

Benzene Exposure, Assessed by Urinary *trans,trans*-Muconic Acid, in Urban Children with Elevated Blood Lead Levels

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A pilot study was performed to evaluate the feasibility of using *trans,trans*-muconic acid (MA) as a biomarker of environmental benzene exposure. A secondary aim was to provide data on the extent of exposure to selected toxicants in a unique population consisting of inner-city children who were already overexposed to one urban hazard, lead. Potential sources of benzene were assessed by a questionnaire. Exposure biomarkers included urinary MA and cotinine and blood lead. Mean MA was 176.6 ± 341.7 ng/mg creatinine in the 79 children who participated. A wide range of values was found with as many as 10.1%, depending on the comparison study, above the highest levels reported in adults not exposed by occupation. Mean MA was increased in children evaluated in the afternoon compared to morning, those at or above the median for time spent playing near the street, and those studied in the first half of the investigation. MA levels were not associated with blood lead or, consistently, with either questionnaire environmental tobacco smoke (ETS) data or cotinine. As expected, the mean blood lead level was elevated (23.6 μ g/dl). Mean cotinine was also increased at 79.2 ng/mg creatinine. We conclude that the use of MA as a biomarker for environmental benzene exposure is feasible since it was detectable in 72% of subjects with a wide range of values present. In future studies, correlation of MA with personal air sampling in environmental exposure will be essential to fully interpret the significance of these findings. In addition, these inner-city children comprise a high risk group for exposure to environmental toxicants including ETS, lead, and probably benzene, based on questionnaire sources and its presence in ETS. **Key words:** biological monitoring, biomarkers, cotinine, environmental benzene exposure, *trans,trans*-muconic acid, urban pollution. *Environ Health Perspect* 104:318–323 (1996)

Environmental exposure to benzene is an important public health concern because it is common (1), particularly in cities (2), and adverse hematologic effects, including leukemia, have occurred in occupationally exposed workers. It is classified as a human carcinogen by numerous public health agencies and regulated as a toxic air pollutant under the Clean Air Act (1). Extensive U.S. EPA monitoring has found that several sources contribute to exposure (1). Mainstream tobacco smoke accounts for about 50% of the total U.S. population burden with environmental tobacco smoke (ETS) contributing 5%. Benzene in gasoline results in about 20% of residential exposure due to attached garages and time spent in automobiles. Vehicle exhaust, certain industrial facilities, and consumer products are other sources.

Personal air sampling and exhaled benzene breath measurements have been used previously to assess environmental exposure (1,2). Exposure assessment that includes measurement of metabolites in biological specimens would add substantially to the knowledge gained in such research by providing information on individual variation in absorption and metabolism. This varia-

tion is an essential factor in the markedly different human susceptibility for toxic outcomes at similar exposure levels. Such data will ultimately allow the identification of high risk individuals and populations.

Urinary phenol has been utilized as a biological monitoring tool for benzene exposed workers; however, phenol is found in many foods and is a product of protein catabolism. As a result, it lacks the specificity needed for exposures below 5 ppm (3). An aliphatic metabolite, *trans,trans*-muconic acid (MA), is both sensitive and specific when correlated with airborne levels in workers exposed to a wide range of benzene concentrations including levels as low as 0.1 ppm (3–5). Research on MA in subjects not exposed by occupation or revealed significantly higher mean levels in smokers compared to nonsmokers (6) as well as a linear correlation with cotinine in smokers (7). MA excretion in a 12-hr post-exposure period has been correlated with short-term ETS exposure (8). Lastly, the metabolite has been detected in varying proportions of nonsmokers, ranging from 23.7% (9) to 100% (5). These results suggest that MA may have the requisite characteristics for use as a biomarker for envi-

ronmental benzene exposure. However, its use in the ppb air level exposures that are characteristic of the environmental setting must be approached carefully since it is not completely specific for benzene; 0.1–0.2% of the food preservative, sorbic acid, is metabolized to MA (10).

Therefore, we conducted a pilot study whose primary goal was to determine the feasibility of using MA as a biomarker in environmental exposure. A secondary aim was to provide data on the extent of exposure to selected toxicants in a unique population consisting of inner-city children in urban Baltimore, MD. Low socioeconomic status is a risk factor for development of many chronic diseases, including cancer (11) and there is mounting concern that this may be due, in part, to environmental toxicant exposures (12). However, data to address this concern are limited, particularly in children. Because individuals who are already overexposed to one environmental hazard may be at greater risk for additional exposures, we recruited subjects from a clinic for lead exposed children. The pilot study assessed potential sources of benzene using a questionnaire and urinary cotinine (as a measure of ETS as well as a benzene source) and compared these results with urinary MA. Blood lead was measured as part of routine clinical care.

