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Original Article

Comparison of personal air benzene and urine *t,t*-muconic acid as a benzene exposure surrogate during turnaround maintenance in petrochemical plants

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Abstract: Previous studies have shown that biomarkers of chemicals with long half-lives may be better surrogates of exposure for epidemiological analyses, leading to less attenuation of the exposure-disease association, than personal air samples. However, chemicals with short half-lives have shown inconsistent results. In the present study, we compared pairs of personal air benzene and its short-half-life urinary metabolite *trans,trans*-muconic acid (*t,t*-MA), and predicted attenuation bias of theoretical exposure-disease association. Total 669 pairs of personal air benzene and urine *t,t*-MA samples were taken from 474 male workers during turnaround maintenance operations held in seven petrochemical plants. Maintenance jobs were classified into 13 groups. Variance components were calculated for personal air benzene and urine *t,t*-MA separately to estimate the attenuation of the theoretical exposure-disease association. Personal air benzene and urine *t,t*-MA showed similar attenuation of the theoretical exposure-disease association. Analyses for repeated measurements showed similar results, while in analyses for values above the limits of detection (LODs), urine *t,t*-MA showed less attenuation of the theoretical exposure-disease association than personal air benzene. Our findings suggest that there may be no significant difference in attenuation bias when personal air benzene or urine *t,t*-MA is used as a surrogate for benzene exposure.

Key words: Benzene, *t,t*-muconic acid, Attenuation, Biomarker, Exposure

Introduction

Benzene is a potent carcinogen that causes acute non-lymphocytic leukemias and is suspected to be associated with lymphocytic leukemias, multiple myeloma and non-Hodgkin's lymphoma[1]. It also suppresses bone marrow, resulting in serious hematological adverse effects such as pancytopenia and aplastic anemia[2–4]. Benzene exposure has occurred across a wide range of industries[5].

In assessing occupational and environmental exposures, personal air samples and biomarkers have been used. Personal air sampling is used to measure external exposures, but it usually does not reflect moderating factors such as respirator use or dermal absorption[6]. Biomarkers are used

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to measure internally absorbed doses accounting for the moderating factors, but these are also affected by personal factors such as cigarette smoking and food intake[7].

In epidemiological analyses, both personal air samples and biomarkers have been used to evaluate associations between exposures and diseases. Many studies have sought better indicators with which to examine exposure-disease association[6, 8–10]. Some studies that analyzed multiple datasets containing both personal air samples and biomarkers have shown that biomarkers may be a less-biasing surrogate than personal air samples in terms of attenuation of theoretical exposure-disease associations, especially in chemicals with long half-lives, such as lead[6, 8]. The attenuation bias represents an underestimation of the true exposure-disease association due to measurement error[11]. However, the notion that biomarker is a less biasing surrogate is inconsistent for chemicals with short half-lives.

t,t-MA is a urinary benzene metabolite with a short half-life (5 h)[12]. Urine *t,t*-MA has been used as a surrogate of benzene exposure in epidemiological studies, but it has not yet been tested for whether personal air benzene samples or urine *t,t*-MA is a better surrogate of benzene exposure in terms of attenuation of the exposure-disease association.

In the present study, we estimated attenuation of the theoretical exposure-disease association for personal air benzene and its urinary metabolite *t,t*-MA to determine which is a better surrogate of benzene exposure for use in future epidemiological analyses.

Methods

Study subjects

The study subjects consisted of 474 male workers who participated in turnaround maintenance operations held in seven petrochemical plants[13, 14]. During turnaround maintenance, workers can be exposed to various carcinogenic chemicals such as benzene and 1,3-butadiene[15]. The plants are located in a refinery/petrochemical complex in Korea and have produced or used benzene. The turnaround maintenance operations were held in manufacturing processes in which benzene exposure can occur: styrene monomer (SM; one plant), cumen (two plants), mononitrobenzene (MNB; two plants), and aromatic processes (BTX; two plants).

