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Commentary
High Background Levels of Urinary Benzene Metabolites Found in a Volunteer Study

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INTRODUCTION

Although benzene exposure is most often assessed via air monitoring, its metabolites can be measured in urine as well. The most commonly measured urinary metabolites for benzene are phenol, t,t-muconic acid (ttMA), and s-phenylmercapturic acid (sPMA). Biological monitoring for benzene can be done to assess both peak and chronic occupational exposures. The Occupational Safety and Health Administration (OSHA) benzene standard (29 CFR 1910.1028) requires that in an emergency benzene exposure incident, an employee's urine be collected at the end of the shift and analyzed for phenol adjusted for specific gravity. If the urinary phenol exceeds 75 mg/L, further medical evaluation and monitoring is required. In addition, the American Conference of Governmental Industrial Hygienists (ACGIH®) has established recommended biological exposure indices (BEIs) for benzene exposure of 500 μg ttMA/g creatinine and 25 μg sPMA/g creatinine measured at the end of a work shift.[1] The BEI for both metabolites is based on the current threshold limit value (TLV) of 0.5 ppm. Studies show that urinary phenol levels are not reliable indicators of airborne benzene exposures below approximately 5 ppm.[1–6]

This commentary presents the urinary phenol and ttMA results of one volunteer (first author) exposed to low levels of airborne benzene while simulating historical occupational exposure to Liquid Wrench. These results were used to evaluate the reliability of a single person's urinary excretion levels of phenol and ttMA to accurately portray low level exposure to benzene.

METHODS

A simulation study was performed to assess historical occupational exposure to aromatic hydrocarbons from using past formulations of Liquid Wrench, a product used to loosen rusty metal parts. Four historical products that contained varying concentrations of benzene were developed by Datachem Laboratories Inc. that may have been similar in physical and chemical composition to that of Liquid Wrench in some formulations used from 1960 to 1978.

The simulation study occurred during a 4-day period from July 18–21, 2005, in a residential two-car garage in Boulder, Colorado. Eleven different product-use scenarios were evaluated that varied according to the following parameters: amount of benzene in each product, the type of product used, the quantity of product used, and the ventilation rate. Four scenarios were assessed that varied only in terms of the benzene content in the historical Liquid Wrench formulation (i.e., 1%, 3%, 14%, 30%), while all other parameters were held constant. The seven remaining scenarios were chosen to evaluate the sensitivity of the air sampling results to each of these parameters by varying one parameter at a time while holding the benzene content constant at 3% or 14% by volume. The parameters included the quantities used (10 mL vs. 20 mL), the product type, and the ventilation rate (low, average, high, outdoors). The quantity of past formulations of Liquid Wrench used in the study was determined *a priori* by applying different amounts of product to rusted nuts, bolts, and pipe threads and determining how much liquid on average was required to complete various work tasks.

Personal and bystander air samples for benzene were collected for 15 min and 60 min during each scenario in the simulation study. The samples were analyzed by NIOSH Method 1501 by an an accredited industrial hygiene laboratory. Each sample was also analyzed for ethyl benzene, toluene, total xylenes, cyclohexane, and n-hexane. More details regarding the simulation study and the air sampling are presented in Williams et al. in this issue.[7]

The volunteer worker of interest in this study used Liquid Wrench to loosen rusty bolts during the first 3 days of the 4-day study. The total duration of benzene exposure was 5 hr, 6 hr, and 7 hr, respectively. The volunteer is a nonsmoker and wore no personal protective equipment (i.e., gloves, respirator,

etc.) during the study. Twenty-nine consecutive urine samples were collected from the volunteer worker during the 3 days of the simulation study and for 36 hours after the completion of his involvement in the study. The 29 samples represented the total urine void during this time period. Additionally, 30 consecutive background urine samples were collected after the study period in the same manner, such that total urine void was collected. Although the diet of the volunteer was comparable between the sampling and background periods, it was not held constant.

Urine samples (N = 59) were collected in polyethylene containers per the laboratory's sampling and shipping instructions. The samples were analyzed for specific gravity, creatinine, phenol, and ttMA. Phenol levels were quantified via high-performance liquid chromatography (HPLC) with ultraviolet detection with a limit of detection (LOD) of 0.1 mg/L. Urinary phenol was reported in three ways: unadjusted, adjusted for specific gravity, and creatinine adjusted. Samples were analyzed for ttMA using HPLC with ultraviolet detection with an LOD of 0.1 mg/L and reported unadjusted and adjusted for the creatinine level in the sample. The purpose of adjusting urinary phenol for specific gravity was to compare the urine results with the OSHA benzene standard. Likewise, ttMA was adjusted for creatinine so results could be compared with the ACGIH BEI.