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Methods

Study population and design. All children who were seen at the Kennedy Krieger Lead Poisoning Prevention Clinic during a 4-week study period in September and October of 1994 were eligible for enrollment. These children were originally referred to the clinic for evaluation of elevated blood lead levels; some were receiving ongoing follow-up for persistently elevated blood leads or other lead related concerns such as learning disorders. The parent/guardians of 117 children were informed about the study during the clinic visit. Thirty-one of the children were excluded for one of the following reasons: not toilet trained, could not produce a specimen during the clinic visit, or the guardian was not present. The parents of 79 of the remaining 86 children (91.9%) agreed to participate. In a few instances, siblings were included in our study when more than one child in a family was seen as a patient in the clinic.

Explanations to the adults and children (appropriately age-modified) were provided, and informed consent, approved by the Joint Committee on Clinical Investigation (Johns Hopkins University School of Medicine and the Johns Hopkins Hospital) was obtained from all participants. A questionnaire, administered to the parents, elicited basic demographic information on the child as well as medical and environmental histories. Sources of benzene exposure were noted, including ETS, gasoline (attached garage, time spent in a car or bus, living near a gas station or on a main street, time spent playing near street), and presence of a neighborhood industrial benzene source. Because the half-life of MA is relatively short [approximately 5 hr (9)], most questions regarding benzene exposure were directed toward the 24-hr period preceding urine collection. However, questions pertaining to ETS assessed exposure in the preceding 48 hr, due to the longer half-life of cotinine. A urine sample for MA, cotinine, and creatinine was obtained and stored at -80°C until analyzed. Venous blood lead levels were obtained as part of routine clinical care.

Chemicals. MA for standard preparation was purchased from Aldrich Chemical Co. (Milwaukee, Wisconsin). A stock solution of MA was prepared as described by Lee et al. (4) and quantified with a Beckman DU-7 spectrophotometer (Beckman Instruments, Inc., Fullerton, California) using a molar extinction coefficient of 21,900 at 261 nm. Stock standard solution was stored in 80% methanol/water at -20°C and was found to be stable for 4–6 weeks. Working dilutions of 100 mg/l and 1 mg/l in 1% acetic acid were also stored at -20°C and were stable for at

least 2 months. All water used was purified by a MilliQ Water System (Millipore Corporation, Bedford, Massachusetts). All other chemicals used were of the highest quality obtainable commercially.

HPLC assay. We adapted an HPLC method from that of Ducos et al. (10). Sodium acetate was added to the mobile phase to stabilize pH and reduce variation in retention times (4), the organic and aqueous components of the mobile phase were premixed to reduce air bubble generation (13), and diode array detection was used to increase specificity. Urine pH was adjusted before strong anion exchange (SAX) extraction to ensure reproducible recovery of MA (14), and the final eluate volume was reduced to 3 ml (6). In a routine analysis, a 1-ml sample of urine was adjusted to pH 4.5–5.7 with concentrated HCl. The sample was then extracted on a PrepSep SAX cartridge (Fisher Scientific, Fair Lawn, New Jersey) (preconditioned with 3 ml of methanol and 3 ml of water) by application at a flow rate of 1 ml/min using a Gilson minipulse peristaltic pump (Middleton, Wisconsin). After application of the urine, the cartridge was washed with 3 ml of 1% acetic acid at 2 ml/min and the MA was eluted with 3 ml of 10% acetic acid at 1 ml/min. Twenty microliters of eluate was injected onto the HPLC. This instrument consisted of a Hewlett-Packard 1090M gradient machine equipped with a diode-array detector. The HPLC column was an Altima C18 5 μ m (25 cm $\times$ 4.6 mm) analytical column (Alltech Associates Inc., Deerfield, Illinois). The analytical column was preceded by a Brownlee guard cartridge system with a 5 μ m (30 mm $\times$ 4.6 mm) cartridge (Rainin Instrument Co., Woburn, Massachusetts). Chromatography was isocratic in a mobile phase consisting of glacial acetic acid, 1 M sodium acetate, and methanol (4.5/1.8/100 ml) brought up to 1 l at pH 3.0, at a flow rate of 1 ml/min. The column temperature was maintained at 40°C. The mobile phase was filtered and sparged with helium before use. We quantified peak area by the area-under-the-curve method of the supplied software. We calculated concentration from a standard curve regression line.