Study subjects were workers who participated in the benzene-exposed turnaround maintenance operations in the seven petrochemical plants. The study subjects can be largely classified into temporary maintenance workers and employees of petrochemical plants. Temporary maintenance workers were hired on fixed-term contracts and engaged in petrochemical plant maintenance operations[16]. Jobs of temporary maintenance workers were categorized into eight jobs: insulation fitter, plumber, mechanical engineer, scaffold worker, drain fitter, construction craftsman, electrical fitter, welder, painter, and tank fitter. Jobs of employees of petrochemical plants were categorized into three jobs: board man, field man, and maintenance engineer. Overall the jobs of the study subjects were categorized into 13 groups *a priori*. The major activity of each job is described in detail elsewhere[13]. The study subjects were supposed to be directly or indirectly exposed to benzene during turnaround maintenance operations.

During turnaround maintenance operations, workers were randomly selected and asked to wear personal air samplers and provide urine samples, which were used to evaluate their benzene exposure levels. Shift-long personal air benzene sampling and post-shift urine sampling started in October of 2007 and ended in January of 2009. Personal air samples and urine samples were collected simultaneously at the work sites immediately after the work has finished. Overall, 669 pairs of personal air benzene and urine *t,t*-MA samples were collected from 474 workers. Among them, 122 workers wore personal air samplers and provided urine samples two or more times on different days. The time differences between the first and last measurements for the 122 workers were skewed with a median of 2 d (interquartile range (IQR): 1–24 d, max 215 d). The average number of repeated measurements was 2.6 times (max: 6).

Personal air benzene measurements

Shift-long passive sampling by organic vapor monitors (OVM 3500, 3M, USA) was employed to measure personal air benzene concentrations during turnaround operations. Personal air benzene samples were collected immediately after the work has finished at the work sites from the workers. The mean sampling time and standard deviation (SD) were 441 min and 78 min, respectively, with a range of 198 to 693 min. All personal air benzene samples were analyzed according to the NIOSH Manual of Analytic Methods 1500 and 1501 using GC/FID (Gas chromatography/flame ionization detector) in a laboratory at the Occupational Safety and Health Research Institute (OSHRI). OSHRI is an accredited laboratory from PAT (Proficiency Analytic Testing) program of the AIHA (American Industrial Hygiene Association).

To validate the performance of the passive sampler,

active sampling using charcoal tubes (SKC 26–01, SKC Inc., USA) was carried out in a pilot study. A pump (Gillian LFS-113, USA) calibrated to <0.2 l/min was used to estimate external inhalation benzene exposure during turnaround operations. These two sampling methods showed a strong correlation (r=0.95, p<0.001)[13]. Details of the sampling and analytical procedures used in this study are described in the literature[13].

t,t-Muconic Acid measurements

Post-shift urine samples were collected in high-density polyethylene (HDPE) containers at the work sites immediately after the work has finished, and stored in a refrigerator at 4°C. All urine *t,t*-MA samples were analyzed in a laboratory of the OSHRI. Urine *t,t*-MA analyses were performed by the following processes. Collected samples and standard solutions were purified by solid phase extraction using Bond Elut SAX (500 mg, Varian, USA) as an anion exchange cartridge. After applying 1,000 µl of sample or standard solution mixed with 5 µl of 10 mM sodium hydroxide solution, the cartridges were washed with 2 ml of 1% aqueous acetic acid twice. *t,t*-MA was eluted with 3 ml of 10% aqueous acetic acid and collected into 5-ml measuring test tubes, which were then filled with an eluent of water washed through the cartridge. An aliquot (80 µl) of eluent filtered through a 0.22-µm membrane filter was directly injected into an HPLC system for determination of *t,t*-MA levels. A Capcell Pak MF Ph-1 SG80 column (150 × −4.6 mm I.D., 5 µm, Shisheido, Japan) was employed for the separation process. The mobile phase used was 10 mM KH2PO4 containing 0.1% $H3PO_4$ at a flow rate of 0.5 ml/min. The column temperature was held constant at 40°C, and UV detection was performed at 259 nm. G-EQUAS urine samples of round robin 26, 29 and 32 were analyzed as a reference sample for *it, t*-MA. The accuracy was calculated from the recovery of *t,t*-MA, which was 82−129% of reference samples in the range of 0.41–2.91 mg/l. The linearity was good with the correlation constant of 0.9999 in the range of 0.1−5.0 g/l. Limit of detection (LOD) was calculated from calibration data of standard solutions of t,t-MA in the range of 0.5–5.0 mg/l[17].