Background concentrations of total daily and spot urinary excretion of phenol and ttMA were compared with the excretion levels measured in the urine collected during the simulation study period. Phenol and ttMA results reported by the lab to be below the LOD were treated as LOD/$\sqrt{2}$ for analysis purposes. A nested ANOVA test with sampling day as the nested variable was performed to determine whether there was a statistical difference between the spot urine levels of uncorrected phenol, phenol adjusted for specific gravity, creatinine-adjusted phenol, uncorrected ttMA, and creatinine-adjusted ttMA collected during the background and study sampling periods.

RESULTS

During the simulation study, the calculated 8-hr-time weighted average (TWA) airborne concentrations of benzene ranged from 0.1 ppm to 0.83 ppm (mean = 0.47 ppm). The 8-hr TWAs were calculated using the 1-hr personal samples collected over the course of each day. During periods of nonexposure, benzene concentrations were assumed to be zero. Total daily excretion of phenol adjusted for both specific gravity and creatinine and ttMA adjusted for creatinine are presented in Figures 1 and 2, respectively. Figures 3 and 4 present the concentration of phenol corrected for both creatinine and specific gravity and ttMA corrected for creatinine measured in the spot urine samples. Of the 29 urine samples collected during the simulation study period, approximately 28% contained levels of phenol greater than 75 mg/L (Figure 3), and 31% of the samples had levels of ttMA greater than 500 μg/g creatinine (Figure 4). Thirty background urine samples were also collected from the worker; approximately 7% had levels of phenol over 75 mg/L (Figure 3), and 30% had levels of ttMA over the 500 μg/g (Figure 4).

The mean concentrations and standard deviations of phenol, phenol adjusted for creatinine, phenol adjusted for specific gravity, ttMA, and ttMA adjusted for creatinine are presented in Table I. Only total phenol corrected for specific gravity was significantly greater in the urine samples collected during the study period than in the urine collected during the background period (p = 0.0002). The levels of uncorrected total phenol, total phenol corrected for creatinine, uncorrected ttMA, and ttMA corrected for creatinine were not statistically different (p = 0.50, 0.39, 0.32, 0.48, respectively).

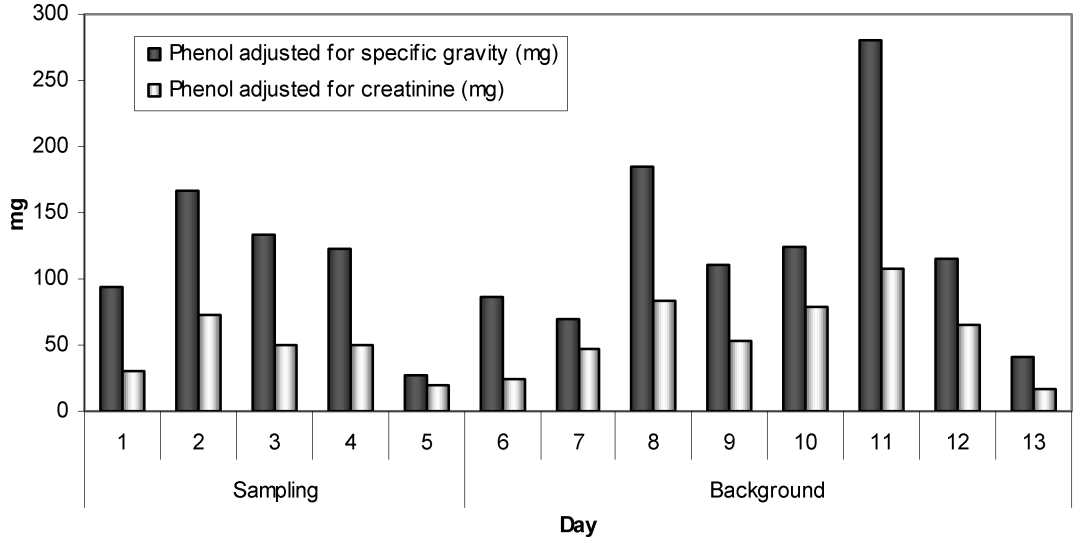

FIGURE 1. Total urinary phenol results adjusted for specific gravity and creatinine by day during sampling and background periods