GC/MS assay. We obtained structural confirmation of elevated MA levels by a gas chromatography/mass spectroscopy (GC/MS) method adapted from that described by Bechtold et al. (15). Urine samples were prepared using the PrepSep SAX method, described above. 500 μ l of the eluate was dried in a Speed Vac (Savant Instruments Inc., Farmingdale, New York). 100 μ l of Tri-Sil BSA in dimethylformamide (Pierce, Rockford, Illinois) was added to the dried sample which was then derivatized for 15

min at 60°C. A 1- μ l aliquot of the derivatized sample was injected onto the GC/MS, a Hewlett-Packard 5890 Series II gas chromatograph equipped with a 5971 MSD detector (Hewlett-Packard Company, Palo Alto, California). The GC column was an HP-1 fused silica capillary column, 25 m by 0.20 mm, 0.11- μ m film thickness. The initial temperature was set at 100°C for 1 min followed by a 10°C/min increase to a final temperature of 265°C. Selected ion monitoring (ion 271) and scan modes on standards and human samples were used to confirm the presence of derivatized MA.

Urinary cotinine assay. Cotinine values were measured by radioimmunoassay at the American Health Foundation, Valhalla, New York. The assay is based on the Langone et al. method (16) which involves producing a specific antiserum in rabbits through injection of *trans*-4-carboxycotinine bound to albumin (17). The inter- and intra-assay variations are reported as less than 6% (17). The limit of detection is 2 ng/ml.

Urinary creatinine assay. We measured creatinine using the Sigma kit (St. Louis, Missouri). This colorimetric assay is based on difference in absorbance at 500 nm of the creatinine-picric acid chromogen before and after acidification. Absorbance was measured in a Beckman DU-7 spectrophotometer. The assay described in the kit was followed using a 10-fold urine dilution; however, each sample was modified to use a third of the suggested sample and reagents, thus 1 ml of alkaline picric acid solution was added to 100 μ l of diluted urine and 33 μ l of acid reagent was subsequently added. An automated creatinine assay was also performed on 10 specimens, using a Kodak Ektachem 500 Computer-Directed Analyzer (Eastman Kodak Company, Rochester, New York). A correlation of 0.98 was found between the two assays.

Blood lead analysis. Anodic stripping voltammetry was used to measure blood lead levels. An Environmental Science Associates 3010A Trace Metals Analyzer with mercury coated graphite electrode, Ag/AgCl reference electrode and platinum counter electrode was used (ESA). The limit of detection is approximately 2 μ g/dl.

Statistical analysis. Because the MA distributions were rightward skewed, we used the natural logarithmic transformations, both with and without creatinine adjustment. Values below the limit of detection (LD) were set at half its level for data analysis. In *t*-test and regression analyses, nondichotomous questionnaire exposure source variables were categorized by median value; clinic visit time was divided into morning and afternoon. The number of nondetectables resulted in an inability to completely

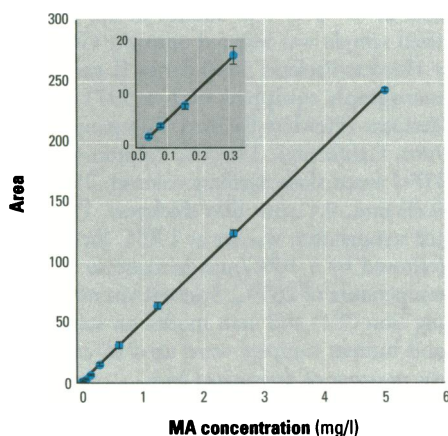


Figure 1. The *trans,trans*-muconic acid (MA) spiked urine concentration curve performed in triplicate. The error bars indicate the standard deviation from the mean. The curve range is 0.26–33.3 ng MA per injection; $r = 0.99$.

normalize the creatinine unadjusted MA results through logarithmic transformation. Therefore, we performed nonparametric statistical testing for this variable with Mann-Whitney rank sum for mean comparisons and Spearman correlation coefficient for continuous variables. Standard parametric analyses were used for the creatinine adjusted MA variable which was normalized by $\ln$ transformation. Multiple linear regression models were fitted to assess independent associations while adjusting for other possibly confounding variables. Again, to address non-normality concerns, logistic regression using a dichotomous MA outcome of detectable versus nondetectable and linear regression using only detectable MA values were also performed for both MA variables. All analyses were performed in BMDP (BMDP Statistical Software, Inc., Los Angeles, California), except logistic regression which was performed in EGRET (EGRET Statistical Software, SERC and Cytel Corp., Seattle, Washington). Sigmaplot (Jandel Scientific, San Rafael, California) was used for graphics.