In a pilot study, pairs of pre-shift and post-shift urine samples from 15 workers were analyzed, and post-shift *t,t*-MA levels were significantly higher than pre-shift *t,t*-MA levels (mean difference 1.18 mg/g creatinine, p<0.001).

Statistical analysis

To examine which is a less-biasing surrogate of benzene exposure for epidemiological analyses, we computed attenuation of the theoretical exposure-disease association both for personal air benzene and its urinary metabolite *t,t*-MA[18, 19].

Generation of complete data sets using a multiple imputation technique

Of the personal air benzene samples, 34.1% were below the 0.012 ppm of LOD. Of the urine *t,t*-MA samples, 10.9% were below the 0.06 mg/l of LOD. In the first step, we generated complete data sets for subsequent analyses using a multiple imputation technique[20]. We created a bootstrap data using random sampling with replacement. Then, Tobit regression was performed to obtain distribution parameters of measurements. We imputed values below the LOD assuming that measurements were log-normally distributed defined by estimates of distribution parameters from the Tobit regression. This imputation procedure was repeated five times to obtain five complete data sets, consisting of imputed values for measurements below the LOD and real values above the LOD. The imputation process was conducted for personal air benzene and urine *t,t*-MA separately. Tobit regression was performed using 'AER' package[21] of statistical software R[22].

Calculation of variance components

In the second step, we calculated the pooled variance components across five imputed data sets to be used for estimating attenuation of the theoretical exposure-disease association. Personal air benzene and urine *t,t*-MA levels in the five complete data sets were natural log-transformed (Ln*(Y)*) to approximate normal distributions. Mixed-effects models were developed separately to calculate variance components for personal air benzene and urine *t,t*-MA. Mixed-effects models for group-based and individual-based exposure assessment were developed separately[23].

For group-based analyses in which a group mean is assigned to workers within the group as an exposure estimate in epidemiological analyses, we developed the following mixed-effects model:

$$\mathrm{Ln}(Y_{ijk})=\alpha_0 + Job_i + Worker_{ij} + \varepsilon_{ijk}$$

In this equation, α_0 represents the intercept of the model or the mean exposure. Job_i is a random job group effect [Job_i to N (0, σ^2_{BG})] (between-group variance), $Worker_{ij}$ is a random effect for the j^{th} worker in the i^{th} job group [$Worker_{ij}$ to N (0, σ^2_{WG})] (within-group variance), and ε_{ijk} is a random effect for the k^{th} measurement of the j^{th} worker in the i^{th} job group [ε_{ijk} to N (0, σ^2_{WW})] (within-worker variance). We assumed that Job_i , $Worker_j$, and ε_{ijk} were

independent of one another.

For individual-based analyses in which individual means are assigned to workers as an exposure estimate in epidemiological analyses, we developed the following mixed-effects model across job groups:

$$\mathrm{Ln}(Y_{kn})=\alpha_0 + Worker_j + \varepsilon_{jk}$$

Here, $Worker_j$ is a random effect for the j^{th} worker [$Worker_j$ to N (0, σ^2_{WG})] and ε_{jk} is a random effect for the k^{th} measurement of the j^{th} worker [ε_{jk} to N (0, σ^2_{WW})]. We assumed that $Worker_j$ and ε_{jk} were independent of one another.

We fitted each model for five imputed data sets with a restricted maximum likelihood estimation method using 'lme4' package[24] of statistical software R. Then, we combined the variance components across five complete data sets to obtain pooled variance component estimates, using 'mitml' package of statistical software R.

