Limited Validity of *o*-Cresol and Benzylmercapturic Acid in Urine as Biomarkers of Occupational Exposure to Toluene at Low Levels

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Abstract: This study was initiated to evaluate *o*-cresol and benzylmercapturic acid in urine in comparison with other biomarkers, as tools to estimate the intensity of occupational exposure to toluene at low levels. In total, 108 solvent exposed workers (engaged in tape production) and 17 non-exposed controls (all men) participated in the study. The surveys were conducted in the second half of working weeks. Diffusive sampling was conducted to measure 8-h time-weighted average intensity of occupational exposure to toluene. Blood and urine samples were collected at the end of a working shift. Blood samples were subjected to analysis for toluene (Tol-B), and urine samples were analyzed for benzyl alcohol (BeOH-U), benzylmercapturic acid (BMA-U), *o*-cresol (*o*-CR-U), hippuric acid (HA-U) and toluene (Tol-U) by the methods previously described. The toluene concentrations in workplaces were low in general, with a geometric mean (GM) and the maximum concentration of 1.9 ppm and 8.8 ppm, respectively. The statistical analyses of the six biomarkers for correlation with air-borne toluene showed that both Tol-B and Tol-U gave a high correlation coefficient of 0.58 to 0.61 ($p < 0.01$), whereas the coefficients for BeOH-U and BMA-U together with HA-U were all low (up to 0.22, depending on the correction for urine density) and statistically insignificant ($p > 0.10$) in most cases. *o*-CR-U had an intermediary coefficient of 0.20 ($p < 0.05$). Comparison with previous publications disclosed that BeOH-U, BMA-U and HA-U correlate with toluene in air when the exposure is intense (e.g., 50 ppm or above), but no longer proportional to air-borne toluene when the exposure is low, e.g., 2 ppm. Such appeared to be also the case for *o*-CR-U. In over-all evaluation, the validity of *o*-CR-U in monitoring occupational exposure to toluene at low levels (e.g., 2 ppm) appear to be limited, and BMA-U is not an appropriate biomarker. BeOH-U and HA-U are also inappropriate for this purpose. Only Tol-B and Tol-U may be employed to estimate toluene exposure at low levels.

Key words: Benzyl alcohol, Benzylmercapturic acid, Biomarkers, *o*-Cresol, Hippuric acid, Occupational exposure, Toluene, Urinalysis

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Introduction

Although the use of toluene in occupational setting has been gradually decreasing¹⁻⁴⁾, this aromatic solvent still maintains the leading position among solvents in various workplaces. To evaluate the exposure, biological monitoring has been conducted traditionally in terms of measuring hippuric acid concentration in the end-of-shift urine samples⁵⁻⁹⁾. The focus of current concern is if the traditional biomarker of hippuric acid is still valid to monitor the exposure when the air-borne toluene concentration has been decreasing in recent years in accordance with improvements in industrial hygiene.

Comparative evaluation of recently developed biomarkers to detect occupational toluene exposure was conducted in previous two analyses to show that, whereas the traditional biomarker of hippuric acid is valid when the time-weighted average (TWA) toluene exposure is rather intense¹⁰⁾, this marker only poorly correlates with the exposure when toluene exposure is low, e.g., at less than 10 ppm^{10, 11)}. It was also observed that un-metabolized toluene both in blood and urine is a better marker of the exposure to toluene not only at moderate levels (e.g., at 50 ppm) but at lower levels (e.g., 2 ppm)¹¹⁾.

The validity of the two urinary biomarkers of *o*-cresol and benzylmercapturic acid at low toluene exposure was, however, not examined in these previous studies. It is the purpose of the present study to examine if *o*-cresol and benzylmercapturic acid are as valid as other biomarkers such as toluene in urine, when toluene exposure intensity is low.

Subjects, Materials and Methods

Ethical issue

The Ethics Committee of Kyoto Industrial Health Association approved the study protocol. The participating workers were 108 solvent-exposed workers and 17 occupationally non-exposed controls (all men) at the ages of 20 to 60 yr. They engaged in the production of adhesive tapes, and volunteered to participate in the study.