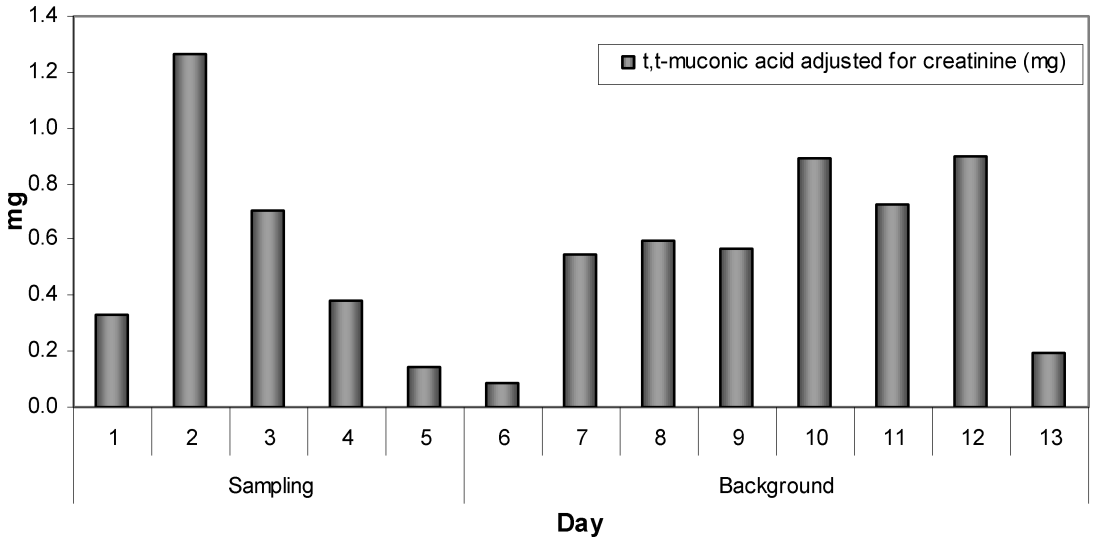

FIGURE 2. Total urinary ttMA results adjusted for creatinine by day during sampling and background periods

DISCUSSION

The results of this study support earlier research that reported that urinary phenol and ttMA are not reliable indicators of low level benzene exposures.[7] Although personal air samples were not collected during the background study period, based on the volunteer subject's activities during those days he was most likely exposed to typical, urban background benzene air concentrations that have been reported in the literature to range from approximately 2 $\mu g/m^3$ to 11 $\mu g/m^3$.[8–10] The biomonitoring results show that for this subject, the average background levels of urinary phenol and ttMA were not significantly different from those measured during the simulation study period when the airborne concentrations of benzene were most likely an order of magnitude higher.

There are limitations to this study. The main limitation is that this study reports the results of a single volunteer. The results of this study would be more informative if there were data on multiple individuals for several exposure scenarios. Furthermore, the probability of actually being overexposed to benzene when a biomarker is above the BEI or OSHA standard cannot be estimated with the results of a single individual with a few days of exposure. To produce that probability, several volunteers and several simulated exposure runs would be needed. This would be a worthwhile area of further study.

Another limitation to this study exists in the fact that urine samples collected throughout the day by the volunteer during both the sampling and background periods are compared with the OSHA benzene standard and the ACGIH BEI, which are designed to be compared with urine collected at the end of an 8-hr workshift. In comparing only "end-of-shift" urine samples with these reference values, Figure 4 shows that during the sampling period almost all urine samples collected at the end of the exposure scenarios had ttMA concentrations above the BEI. However, there were also urine samples collected before and during the exposure scenarios above the BEI. Furthermore, 5 of the 8 background days had end-of-the-day urine samples containing ttMA concentrations above the BEI.

Despite the limitations, this study provides additional data regarding the reliability of single spot urine sampling such as those typically collected at the end of the workshift. In this study, the volunteer subject collected numerous spot urine samples throughout the day during both the background and simulation study time periods. Examination of the individual spot samples collected during the background study shows that, occasionally, single spot samples had phenol and ttMA concentrations greater than the OSHA guideline for phenol and the ACGIH BEI for ttMA. Although it cannot be confirmed that the volunteer was not exposed to benzene over typical background concentrations, the results of this study suggest that the evaluation of single spot urine samples may lead to false positive results in terms of the external benzene exposure concentration. These results support the current literature that spot urine samples are not reliable indicators of exposure when 8-hr TWA benzene exposures do not exceed 1 ppm and confounding factors (i.e., smoking, environmental sources, diet, genetics, etc.) are not considered.[1,2,11–18]