Estimation of attenuation of the theoretical exposure-disease association

In the third step, we estimated the attenuation of the theoretical exposure-disease associations both for personal air benzene and urine *t,t*-MA, using pooled estimates of variance components[19]. We calculated estimates separately for the group-based analysis and the individual-based analysis.

For group-based analysis, the expected value of the true exposure-disease coefficient ($\hat{\beta}_1$) can be calculated as follows:

$$E(\hat{\beta}_1)=\left(\frac{\sigma^2_{BG}+\frac{\sigma^2_{WG}}{k}}{\sigma^2_{BG}+\frac{\sigma^2_{WG}}{k}+\frac{\sigma^2_{WW}}{kn}}\right)\beta_1$$

Here, k and n represent the number of workers for a job group and the number of repeated measurements for a worker, respectively. β_1 represents true exposure-disease coefficient.

For individual-based analysis, the expected value of $\hat{\beta}_1$ can be calculated as:

$$E(\hat{\beta}_1)=\left(\frac{\sigma^2_{WG}}{\sigma^2_{WG}+\frac{\sigma^2_{WW}}{n}}\right)\beta_1$$

In addition, we repeated above calculations, restricting the analysis to repeated measurements and/or values above LODs of personal air benzene and urine *t,t*-MA.

Correlation between personal air benzene and urine t,t-MA

We calculated correlation coefficients between natural log-transformed personal air benzene and natural log-transformed *t,t*-MA from the five complete data sets. Fisher's Z-transformation was used to estimate the pooled correlation coefficient because the distribution of a sample correlation coefficient is known to be skewed[25]. Pooled correlation coefficients were calculated for complete data sets and subset consisting of repeated measurements. In addition, correlation coefficients were further calculated for other subsets, consisting of values above LODs of personal air benzene and urine *t,t*-MA.

Results

Distributions of personal air benzene and urine *t,t*-MA by job and manufacturing process were presented in Table 1. The median and IQR of overall personal air benzene measurements were 0.04 and <LOD-0.19 ppm, respectively (max: 137 ppm). The median and IQR of overall *t,t*-MA measurements were 0.38 and 0.19–0.65 mg/g creatinine, respectively (max: 15.8 mg/g creatinine). Of the personal air benzene measurements, 9% exceeded 1 ppm of the Korean occupational exposure limit (OEL)[26]. Of the urine *t,t*-MA measurements, 37% exceeded 0.5 mg/g creatinine of the biological exposure index (BEI)[27].

Between-group variance, within-group variance and within-worker variance components, and estimates of attenuation of the theoretical exposure-disease association for personal air benzene and urine *t,t*-MA were presented in Table 2. Personal air benzene showed a similar degree of attenuation with urine *t,t*-MA in both group-based (0.91 vs. 0.87) and individual-based (0.40 vs. 0.48) analyses. Group-based analyses showed less attenuation than individual-based analyses. In analyses for subset consisting of only repeated measurements, in general, similar patterns were found with complete data set.

In analyses for subset consisting of values above LODs of personal air benzene and urine *t,t*-MA, attenuation was substantially increased for personal air benzene (group-based analysis 0.68; individual-based analysis 0.11), while a relatively small increase was found in urine *t,t*-MA (group-based analysis 0.82; individual-based analysis 0.45). When further restricting analyses to repeated measurements, similar patterns were found with the subset

Table 1. Summary statistics of personal air benzene and urine *t,t*-muconic acid by job and manufacturing process

