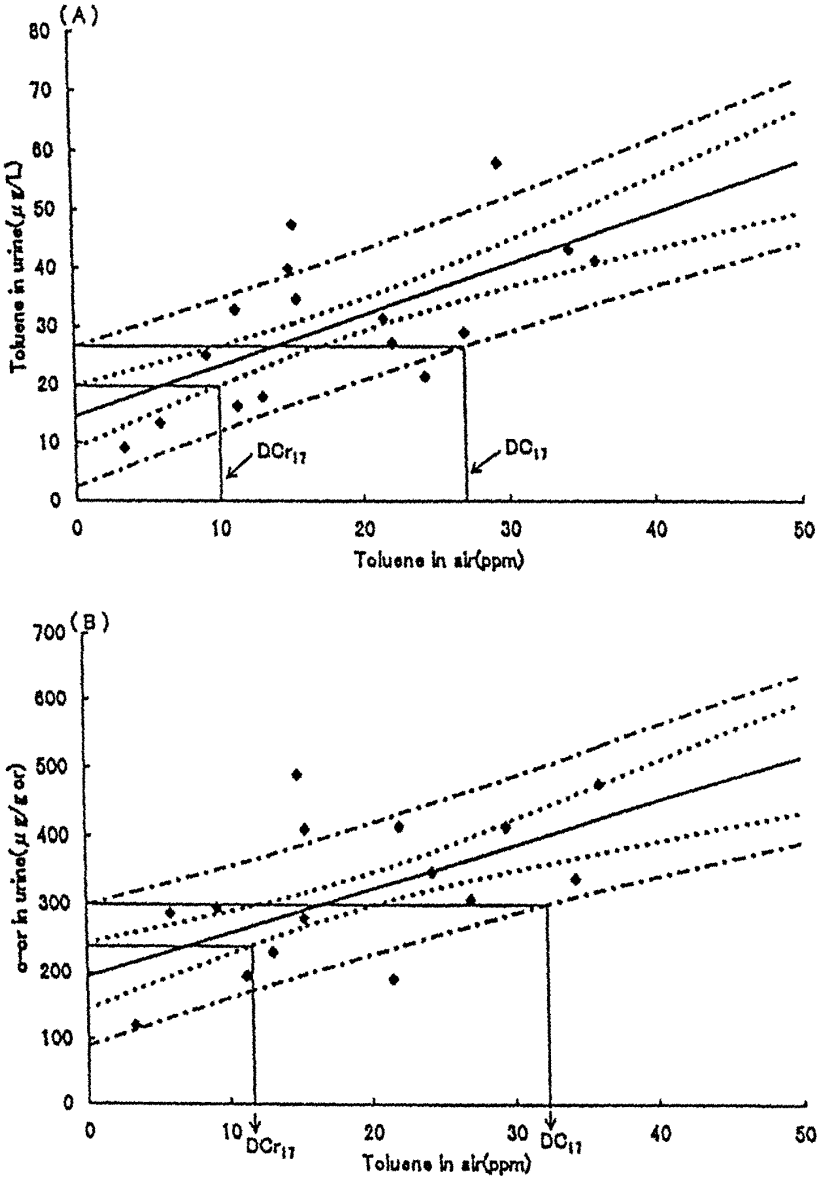


Fig. 4 Regression lines with a 66percent confidence and predictive intervals between toluene concentrations in the breathing zone air taken during the whole eight hours and the concentration of biological exposure indicators of toluene collected during the whole eight hours.
Dotted lines indicate the 66confidence intervals of the regression line and chain lines indicate the 66predictive intervals of the individual samples.
Fig. A = toluene in air versus toluene in the urine. Fig. B = toluene in air versus o-cresol in the urine. DC_{17} = Discriminant concentration of toluene at a 17percent level of error from individual samples. DCr_{17} = The discriminant concentration of toluene at a 17percent level of error from the 66percent confidence intervals of regression lines.

respectively. Toluene concentrations in the breathing zone of workers was 15.1 ppm during the first four hours and 21.7 ppm during the last four hours.

The results of other scientists for toluene in blood, urinary toluene, urinary hippuric acid and urinary o-cresol were 0.64 ± 0.150 mg/L ($n = 9$)[9-11], 71.5 μg/L, ($n = 2$)[17,18], 1.61 ± 0.15 g/gcr. ($n = 4$)[15,19-21] and 0.51 ± 0.053 mg/gcr. ($n = 5$)[19,20,22,23], respectively.

A comparison of the present data with the previous results of other scientists indicated the following: ① Urinary toluene concentrations taken during the last four hours was 74.5 μg/L, which is similar to results of other scientists. ② Urinary hippuric acid concentrations taken during the last four hours was 1.98 g/gcr. and the concentration during the whole eight hours was 1.44 g/gcr. The values of other scientists are between these values and were done at different times (4hrs and 8hrs). ③ Urinary o-cresol concentrations taken during the last four hours was 514 μg/gcr., which is similar to the results of other scientists.