Air-borne toluene monitoring, and biological monitoring by means of urinalysis and blood analysis

The survey was conducted on the second half of working weeks following the design as previously described^{10, 11)}. In short, TWA toluene exposure concentration was monitored by use of diffusive samplers¹²⁾ which were placed on the working cloth (at the chest pocket level) of each worker for approximately 8 h (i.e., typically from 09:00 to 17:00). The carbon disulfide-extract of the exposed carbon cloth was analyzed by gaschromatography⁴⁾; the limit of detection (LOD) for

various solvents was 0.1 ppm in common. A spot urine sample and a peripheral venous blood sample were collected from each worker at the end of the shift of the survey day. Blood samples were subjected to toluene (Tol-B) analysis by head-space gaschromatography with a LOD of 2 $\mu\text{g/l}$ ¹³⁾. Urine samples were analyzed for benzyl alcohol (BeOH-U, U standing for urine; LOD, 5 $\mu\text{g/l}$ ¹⁴⁾), benzylmercapturic acid (BMA-U; LOD, 0.2 $\mu\text{g/l}$ ^{15, 16)}), *o*-cresol (*o*-CR-U; LOD, 10 $\mu\text{g/l}$ ¹⁴⁾), hippuric acid (HA-U; LOD, 2 mg/l¹⁷⁾), and toluene in urine (Tol-U; LOD, 2 $\mu\text{g/l}$ ¹³⁾). Creatinine concentration (CR) and specific gravity (SG) were measured by colorimetry and refractometry, respectively. Biomarker levels were corrected in some cases either for creatinine concentration¹⁸⁾ or for a specific gravity of urine of 1.016¹⁹⁾. Specific gravity was expressed in terms of factor $G^{20)$, which was defined as factor $G = (\text{Specific gravity} - 1.000) \times 1000$.

The additiveness formula^{8, 21)} was employed to calculate the SUM as an indicator of combined exposure to multiple solvents. For this purpose, occupational exposure limits²²⁾ of 50, 50, 200 and 40 ppm were taken for toluene, xylenes, ethyl acetate and hexane, respectively.

Statistical analysis

CR and SG distributed normally so that the distributions were expressed in terms of arithmetic means (AM) and arithmetic standard deviations (ASD). Toluene in air and biomarkers in blood and urine distributed log-normally, and geometric means (GM) and geometric standard deviations (GSD) were taken as representative distribution parameters. In calculating GM and GSD, the values below LOD were taken as if they were LOD/2.

Regression analyses were conducted by use of STATVIEW Version 5. When necessary, the statistical significance of the difference in two regression lines was examined by comparing paired intercepts, slopes and correlation coefficients after Ichihara²²⁾.

Results

Levels of exposure to toluene, and that of exposure biomarkers

The exposure level of toluene in air was low with a GM of 1.9 ppm, although the GSD was large, 2.7, and the maximum toluene in air on an individual basis was nearly 9 ppm. There were co-exposures to other solvents (Table 1). However, only the exposure to ethyl acetate exceeded that to toluene when GM values were compared (i.e., 10.5 ppm vs. 1.9 ppm). The GM (and the maximum) of the SUM was 0.15 (1.6). The biomarker levels among the exposed group were also low, e.g., 307 mg/l HA-U_{ob} and 91 $\mu\text{g/l}$ *o*-CR-U_{ob} (Table 2). When compared

Table 1. Solvent exposures

Parameter	Ethyl acetate	Ethylbenzene	Hexane	Toluene	Xylenes	SUM
OEL ^{a, b}	200	50	40	50	50	1 ^c
GM ^b	10.5	0.34	0.50	1.89	0.65	0.15
GSD	4.69	3.78	3.36	2.67	3.50	2.86
Maximum ^a	280.3	11.5	25.1	8.8	15.9	1.55

^aOccupational exposure limits by Japan Society for Occupational Health²¹⁾.

^bUnit; ppm except for the SUM which is dimensionless.

^cUnity.

with the levels among the non-exposed subjects, the minimum biomarker levels among the exposed subjects were smaller than the maximum levels of the corresponding biomarkers among the non-exposed subjects in cases of BeOH-U, *o*-CR-U and HA-U, irrespective of correction for CR or SG. It should be added that no BMA-U, Tol-U or Tol-B were detected (i.e., <LOD) among the non-exposed whereas they were detectable in almost all cases of the exposed subjects.