			Personal air benzene (ppm)						Urine *t,t*-muconic acid (mg/g creatinine)					
		N	N<LOD (%)	Min	Q1	Median	Q3	Max	N<LOD (%)	Min	Q1	Median	Q3	Max
Job	Insulation fitter	58	34	<LOD	<LOD	0.06	0.12	1.48	10	<LOD	0.17	0.29	0.54	1.24
	Field man	164	23	<LOD	0.02	0.09	0.43	42.1	7	<LOD	0.28	0.51	1.17	15.8
	Plumber	101	25	<LOD	0.02	0.08	0.21	6.15	12	<LOD	0.15	0.34	0.56	4.38
	Mechanical engineer	149	38	<LOD	<LOD	0.04	0.24	137	7	<LOD	0.24	0.43	0.86	9.73
	Scaffold worker	44	30	<LOD	<LOD	0.04	0.15	5.24	30	<LOD	<LOD	0.24	0.36	2.15
	Drain fitter	10	60	<LOD	<LOD	<LOD	0.03	1.32	0	0.07	0.14	0.22	0.42	0.53
	Board man	36	53	<LOD	<LOD	<LOD	0.05	0.89	8	<LOD	0.25	0.37	0.53	3.65
	Construction craftsman	12	8	<LOD	0.16	0.23	0.76	1.58	0	0.18	0.39	0.67	0.89	2.45
	Electrical fitter	36	42	<LOD	<LOD	0.02	0.07	0.84	11	<LOD	0.21	0.34	0.47	1.29
	Welder	21	62	<LOD	<LOD	<LOD	0.03	1.22	10	<LOD	0.12	0.32	0.54	0.70
	Maintenance engineer	20	55	<LOD	<LOD	<LOD	0.05	0.48	10	<LOD	0.24	0.37	0.67	5.87
	Painter	13	77	<LOD	<LOD	<LOD	<LOD	0.17	0	0.07	0.16	0.21	0.27	0.51
	Tank fitter	5	20	<LOD	0.05	0.20	0.35	0.47	40	<LOD	<LOD	0.08	0.76	0.87
Process	Cumen	97	24	<LOD	0.02	0.11	0.41	7.11	14	<LOD	0.14	0.34	0.85	15.8
	MNB	83	61	<LOD	<LOD	<LOD	0.03	6.15	11	<LOD	0.16	0.29	0.43	5.87
	BTX	474	32	<LOD	<LOD	0.05	0.18	137	9	<LOD	0.21	0.40	0.64	9.73
	SM	15	0	0.13	0.20	0.43	0.96	2.38	7	<LOD	0.52	1.04	2.05	3.57
Total		669	34	<LOD	<LOD	0.04	0.19	137	11	<LOD	0.19	0.38	0.65	15.8

N: number of measurements; LOD: limit of detection; Min: minimum; Q1: the first quartile; Q3: the third quartile; Max: maximum; MNB: mono-nitro-benzene; BTX: benzene-toluene-xylene; SM: styrene monomer.

consisting of values above LODs of personal air benzene and urine *t,t*-MA.

The pooled correlation between personal air benzene and urine *t,t*-MA showed weak correlations (all measurement 0.24; repeated measurements 0.26) (Table 3). The correlation became stronger when the analyses were restricted to values above LODs of personal air benzene and urine *t,t*-MA (all measurement 0.37; repeated measurements 0.43).

Discussion

In this study, we estimated attenuation of the theoretical exposure-disease association for personal air benzene and its urinary metabolite *t,t*-MA to examine which is a better surrogate for benzene exposure in epidemiological analyses from a viewpoint of exposure variability. Previous studies[6, 8] examining measurement errors of personal air samples and biomarkers showed that, in general, biomarkers of chemicals with long half-lives present less attenuation than air samples. However, because chemicals with short half-lives have shown inconsistent results, and furthermore *t,t*-MA has not been examined before, here we attempted to compare the theoretical attenuation using both personal air benzene and its short-half-life urinary metabolite *t,t*-MA[19, 28]. According to our results, personal air benzene and urine *t,t*-MA showed a similar degree of attenuation of the theoretical exposure-disease association, which suggests that there may be no significant difference in attenuation bias when personal air benzene or urine *t,t*-MA is used as a surrogate for benzene exposure in epidemioloigical studies. Group-based analyses showed less attenuation than individual-based analyses, which is consistent with the results of previous studies examining measurement errors[19, 23].

During a maintenance operation, workers held different jobs work together around benzene exposure sources, but some workers may work far outside the exposure sources. Only including workers near exposure sources may lose variabilities among jobs and among workers, which may explain the decrease in between-group and within-group variance components in analyses for subsets consisting of values above LODs of personal air benzene and urine t,t-MA, thus resulting in an increase of attenuation. Our findings may suggest that assigning mean of only high exposure values for a job or a worker would lead to an increase of attenuation bias.