The elevated metabolites identified in the volunteer subject during the background study are noteworthy, since the percentage of the population who are "high excretors" for these biomarkers is not known. However, the ttMA results are consistent with other research findings showing elevated background levels of benzene metabolites when airborne benzene exposures are low.[1,2,7,15,18,19] Studies[2,19] have shown high background levels of ttMA ranging from 710–880 μg ttMA/g creatinine in unexposed and nonsmoking study populations. The results may be related to personal dietary habits (i.e., consumption of sorbic acid preserved foods), exercise, pharmaceutical use, and/or interindividual differences in metabolism.[1,16–18]

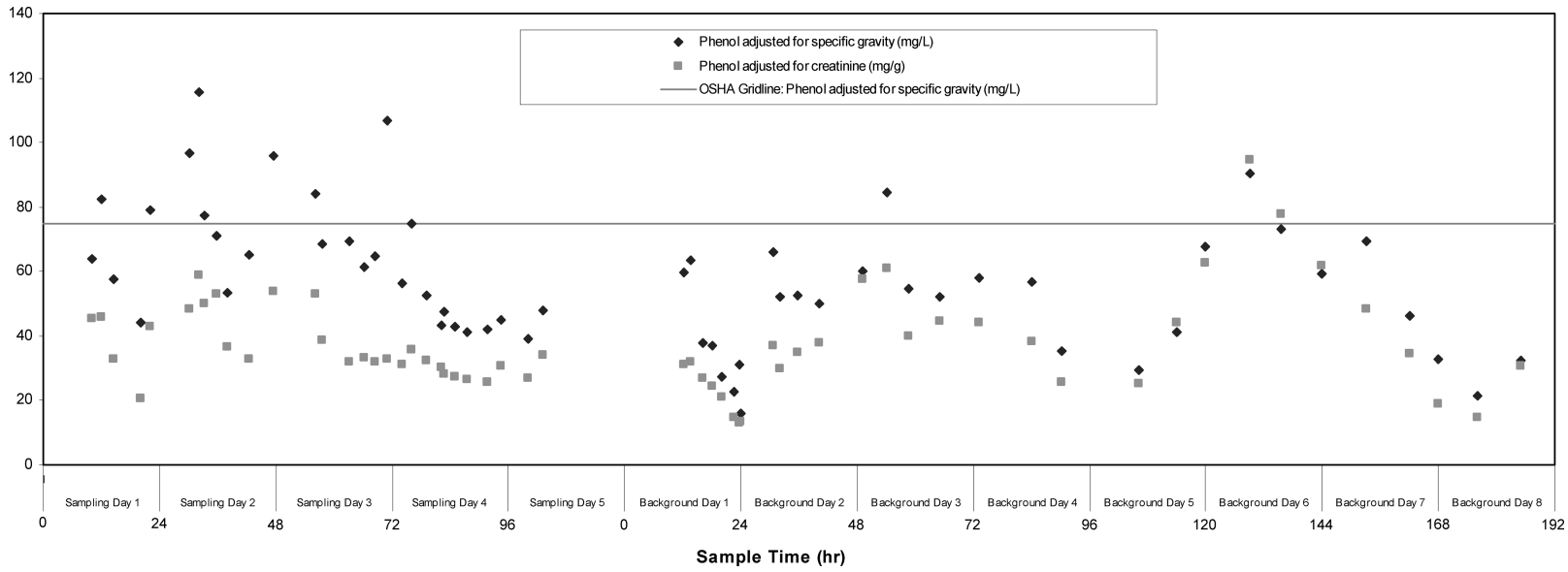

FIGURE 3. Spot urinary phenol results adjusted for specific gravity and creatinine by day during background and sampling periods as compared with OSHA emergency standard for benzene