Results

Initial experiments were performed to optimize the HPLC method for the detection and measurement of MA in human urine samples. The key parameter in optimizing the method was pH control of both the HPLC mobile phase and the urine sample before application to the preparative anion-exchange column. By carefully controlling the pH, the mean recovery of MA spiked into a urine sample was $95.0 \pm 3\%$ (SD) over an MA concentration range of 0.039–5.0 mg/l. A spiked human urine sample, rather than the pure compound, was used to generate a standard curve,

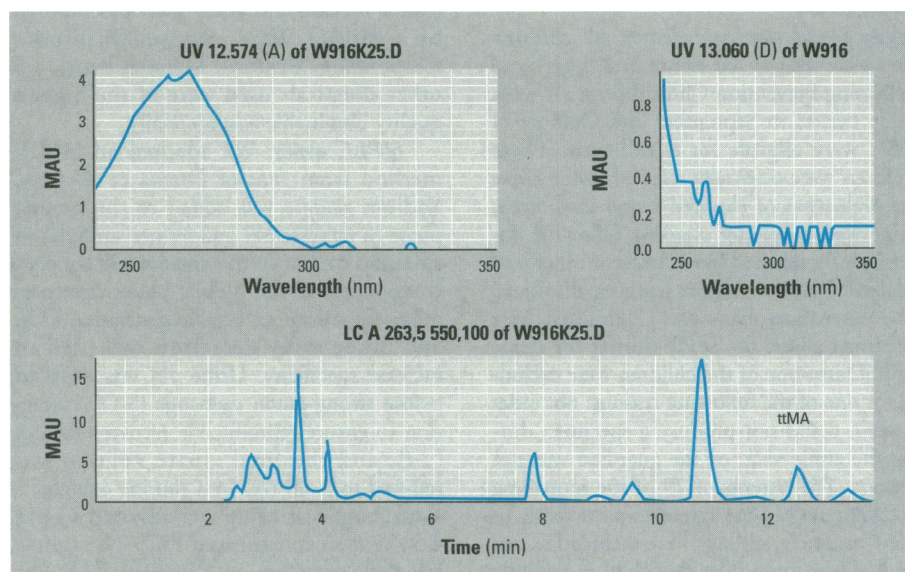


Figure 2. HPLC chromatogram from one of the study participants. The UV spectrum is displayed in the upper left corner with the baseline reference spectrum in the upper right.

Table 1. Characteristics of the 79 study participants

Age in years (mean $\pm$ SD)	4.3 $\pm$ 1.6
Range	1.7–10.5
Race	
% Black	96.2
% White	2.5
% Asian	1.3
Gender	
% Male	48.1
% Female	51.9
Blood lead (μ g/dl)	
(mean $\pm$ SD; $n = 77$)	23.6 $\pm$ 8.51
Range	5–45

Table 2. Urinary levels of *trans,trans*-muconic acid (MA) and cotinine

Urinary metabolites	ng/ml	ng/mg creatinine
MA (mean $\pm$ SD)	144.5 $\pm$ 296.1	176.6 $\pm$ 341.7
Range ^a	8–2001.2	7.1–2579.2
Geometric mean	49.4	75.2
Median	59.5	78.8
≥ 500 ng/ml	6 (7.6%)	—
$\geq$ Limit of detection	57 (72.2%)	—
Cotinine	54.7 $\pm$ 45.6	79.2 $\pm$ 70.0
(mean $\pm$ SD; $n = 78$)		

^aNondetectables set at 1/2 limit of detection.