The correlation coefficient between personal air benzene

Table 2. Variance components and estimated attenuations of theoretical exposure-disease association for personal air benzene and urine *t,t*-MA

Dataset		Personal air benzene		Urine *t,t*-muconic acid	
		Group-based analysis	Individual-based analysis	Group-based analysis	Individual-based analysis
All measurements (N=667)	*k*	36.4	-	36.4	-
	n	1.4	1.4	1.4	1.4
	G	13	474	13	474
	Pooled σ^2_{BG}	0.65	-	0.08	-
	Pooled σ^2_{WG}	1.96	2.06	0.48	0.47
	Pooled σ^2_{WW}	3.77	4.35	0.74	0.74
	Attenuation	0.91	0.40	0.87	0.48
Repeated measurements (N=317)	*k*	10.2	-	10.2	-
	n	2.6	2.6	2.6	2.6
	G	12	122	12	122
	Pooled σ^2_{BG}	0.63	-	0.13	-
	Pooled σ^2_{WG}	1.91	1.84	0.49	0.52
	Pooled σ^2_{WW}	3.74	4.21	0.74	0.77
	Attenuation	0.85	0.53	0.86	0.64
All measurements & > LODs (N=395)	*k*	23.0	-	23.0	-
	n	1.3	1.3	1.3	1.3
	G	13	299	13	299
	σ^2_{BG}	0.14	-	0.07	-
	σ^2_{WG}	0.27	0.23	0.23	0.30
	σ^2_{WW}	2.20	2.35	0.52	0.53
	Attenuation	0.68	0.11	0.82	0.45
Repeated measurements & > LODs (N=155)	*k*	7.4	-	7.4	-
	n	2.6	2.6	2.6	2.6
	G	8	59	8	59
	σ^2_{BG}	0.21	-	0.10	-
	σ^2_{WG}	0.38	0.29	0.22	0.31
	σ^2_{WW}	2.49	2.70	0.52	0.53
	Attenuation	0.67	0.22	0.83	0.60

k: Average number of workers per job group ; *n*: average number of measurements per worker; *G*: the number of groups for group-based analysis or the total number of workers for individual-based analysis; σ^2_{BG}: between-group variance; σ^2_{WG}: within-group variance; σ^2_{WW}: within-worker variance; LODs: limits of detection for personal air benzene and urine *t,t*-MA.

Table 3. Correlation between log-transformed personal air benzene and log-transformed urine *t,t*-muconic acid

Dataset	N	r	SE
All measurements	669	0.24	0.04
Repeated measurements	317	0.26	0.05
All measurements, personal air benzene>LOD & urine *t,t*-muconic acid >LOD	395	0.37	0.05
Repeated measurements, personal air benzene>LOD & urine *t,t*-muconic acid >LOD	155	0.43	0.05

N: number of measurements; r: pooled correlation coefficient; SE: standard error; LOD: limit of detection.

and urine *t,t*-MA, excluding values below LODs was 0.37, but the correlation using all data resulted in a decreased correlation (0.24). The decrease might be caused mainly by the introduction of the multiple imputation technique, because the imputation process was performed independently for personal air benzene and for urine *t,t*-MA; thus, imputed values for personal air benzene and urine *t,t*-MA bear no correlation. Including uncorrelated imputed values in the analysis might lead to decrease in the correlation coefficient.

One of the causes of the low correlations may be due to the low benzene exposures. In previous studies, correlation coefficients between personal air benzene and urine *t,t*-MA reported ranged from 0.4 to 0.9[12, 29–38], and these studies

usually examined correlations in high-exposure environments. Personal air benzene and urine *t,t*-MA are well correlated in high-exposure circumstances; by contrast, if personal air benzene concentrations are low; i.e., <1 ppm, urine *t,t*-MA may show less-strong correlations[36, 39–42] or not be linearly correlated with air benzene concentration, instead showing a constant level[43]. Other studies reported that urine concentrations of *t,t*-MA were consistently elevated at or above an air benzene concentration of 0.2 ppm[43], or were useful at levels as low as 0.5 ppm[44, 45]. In our data set, only 9% of personal air benzene samples exceeded 1 ppm; such a high proportion of low concentrations might lead to a poor correlation between personal air benzene and urine *t,t*-MA. When excluding values below LODs of personal air benzene and urine *t,t*-MA, the correlation coefficient improved to 0.37, but not substantial.