Regression of biomarker levels with air-borne toluene

When possible correlation of biomarker levels (as observed) with air-borne toluene was examined among the exposed subjects, the correlation coefficients were statistically significant for Tol-U_{ob} and Tol-B ($p < 0.01$ for both) (the left half in Table 3). The coefficients for BeOH-U_{ob}, BMA-U_{ob} and HA-U_{ob} were however insignificant ($p > 0.10$, or ns). The coefficient for *o*-CR-U_{ob}, 0.202, was statistically significant ($p < 0.05$), but this might owe to the large number of cases (i.e., $n = 108$). Correction for CR or SG resulted in minimum improvement in the coefficients, with no change in the stratified p values. It should be added that no correction for CR or SG was applicable to Tol-B. Combination of the 17 non-exposed cases with the 108 exposed cases (the right half in Table 3) induced essentially no change; not only the correlation coefficients but the slopes and even the intercepts on the vertical axes stayed almost unchanged.

Classification of biomarkers through comparison of correlation coefficients

When the statistical significance of the difference between any two correlation coefficients for the exposed group were examined for 15 pairs among the six biomarkers (Table 4), it was possible to classify the six biomarkers into two groups, i.e., two (Tol-U_{ob} and Tol-B) with high coefficients and four (*o*-CR-U_{ob}, BeOH-U_{ob}, BMA-U_{ob} and HA-U_{ob}) with low coefficients. For example, 'ns' at the cross of the 'Tol-B' column and the 'Tol-U_{ob}' line means that the difference between the two coefficients of correlation with air-borne toluene, 0.613 for Tol-U_{ob} and 0.575 for Tol-B, was statistically insignifi-

cant ($p > 0.10$), whereas '<0.01' at the cross of the '*o*-CR-U_{ob}' column and the 'Tol-U_{ob}' line means that the difference between the two coefficients, 0.613 for Tol-U_{ob} and 0.202 for *o*-CR-U_{ob}, was in fact significant ($p < 0.01$). Thus, no significant difference ($p > 0.10$) was detected between the former two or among the latter four. The analyses with 10 pairs each of CR- or SG-corrected biomarkers (no correction was applicable to Tol-B) gave exactly the same results.

For visual understanding of the difference between biomarkers with high and low correlation coefficients, Tol-U_{ob} (with a high correlation coefficient) *o*-CR-U_{ob} and BMA-U_{ob} (with low coefficients) were selected as typical cases, and scatter diagrams (with 95% confidence intervals) against air-borne toluene were depicted in Fig. 1. It is clear that the 95% confidence interval was narrower for Tol-U_{ob} than that for *o*-CR-U_{ob} or BMA-U_{ob}.

Discussion

The present analysis on 108 workers occupationally exposed to toluene at 1.9 ppm as GM (8.8 ppm as the maximum; Table 1) made it clear that both Tol-U_{ob} and Tol-B correlate closely ($r = 0.61$ and 0.58 , respectively; $p < 0.01$ for both) with toluene exposure concentration even at such low levels. The correlation of *o*-CR-U_{ob} was less significant with $r = 0.20$ and $p < 0.05$, whereas BeOH-U_{ob} and BMA-U_{ob} did not correlate significantly ($r = 0.16$ and 0.11 , respectively, with $p > 0.10$ for both; Table 3). In the two previous studies, one¹⁰⁾ with toluene exposure at moderate levels (at 10 ppm as GM and at 121 ppm as the maximum) and the other¹¹⁾ at low levels (at 1.6 ppm as GM and 26.5 ppm as the maximum), Tol-U correlated with TWA toluene concentrations in air not only at moderate levels but at low levels. No Tol-B was examined in the moderate exposure study¹⁰⁾, but separate studies showed that Tol-B significantly correlates with air-borne toluene at low levels¹¹⁾ as well as at high levels (the maximum level being 132 ppm)²³⁾. Nise²⁴⁾ also reported a high coefficient (0.84) for correlation of Tol-B with air-borne toluene. Thus, the close relationship of Tol-U_{ob}