A comparison of the correlations between exposure and concentrations of determinants is as follows: In the present study, correlation coefficients between exposure concentrations and five biological determinant concentra-

Table 5 The constants for the regression equations between toluene vapor concentrations in the breathing zone and concentrations of determinants derived from toluene in the biological specimens of workers exposed to toluene. Conditions and abbreviations in this tables are the same as those in Table 4.

cor	air(x)	Ta		Tp		Ta+Tp	
	deter.(y)	a	b	a	b	a	b
ob.	Tol-EHa	0.593	0.155	1.43	0.069	0.836	0.114
	Tol-EHp	1.805	0.172	0.342	0.219	0.312	0.256
	Tol-EHa+p	1.270	0.159	0.599	0.143	0.315	0.182
	Tol-Bp	177	9.42	-5.19	15.0	11.0	16.7
	Tol-Ua	14.4	0.758	20.0	0.269	16.7	0.494
	Tol-Up	24.7	0.685	10.8	1.13	12.1	1.25
	Tol-Ua+p	19.6	0.721	15.4	0.696	14.5	0.871
cr.	HA-Ua	0.501	0.195	0.39	0.007	0.316	0.012
	HA-Up	0.379	0.033	0.295	0.027	0.228	0.035
	HA-Ua+p	0.325	0.025	0.345	0.017	0.274	0.023
	o-Cr-Ua	120	6.58	180	1.83	150	3.83
	o-Cr-Up	288	7.65	251	7.09	239	8.97
	o-Cr-Ua+p	204	7.11	216	4.46	194	6.40
sp.	HA-Ua	0.504	0.016	0.649	0.005	0.573	0.010
	HA-Up	0.637	0.031	0.583	0.024	0.513	0.032
	HA-Ua+p	0.573	0.024	0.617	0.015	0.544	0.021
	o-Cr-Ua	206	6.85	286	1.09	250	3.24
	o-Cr-Up	449	5.25	417	5.13	411	6.38
	o-Cr-Ua+p	327	6.05	351	3.11	330	4.81

$y = a + bx$, where x = toluene concentration breathing zone air [air(x)]
y = determinant concentrations in biological specimens [deter.(y)]
cor. = correlation
When concentration of toluene in urine corrected for specific gravity and concentration of toluene in the air taken during the whole eight hours ($y = Ta + Tp$) were utilized, values for a and b were calculated. When Tol-Up(x) was used, $a = 4.70$, $b = 1.76$ were obtained. When Tol-Ua+p(x) was used, $a = 6.77$, $b = 1.48$ were obtained

tions taken at shift-end showed that HA-U had the highest correlation followed in descending order by HA-U, TOL-EX, TOL-BL, TOL-U and CR-U. Data suggest that the order is useful for recommending suitable indicators. We should keep in mind that the TOL-U corrected for specific gravity showed a high correlation coefficient.

The discriminative concentrations[3,24] of toluene which discriminate exposure from non-exposure were 49 ppm for toluene in the blood, 40.4 ppm for toluene in exhaled air and 31.9 ppm for hippuric acid in urine, within a five percent of margin of error. When these values for biological indicators are less than 50 ppm TLV-TWA of toluene, the indicators can be useful as toluene exposure indices of toluene. More precise research will be reported in the near future for urinary toluene and o-cresol.

The advantages and disadvantages of each of the biological indicators for toluene exposure are described in Table 8.

① Toluene in blood, exhaled air and urine are superior from the standpoint of agent specificity, because inhaled toluene itself is determined in biological specimens.

② Background concentrations of hippuric acid

Table 6 Values of determinants in biological specimens taken at the last four hours (Table A) and the whole exposure period of eight hours (Table B) which corresponds to 50 ppm of the TLV-TWA of toluene (mean values), 90 percent confidence range of mean (CRM), 90 percent predictive ranges of individual specimens (PRI). Discriminant concentrations of toluene in air calculated from 90 percent or 66 percent confidence ranges of regression equations (DCr_5[25] or DCr_{17}[25]) and discriminant concentrations of toluene in air considered to discriminate exposure from the non-exposure at 5 percent levels (DC_5) [3,4,24] or 17 percent level of error (DC_{17}) which was calculated from 90 percent or 66 percent predictive ranges of individual specimens.

Table A.

determinants	values. corre. 50ppm tol	CRM	PRI	unit	DCr_5 (ppm)	DCr_{17} (ppm)	DC_5 (ppm)	DC_{17} (ppm)
Tol-EH	13.1	16.3	18.4	ppm	14.1	8.8	40.4	21.8
Tol-B	846	1136	1288	μg/L	16.0	9.6	49.0	24.6
Tol-U (actual.)	74.5	98.8	113.6	μg/L	18.5	11.5	···	30.9
HA-U (corr, cr)	1.98	2.40	2.63	g/g cr	12.1	7.4	31.9	17.6
o-Cr-U (corr, cr)	688	902	1014	μg/g cr	23.1	11.6	···	34.2
Tol-U (corr, SG)	92.7	113.4	126.1	μg/L	11.7	12.9	31.2	18.4