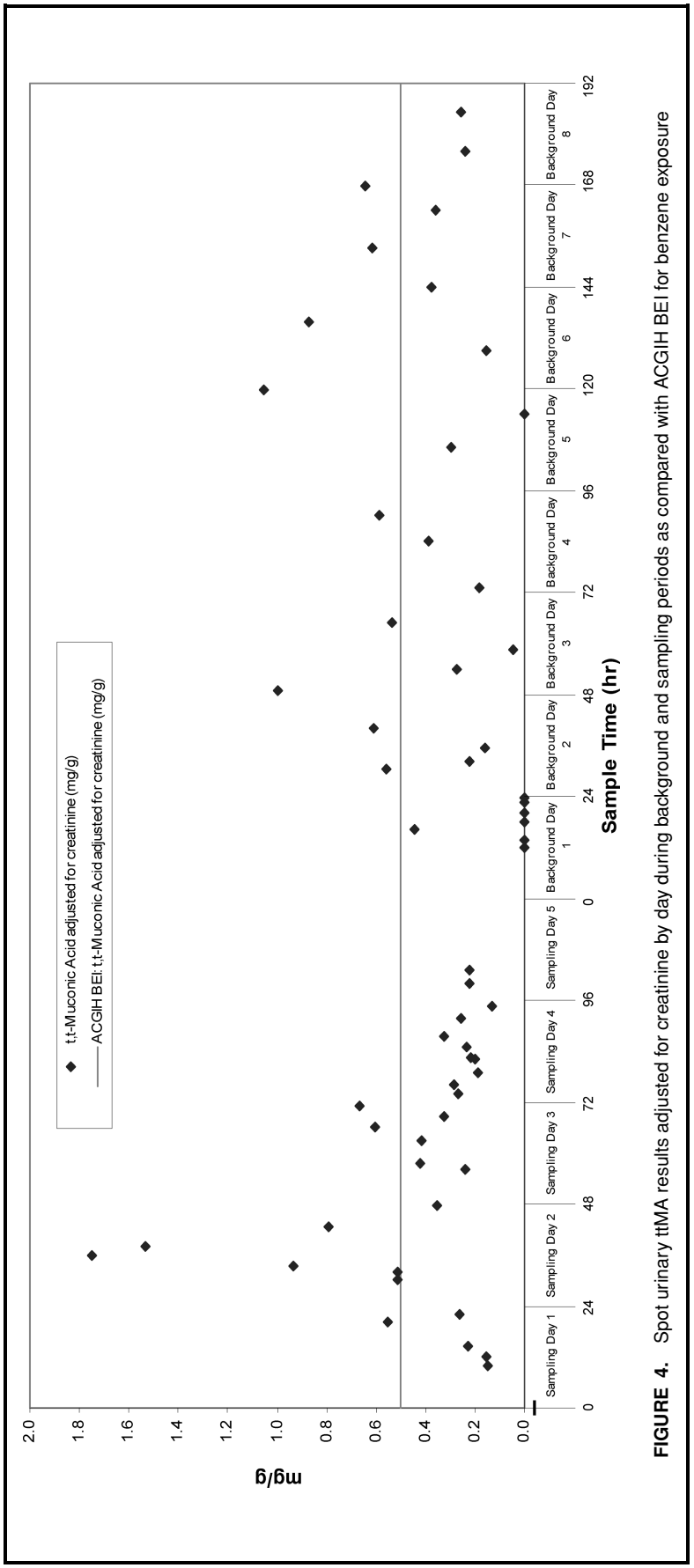

FIGURE 4. Spot urinary ttMA results adjusted for creatinine by day during background and sampling periods as compared with ACGIH BEI for benzene exposure

TABLE I. Results of Spot Urine Analysis

Metabolite	Time Period	Sample Size	Mean	Standard Deviation	Nested ANOVA p-value
Phenol uncorrected (mg/L)					0.50
	Sampling	29	28.00	15.70	
	Background	30	24.09	13.60	
Phenol corrected for creatinine (mg/g cr)					0.39
	Sampling	29	36.96	10.05	
	Background	30	38.02	19.37	
Phenol corrected for specific gravity (mg/L)					0.0002
	Sampling	29	65.07	20.79	
	Background	30	49.36	18.69	
t,t-muconic acid uncorrected (mg/L)					0.32
	Sampling	29	0.36	0.36	
	Background	30	0.26	0.28	
t,t-muconic acid corrected for creatinine (mg/g cr)					0.48
	Sampling	29	0.45	0.39	
	Background	30	0.33	0.31	

Note: Results compare urinary levels of phenol and ttMA during background period with levels found in urine during sampling period.

In addition, co-exposure to five other chemicals at lesser airborne concentrations could have had some measurable impact on the urine values observed during the study. For example, the concentration of toluene present during this study ranged from 0.16 ppm to 0.6 ppm collected in 1-hr personal samples, and low-level toluene exposures may increase the levels of urinary phenol and suppress the levels of ttMA found in the urine of benzene exposed workers.[1,19,20] Inoue et al.[21] showed that in the presence of other aromatic compounds, sPMA may be a more reliable biological indicator of airborne benzene exposure than ttMA and/or phenol. However, the impact of the other contaminants measured in the simulation study could not be determined based on the results of this urine study. In conclusion, multiple background urine samples on each individual are essential in any biomonitoring program to determine whether high levels of excreted metabolites correspond to workplace exposure. Without adequate background data over several days, biomonitoring data should not be relied on to determine the level of exposure or any potential risk to individual employees.

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