Table 2. Levels of toluene exposure and resulting levels of biomarkers

Group	Correction for Parameter	Tol in air (ppm)	SUM ^a	CR (g/l)	G	BeOH-U (μ g/l or g cr)	BMA-U (μ g/l or g cr)	<i>o</i> -CR-U (μ g/l or g cr)	HA-U (mg/l or g cr)	Tol-U (μ g/l or g cr)	Tol-B (μ g/l)
Exposed (108 cases)											
None (i.e., as observed)											
	GM ^b	1.89	0.15	1.64	23	46	2.07	91	307	4.22	14.8
	GSD ^b	2.67	2.86	0.85	6	2.47	7.26	3.28	2.58	1.90	2.31
	MED	2.20	0.17	1.47	23	43	2.60	105	330	4.11	13.3
	Min	0.2	0.01	0.32	7	6	<LOD ^c	<LOD ^c	48	<LOD ^c	2.3
	Max	8.8	1.6	4.42	39	453	94.4	1102	2571	17.00	103.0
Creatinine											
	GM					32	1.45	63	214	2.95	
	GSD					2.44	8.34	3.75	2.28	2.09	
	MED					29	1.67	72	213	2.80	
	Min					5	<LOD ^c	<LOD ^c	35	<LOD ^c	
	Max					440	91.6	1192	1546	17.60	
Specific gravity (1.016)											
	GM					34	1.51	66	223	3.08	
	GSD					2.34	7.57	3.45	2.36	1.88	
	MED					30	1.72	77	239	2.99	
	Min					4	<LOD ^c	<LOD ^c	36	<LOD ^c	
	Max					315	75.5	840	1469	12.19	
Non-exposed controls (17 cases)											
None (i.e., as observed)											
	GM ^b			1.45	23	55	<LOD ^c	75	224	<LOD ^c	<LOD ^c
	GSD ^b			0.78	7	2		3	3		
	MED			1.48	23	56		63.2	250		
	Min			0.52	10	16		5	30		
	Max			3.03	39	162		386	1580		
Creatinine											
	GM					44		60	180		
	GSD					1.77		2.86	2.23		
	MED					42		66	187		
	Min					16		10	42		
	Max					161		412	940		
Specific gravity (1.016)											
	GM					41		55	165		
	GSD					1.63		3.07	2.56		
	MED					39		58	187		
	Min					15		5	28		
	Max					109		362	903		

Abbreviations are: BeOH-U, benzyl alcohol in urine; BMA-U, benzylmercapturic acid in urine; *o*-CR, *o*-cresol in urine; HA-U, hippuric acid in urine; Tol-U, toluene in urine and Tol-B, toluene in blood.

^aSum after the additiveness formula (for details, see the Materials and Methods section).

^bAM and ASD in cases of CR (creatinine) and G (Factor G; for details, see the Materials and Methods section).

^cLess than the limit of detection; for the value, see the Materials and Methods section.

Table 3. Correlation of biomarkers with toluene in air^a

Correction for		Exposed group ^b				Exposed group + non-exposed group ^c			
Biomarker	(unit)	Intercept ^d	Slope ^e	<i>r</i> ^f	<i>p</i> ^g	Intercept ^d	Slope ^e	<i>r</i> ^f	<i>p</i> ^g
None (i.e., as observed)									
BeOH-U _{ob}	($\mu\text{g/l}$)	55.4	5.79	0.161	ns ^h	57.4	5.35	0.161	ns ^h
BMA-U _{ob}	($\mu\text{g/l}$)	6.8	0.83	0.114	ns ^h	4.9	1.25	0.184	<0.10
<i>o</i> -CR-U _{ob}	($\mu\text{g/l}$)	111.7	14.92	0.202	<0.05	115.0	14.22	0.202	<0.05
HA-U _{ob}	(mg/l)	454.1	2.70	0.014	ns ^h	431.8	7.46	0.045	ns ^h
Tol-U _{ob}	($\mu\text{g/l}$)	2.5	0.93	0.613	<0.01	1.8	1.08	0.688	<0.01
Tol-B	($\mu\text{g/l}$)	7.9	4.53	0.575	<0.01	5.7	5.01	0.643	<0.01
Creatinine									
BeOH-U _{cr}	($\mu\text{g/g cr}$)	33.2	6.42	0.217	<0.05	39.9	4.01	0.176	<0.10
BMA-U _{cr}	($\mu\text{g/g cr}$)	5.4	0.71	0.118	ns ^h	3.8	0.87	0.173	<0.10
<i>o</i> -CR-U _{cr}	($\mu\text{g/g cr}$)	75.1	17.51	0.228	<0.05	84.5	10.93	0.210	<0.05
HA-U _{cr}	(mg/g cr)	276.5	7.12	0.063	ns ^h	288.2	6.54	0.055	ns ^h
Tol-U _{cr}	($\mu\text{g/g cr}$)	1.4	0.87	0.607	<0.01	1.3	0.79	0.696	<0.01
Specific gravity									
BeOH-U _{sg}	($\mu\text{g/l}$)	37.6	4.50	0.182	<0.10	38.7	5.26	0.190	$\div$ 0.05
BMA-U _{sg}	($\mu\text{g/l}$)	5.3	0.55	0.100	ns ^h	3.7	1.04	0.190	$\div$ 0.05
<i>o</i> -CR-U _{sg}	($\mu\text{g/l}$)	81.8	11.50	0.210	<0.05	81.1	16.24	0.226	<0.05
HA-U _{sg}	(mg/l)	307.9	2.33	0.020	ns ^h	267.0	9.15	0.084	ns ^h
Tol-U _{sg}	($\mu\text{g/l}$)	1.8	0.69	0.623	<0.01	1.0	0.96	0.672	<0.01