Table B

determinants	values. corre. 50ppm tol	CRM	PRI	unit	DCr_5 (ppm)	DCr_{17} (ppm)	DC_5 (ppm)	DC_{17} (ppm)
Tol-EH	9.44	11.3	12.3	ppm	10.0	6.0	26.1	14.7
Tol-B (p)*	846	1136	1288	μg/L	16.3	9.7	49.0	24.5
Tol-U (actual.)	58.0	72.9	82.0	μg/L	15.8	10.1	···	27.1
HA-U (corr, cr)	1.44	1.76	1.93	g/g cr	12.8	8.4	35.1	18.9
o-Cr-U (corr, cr)	514	655	729	μg/g cr	20.8	11.7	···	32.2
Tol-U (corr, SG)	80.7	95.2	104.1	μg/L	9.7	11.3	28.2	15.0

*(p) : PM. Currently, values for 1.6g/g cr of HAU for the BEI are under discussion in the BEI committee.

Table 7 The correlation coefficients and constants of the regression equations among concentrations of determinants derived from toluene in biological specimens of workers exposed to toluene taken at different intervals of exposure.

Table 4A. Specimens representative of the first 4 hours of exposure.

deter.(y) \ deter.(x)		Tol-EH	Tol-Bp	Tol-U	HA-U	o-Cr-U
Tol-EH	r =		0.230	0.628*	0.430	0.347
	a =		2.46	0.678	1.48	1.61
	b =		0.002	0.087	2.73	0.006
Tol-B	r =			0.130	0.043	0.171
	a =			247	296	218
	b =			2.79	42.1	0.460
Tol-U	r =				0.618*	0.305
	a =				10.8	17.4
	b =				28.1	0.038
HA-U	r =					0.266
	a =					0.375
	b =					0.0007
o-Cr-U	r =					
	a =					
	b =					

*p<0.05, **p<0.01

Table 4B. Specimens representative of the last 4 hours of exposure.

deter.(y) \ deter.(x)		Tol-EH	Tol-Bp	Tol-U	HA-U	o-Cr-U
Tol-EH	r =		0.928**	0.832**	0.537*	0.444
	a =		0.174	-0.43	0.657	0.511
	b =		0.013	0.138	4.30	0.010
Tol-B	r =			0.826**	0.543*	0.464
	a =			-16.7	54.0	34.1
	b =			9.57	304	0.705
Tol-U	r =				0.688**	0.555*
	a =				6.14	5.70
	b =				33.3	0.073
HA-U	r =					0.789**
	a =					0.006
	b =					0.002
o-Cr-U	r =					
	a =					
	b =					

*p<0.05, **p<0.01

Table 4C. Specimens representative of the whole period of 8 hours of exposure.

deter.(y) \ deter.(x)		Tol-EH	Tol-Bp	Tol-U	HA-U	o-Cr-U
Tol-EH	r =		0.841**	0.787**	0.533*	0.491
	a =		1.33	-0.19	1.02	0.650
	b =		0.007	0.127	3.76	0.010
Tol-B	r =			0.711*	0.408	0.416
	a =			-80.2	87.1	26.2
	b =			13.1	329	0.938
Tol-U	r =				0.680**	0.363
	a =				9.46	17.6
	b =				29.8	0.042
HA-U	r =					0.659**
	a =					0.130
	b =					0.002
o-Cr-U	r =					
	a =					
	b =					

*p<0.05, **p<0.01

Table 8 A chart showing various qualities of the biological exposure indicators of toluene

Bi. Ind	Specificity	Background	Biol. Half Life	Amount exc.	Storage	Collec.
Tol-EX	Sup.	Sup.	Inf.	Sup.	Inf.	R-Sup.
Tol-BL	Sup.	Sup.	Inf.	Inf.	Inf.	Inf.
Tol-U	Sup.	Sup.	Inf.	Inf.	Inf.	Sup.
HA-U	Inf.	Inf.	Sup.	Sup.	Sup.	Sup.
oCr-U	Inf.	Inf.	Sup.	Inf.	Sup.	Sup.

Bi. Ind = Biological exposure indicators, Bio. Half Life = Biological Half Life Amount exc. = Amount of excretion. Collec. = collection. Sup = superior, R-Sup = rather superior. Inf = inferior. The names of biological exposure indicators are described in Table 2.

are fluctuated by benzoic acid in some foods and soft drinks.

③ From the standpoint of the biological half life, toluene in blood, exhaled air and urine are inferior indicators which can be useful only if concentrations of toluene during the work period are relatively stable, because the biological half life of toluene itself is much shorter than hippuric acid.

④ Hippuric acid is the major bioproduct of absorbed toluene while others are produced in only minor amounts. Therefore, relying on the measurement of metabolites other than hippuric acid can easily lead to significant errors in field surveys.

⑤ There are only minute amounts of toluene in urine and it can be easily lost due to evaporation during preservation.

⑥ Urine and exhaled air are the easiest to collect. In contrast to this, taking blood requires sterilized syringes and needles, is time consuming and can cause pain and incovenience to the workers. However, it is difficult to collect exhaled air accurately.

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