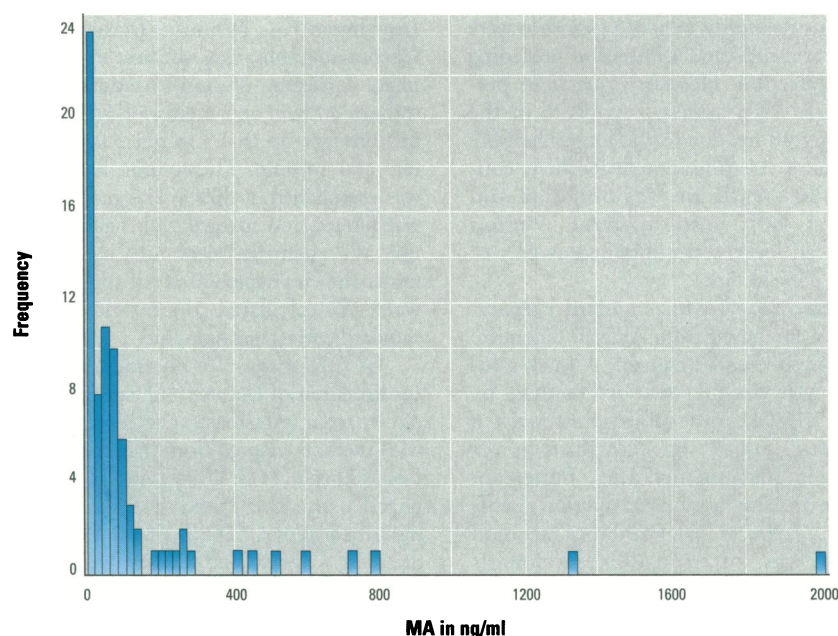


Figure 3. Frequency histogram of *trans,trans*-muconic acid (MA) values.

shown in Figure 1. This decision was made to accommodate the inherently higher background found in real samples and to provide a conservative estimate of the limit of detection of the assay. The coefficient of variation ($n = 3$) was 14% at the lowest point on the concentration curve (0.039 mg/l); the mean variation was $5.6 \pm 3.5\%$ for concentrations from 0.078–5.0 mg/l. The LD, as defined by a 3:1 signal to noise ratio, was 0.016 mg/l or 0.105 ng per HPLC injection. Ultraviolet (UV) spectra of MA were obtainable using the diode-array detector down to a concentration of 0.075 mg/l. Below this level, the identity of MA was determined by the retention time of standard injections. An example of the HPLC profile obtained from one of the subjects is shown in Figure 2.

The characteristics of the 79 children from Baltimore who participated in the study are shown in Table 1. These children were generally young; only five of the children were over 6 years old. The population was predominantly black, reflecting the ethnic composition of the inner-city population cared for at the clinic. As expected, blood lead levels were elevated. The mean of 23.6 $\mu\text{g/dl}$ is well above the 10 $\mu\text{g/dl}$ action level recommended by the Centers for Disease Control and Prevention (18). Only two children had blood lead levels below 10 $\mu\text{g/dl}$.

Table 2 shows levels of the two urinary metabolites measured in this study with and without adjustment for creatinine. Since the data were rightward skewed, geometric means and medians for MA are also given. Quantifiable levels of MA were found in 57 of 79 samples. Dilute urine was likely a contributing factor since creatinine levels were significantly lower ($p < 0.001$) in nondetectables. Figure 3 displays the MA values in a frequency distribution plot. We found 6 values (7.6%) above 500 ng/ml. Although not an absolute cut-off, these values are more consistent with occupational than environmental exposure (10). Structural confirmation was provided by GC/MS analysis on four of these specimens; a chromatogram from one is shown in Figure 4.

Table 3 depicts data on potential sources of benzene exposure and other significant variables found in the study. The sources were diverse. All children were exposed to at least one source with many exposed to two or more. Industrial sources were generally automobile related, such as repair shops. MA mean values from t -test analyses are shown along with the number of subjects in each category. Several statistically significant variables were noted and analyzed further. Figure 5 presents mean

creatinine adjusted MA stratified by time spent near the street (TSNS), morning vs. afternoon evaluation, and study period. Despite small numbers in several of the categories, a consistent pattern is noted; levels are higher in the afternoon, during the first half of the study, and in children who spend one or more hours playing near the street after accounting for the other two variables. These variables are presented in linear regression models in Table 4, thus showing the independent relation of each one with the MA outcomes. In addition, β gives the average MA difference for those in the corresponding category. For example,

playing in the street for one or more hours increases the level of ln MA by 17.5% (0.64/3.65), taking into account the time of day and study date. The additional regression analyses, performed to avoid the non-normality of MA, showed a consistent pattern with the initial linear regression results for time of evaluation and time spent near the street, although with less statistical significance. Study period results, however, were similar only in logistic regression.