Abbreviations are as under Table 1.

^aSimple regression taking toluene in air as a dependent variable and the corresponding biomarker as a dependent variable.^b108 cases.^cA combination of 108 exposed cases and 17 non-exposed cases.^dUnit as given for each biomarker.^eUnit; the unit for each biomarker/ppm.^fCorrelation coefficient.^g*p* for correlation coefficient.^hnot significant (*p*>0.10).**Table 4. Comparison of two correlation coefficients**

Biomarker	<i>r</i>	<i>p</i> for the difference in correlation coefficient (<i>r</i>) between two biomarkers				
		Tol-B	<i>o</i> -CR-U _{ob}	BeOH-U _{ob}	BMA-U _{ob}	HA-U _{ob}
Tol-U _{ob}	0.613	ns	<0.01	<0.01	<0.01	<0.01
Tol-B	0.575		<0.01	<0.01	<0.01	<0.01
<i>o</i> -CR-U _{ob}	0.202			ns	ns	ns
BeOH-U _{ob}	0.161				ns	ns
BMA-U _{ob}	0.114					ns
HA-U _{ob}	0.014					

Abbreviations are as under Table 2.

ns stands for *p*>0.10.

and Tol-B with toluene in air is in a good agreement with previous studies.

A close correlation (*r*=0.80) with toluene in air was reported for BeOH-U_{ob} in a factory survey in which workers were exposed to toluene at 12.6 ppm and 130 ppm as the maximum¹¹. Similarly, the correlation was high when toluene exposure was intense (i.e., 15 ppm as GM and 70 ppm as the maximum), but it was substantially less close otherwise¹⁰. Thus, the present observation of low correlation (*r*=0.16 with *p*>0.10) is on line with the

previous observation of poor correlation when toluene exposure was low¹⁰. With regard to HA-U, it was in fact a highlight of the observations in the previous two studies^{10, 11} that the correlation with air-borne toluene was high and low, respectively, when the GM exposure was in excess of e.g., 10 ppm and less than 2 ppm. The present low correlation at low toluene exposure levels confirms the previous finding after low level toluene exposure, although this biomarker has been long used when toluene in workroom air was high^{5, 6, 25, 26}.

Fig. 1. Scatter diagrams to show correlation of a. toluene in urine as observed (Tol-U_{ob}), b. *o*-cresol in urine (as observed; *o*-CR-U_{ob}) and c. benzylmercapturic acid in urine (as observed; BMA-U_{ob}) with time-weighted average concentrations of toluene in air (Tol in air).

Thick lines in the center of figures are calculated regression lines. The curves on both sides of the lines and the outer-most curves indicate the 95% confidence ranges of the means and the individual values, respectively. Each dot represents an exposed case. For the equations of the regression lines, see Table 3.