The low correlation might also be explained by other factors, such as smoking, food intake, and genetic polymorphisms[39, 46]. Smokers usually show higher urine *t,t*-MA levels than non-smokers, especially in low-level exposure circumstances, which might confound the correlation between air benzene and urine *t,t*-MA[39, 47, 48]. Regarding food intake, *t,t*-MA levels can be substantially influenced by food preservatives and sorbic acid intake, especially at lower air benzene levels (<1 ppm)[7, 30, 49, 50]. However, in the present study, relevant information was not available. For monitoring of extremely low benzene exposure environments, recent studies reported unmetabolized urine benzene would be a more reliable biomarker than S-phenylmercapturic acid and *t,t*-MA[39, 41, 42].

Other causes of the low correlation might be associated with working conditions, such as respirator use and dermal exposure. Most of the workers who participated in the maintenance operations wore respirators. However, in general, highly exposed workers are more likely to wear personal protective equipment properly than less or indirectly exposed workers, which might lead to low urine *t,t*-MA levels in highly exposed workers and, thus, a low correlation.

Our study had several limitations. First, the equations used for estimating attenuation can be applied when the data are balanced; i.e., each job group consists of an equal number of workers and the workers are measured on the same number of occasions[51]. Our measurement data were highly unbalanced in terms of the number of workers per job group and the number of repeated measurements per worker. However, because it was reported that the equations for attenuation are relatively robust despite the violation of the above assumption[52], we assume that our estimates of attenuation are not substantially distorted. Second, the equations used to calculate the attenuation are based on the assumption that no other factors affect the corresponding health outcomes, except for the exposure[53]. This type of assumption may be unrealistic for most health outcomes, considering the multi-factorial nature of the causes of disease. Therefore, the attenuation formulas may be used to determine which surrogate is better, but they should be used with caution for adjusting the exposure-disease association *a posteriori*[51]. Third, we assumed that the first and subsequent measurements are independent, as the half-life of *t,t*-MA is short (5 h). In previous studies with other chemicals, however, various degrees of serial correlation was observed[8, 52]. In general, the strength of the correlation is inversely proportional to the time differences between the first and subsequent measurements[8, 52]. The time differences in our repeated measurement data varied widely. Therefore, we assumed that the correlation structure had compound symmetry. However, considering that introduction of a serial correlation increased attenuation in a previous study[8], our estimates of attenuation can be changed to some degree by adopting different correlation structures. Finally, we could not obtain information on important factors, such respirator use, food intake and smoking, which might affect correlations between personal air benzene and urine *t,t*-MA.

In summary, we aimed to evaluate whether personal air benzene or urine *t,t*-MA is a better surrogate for benzene exposure in terms of attenuation of the theoretical exposure-disease association in epidemiological analysis. Our results suggest that there would be no definitive difference in attenuation bias when personal air benzene or urine *t,t*-MA is used as a surrogate for benzene exposure. Our prediction of attenuation of theoretical exposure-disease association may aid exposure assessment efforts examining the associations between benzene exposure and health outcomes, but our results should also be tested in future epidemiological studies.

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Author Contributions

DHK designed the study and collected urine samples. MYL performed the analysis of urine *t,t*-MA. EKC and

JKJ performed air benzene sampling and analysis. DUP contributed to the analyses and their interpretation.

Ethics approval

This study protocol was reviewed by the Institutional Review Board of the OSHRI. All participants gave signed consent to provide their urine samples.

Conflict of Interests

The authors declare that they have no actual or potential conflict of interest.

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