Discussion

The primary goal of this study was to assess the feasibility of using MA as a biomarker

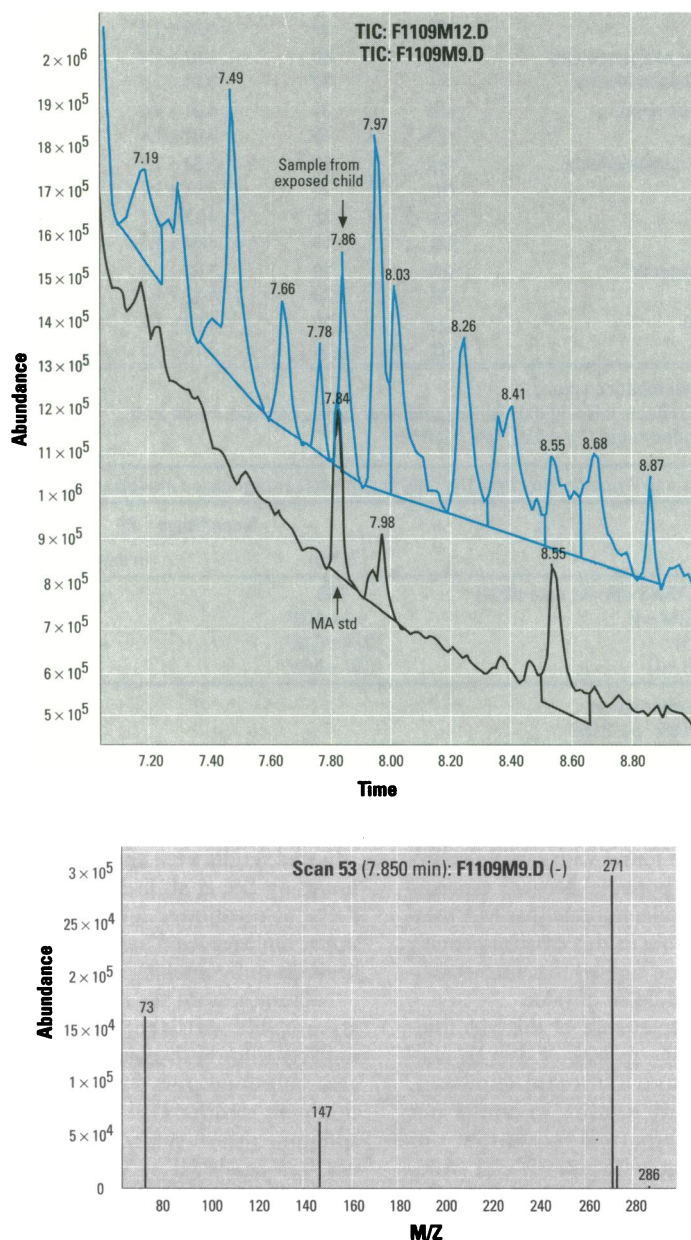


Figure 4. GC/MS chromatogram of a sample from a child with an elevated *trans,trans*-muconic acid (MA) level of HPLC (green line) overlaid with an MA standard injection (black line). The mass spectrum is from the human sample.

Table 3. Mean levels of natural log-transformed *trans,trans*-muconic acid (MA) for exposure sources and selected other variables