BMA-U and *o*-CR-U may need detailed discussion as these two markers were not evaluated in the previous low exposure survey¹¹). Maestri *et al.*²⁷) observed a high correlation coefficient of 0.74 for BMA when they examined 18 workers who were exposed to toluene up to 65 ppm. In a study on 46 solvent-exposed workers (all men) with toluene exposure at 13 ppm as GM (86 ppm as the maximum), Inoue *et al.*¹⁵) observed that the correlation coefficient of BMA-U_{ob} with toluene in air was as high as 0.66 ($p < 0.01$). Inoue *et al.*¹⁶) further observed, in a separate survey with 122 male printers who were exposed to toluene at 8.3 ppm as GM and 86.4 ppm as the maximum, that BMA-U_{ob} correlated closely with toluene in air with a correlation coefficient of 0.73, the coefficient being greater than that for HA-U_{ob} (0.58) or *o*-CR-U_{ob} (0.63). Nevertheless, the coefficient for BMA-U tended to be high (e.g., 0.57) only when toluene exposure was rather intense (e.g., 15 ppm as GM and 70 ppm as the maximum) but low and insignificant after low level exposures¹⁰). The latter negative observation was well reproduced in the present study (Table 3).

High correlation of *o*-CR-U had been attracting attention with the expectation that this metabolite could be a better surrogate of HA-U for toluene biological monitoring^{24, 27}). For example, De Rosa *et al.*²⁹) observed a high correlation coefficient of 0.78 for *o*-CR-U_{cr} among 14 workers exposed to toluene up to 61 ppm. The same group³⁰) obtained a similarly high coefficient of 0.79 for

o-CR-U_{cr} among 18 printers exposed to up to 59 ppm. Nise²³) observed a correlation coefficient of 0.57 for *o*-CR-U_{cr} in a factory survey in which 58 workers were exposed to toluene at 25.6 ppm as a median and 132 ppm as the maximum. The correlation was in fact closer than that for HA-U_{cr} (0.37). Similar correlation coefficients of 0.52¹³), 0.70³¹) and 0.65³²) were observed in studies on solvent workers exposed to 3.9 ppm (as GM; 100 ppm as the maximum), 25 ppm (as GM), and 63 ppm (median), respectively. Recently, Fustinoni *et al.*³³) studied 38 printers exposed to 12.8 ppm as GM (43.2 ppm as the maximum). The regression analysis after logarithmic conversion of both toluene in air and *o*-CR-U gave a coefficient of 0.70 for *o*-CR-U_{cr}. This study group also found even a higher coefficient of 0.88 in a group of 122 solvent workers exposed to toluene at 14 ppm as GM (62 ppm as the maximum)¹⁰). Thus, sufficiently high correlation coefficients were obtained for *o*-CR-U in various studies in which toluene exposure was high. Nevertheless, a very low coefficient of 0.17 was also observed in a plant where the GM toluene in air was 2.1 ppm (9.3 ppm as the maximum)¹⁰). The present observation of $r = 0.20$ (Table 3) is apparently similar to the latter value of 0.17. It appears likely that this biomarker may correlate closely with toluene in air under the condition of intensive exposure, but not necessarily so when the exposure is less intense.

With regard to possible confounders of toluene metabolism, it should be noted that workers in the present study

were not only exposed to toluene but to other solvents although at low concentrations, e.g., below the current occupational exposure limits²¹⁾ (with one exception of ethyl acetate; Table 1). It is known that no BeOH, BMA, HA or Tol will be formed after inhalation of ethylbenzene, hexane or xylenes³⁴⁾, and that ethyl acetate will be quickly hydrolyzed *in vivo* by ubiquitous esterase and then mineralized³⁵⁾. It is also known that no metabolic interaction will take place between organic solvents when the exposures are low, e.g., below occupational exposure limits^{36, 37)}. In fact, no metabolic modification was detected among workers exposed to toluene in combination with xylenes^{38–40)}, hexane⁴¹⁾ or ethyl acetate⁴²⁾ at the concentrations below respective occupational exposure limits. Thus, chances should be very remote for the present study that toluene metabolism was modified by other solvents co-exposed. It was previously observed that metabolism of toluene will not be modified by social drinking³⁸⁾. In contrast, metabolism of toluene and that of xylenes were suppressed by smoking^{38, 39)}. It was not possible, however, to confirm this effect in the present study as no data were available on the smoking habits.

In over-all evaluation, therefore, it appears prudent to conclude that un-metabolized toluene in blood and urine (i.e., Tol-B and Tol-U) correlate with toluene exposure even at low levels such as 1.9 ppm as GM and 8.8 ppm as the maximum. In contrast, BMA-U, BeOH-U and HA-U may not be applicable as exposure biomarkers when toluene exposure is low. The value of *o*-CR-U also appears to be limited under low toluene exposure.

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