Variable		n ^a	ln MA	
			ln MA (ng/μl; mean ± SD)	(ng/mg creatinine; mean ± SD)
Time of evaluation	AM	59	3.55 ± 1.4	4.05 ± 1.2
	PM	20	4.95 ± 1.1 [†]	5.10 ± 1.1 [†]
Study period	<9/21	39	4.16 ± 1.4	4.72 ± 1.1
	≥ 9/21	40	3.65 ± 1.5	3.92 ± 1.2**
Garage attached to residence	Yes	6	3.44 ± 1.7	4.11 ± 1.4
	No	73	3.94 ± 1.4	4.33 ± 1.2
Nearby industrial source	Yes	13	3.61 ± 1.6	4.19 ± 1.3
	No	66	3.96 ± 1.4	4.34 ± 1.2
Residence on a main street	Yes	42	3.61 ± 1.5	4.03 ± 1.2
	No	37	4.24 ± 1.4*	4.64 ± 1.2*
Time spent near the street (min)	<60	33	3.5 ± 1.3	3.92 ± 1.1
	≥60	35	4.32 ± 1.5*	4.62 ± 1.3*
Distance between residence and nearest gas station in blocks	≤5	40	3.87 ± 1.4	4.33 ± 1.2
	>5	39	3.94 ± 1.5	4.30 ± 1.4
Travel in car or bus (min)	≤25	34	4.01 ± 1.6	4.29 ± 1.5
	>25	45	3.82 ± 1.4	4.33 ± 1.0
ETS exposure by questionnaire	Yes	53	3.84 ± 1.6	4.28 ± 1.4
	No	26	4.02 ± 1.1	4.39 ± 1.0
Mother smokes	Yes	23	4.16 ± 1.7	4.58 ± 1.5
	No	56	3.80 ± 1.3	4.21 ± 1.1
Urinary cotinine (ng/ml)	≤44	39	3.53 ± 1.4	4.16 ± 1.2
	>44	39	4.32 ± 1.4*	4.51 ± 1.2
Blood lead (μg/dl)	≤22	39	4.08 ± 1.4	4.46 ± 1.3
	>22	38	3.74 ± 1.5	4.18 ± 1.2

ETS, environmental tobacco smoke.

^aCombined n < 79 reflects missing data (time spent near street, cotinine, blood lead).*p < 0.05 for the difference; **p ≤ 0.01; [†]p ≤ 0.001.**Table 4.** Variables and β coefficients from the final multiple linear regression models (n = 68)

Variable	β coefficient ± SE	
	ln MA	ln creatinine-adjusted MA
Intercept (AM visit, TSNS <60 min, date ≤9/21)	3.65	4.3
Time of clinic visit (PM = 1)	1.2 ± 0.38*	0.86 ± 0.33*
TSNS (≥ 60 min = 1)	0.64 ± 0.32*	0.52 ± 0.28 [†]
Study period (≥9/21 = 1)	-0.57 ± 0.32**	-0.8 ± 0.28*

TSNS, time spent near street.

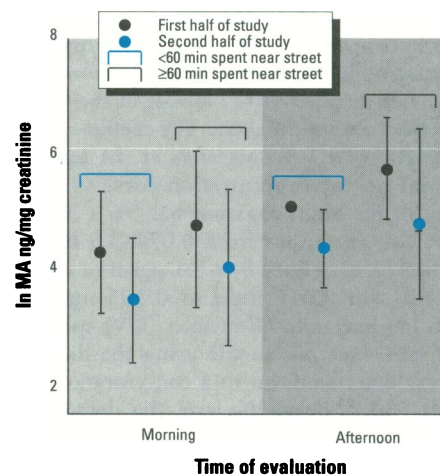
*p < 0.05; **p = 0.085; [†]p = 0.067.

for environmental benzene exposure. MA was detectable in 72% of subjects with a wide range of values present. In addition, associations were found with sample collection times and a potential benzene exposure source. These results indicate that MA measurement is feasible in this environmentally exposed group and suggest that further validation studies would be of value.

Further interpretation of the MA findings is limited due to lack of data in children and in environmental exposure. Comparison with studies in adults not exposed by occupation shows that our results in children (with and without creatinine adjustment) generally fall between the means reported for smokers and nonsmokers (4–6,19). We also found several elevated MA values. Although such levels have been measured previously in adults with no occupational sources of benzene, our find-

ings were high even in relation to upper limits noted by several other researchers. For instance, 10.1% of our creatinine adjusted results were above the upper value found by Lee et al. in 35 smokers (4) and 7.6% of unadjusted levels were above the upper limit reported in 49 controls without breakdown by smoking (10).

Several reasons for these elevated values are possible. Misidentification of MA is unlikely since both retention time and UV absorbance spectrum by diode array detection were used for HPLC confirmation. In addition, a small subset of our highest values were analyzed by GC/MS. However, interindividual variation in metabolism is likely to be a factor. Age-related variation may be important as well; children could metabolize a higher proportion of benzene to MA than adults. In addition, this highly select group of children could have meta-

**Figure 5.** Means and SDs (error bars) of *trans,trans*-muconic acid (MA) data stratified by dichotomized variables including time of evaluation, time spent near street (TSNS), and study period.

bolic differences resulting from enzyme responses (induction or inhibition) to multiple toxicant exposures. It is also possible, as discussed by Johnson and Lucier (20), that certain members of the general population are exposed at levels similar to those in industrial settings. Workers are exposed for 8-hr shifts whereas environmental exposure is continuous. Noninhalational routes of exposure could also be contributory.

However, the food preservative sorbic acid may have influenced our results since approximately 0.1–0.2% is metabolized to MA (10). The extent of current sorbic acid use is difficult to estimate; because it is considered "generally regarded as safe" by the Food and Drug Administration, few data are available regarding sorbic acid content in foods. Our questionnaire included one question on intake of fruit juice which we originally thought would be a measure of sorbic acid intake. Statistically significant correlations were noted for both adjusted and unadjusted MA with intake of juice ($r_s = 0.33$, $p = 0.003$ for the unadjusted MA value). However, a subsequent review of content labels to assess this finding revealed that currently the only juice or fruit drink product to contain sorbates is Kool-Aid Bursts. According to a recent review, processed cheese, baked products, and possibly soft drinks would be common sources of sorbic acid in low levels (0.05–0.1%), but the use of sorbates in juice has declined in the past few years (21). Therefore, the significance of this finding is unclear. Due to limited data on use, we were unable to quantify sorbic acid ingestion in these children.

Other authors have shown that MA correlates rather well with air level or another specific metabolite, *S*-phenylmer-

capturic acid, in workers exposed to benzene at levels as low as 0.1 ppm (4,5,9). It also correlated with cotinine in nonoccupationally exposed smokers (7). Furthermore, unlike phenol, which is routinely present in the urine of subjects not exposed by occupation, MA is below the LD in a variable proportion of those studied. However, given the ppb benzene levels in environmental exposure, MA correlation with personal air sampling in this setting will ultimately be necessary to determine the extent of sorbic acid effect on MA and understand the elevated values found in individuals not exposed by occupation.

Due to the variety of benzene sources and the small size of the study, associations of MA with questionnaire exposure data were expected to be limited. However, a few interesting findings were noted. The amount of time spent near the street was a predictor of MA level. This association was clear in spite of the fact that these data were collected on only 68 subjects. Inner-city children often play in close proximity to the street for lack of better play areas. Our findings suggest that this may expose them to benzene from gasoline and automobile exhaust and, if confirmed in future studies, provide evidence to support the use of reformulated gasoline. The increase noted in levels between morning and afternoon evaluations may be related to exposure differences because the half-life of this metabolite is short. The recent exposure of children who arrive at the clinic early in the morning and remain there is likely to be less than that of children who spend the morning in their homes and are evaluated later in the day. Lauwerys et al. (5) obtained morning and afternoon specimens in the same individuals; they found a significant increase only in smokers who presumably had exposure during the course of the day. Alternatively, diurnal variation in urine concentration may be more important in children. Variation by study period is less clear and may be a chance finding because it did not have a consistent relation in all regression analyses.

We did not find a consistent relation between MA and either questionnaire measures of ETS exposure or cotinine levels. As noted in Table 3, mean MA was higher in children whose mothers smoked and those with cotinine values above the median, but not those with household ETS exposure. Cotinine was a statistically significant variable in the linear regression model for creatinine-unadjusted MA; however, no relation remained if either variable was adjusted for creatinine. This may indicate that, unlike the situation with smokers who receive a substantial benzene dose from

mainstream smoke, ETS makes a smaller contribution to urinary MA in comparison with other exposure sources. Alternatively, timing of sample collection may be an issue given the significantly higher mean MA in subjects evaluated in the afternoon. No relations were found between MA and the clinical measures obtained for overexposure to lead (blood lead, hematocrit, and free erythrocyte protoporphyrin).

The second aim of the study was to obtain data on the extent of exposure to various toxicants in urban children thought to be at higher risk due to known overexposure to lead. As expected, these children had elevated blood lead levels. ETS exposure was also excessive. Mean cotinine was well above 30 ng/mg creatinine, a level thought to be consistent with household ETS (22). Sixty-seven percent of children were exposed to ETS in their homes according to questionnaire data. Environmental benzene exposure currently cannot be assessed solely by MA. However, benzene is a component of ETS and a variety of other benzene sources were present in these children based on questionnaire data so exposure to this toxicant is likely to be substantial as well.

In conclusion, this pilot study suggests that MA may be useful in the evaluation of environmental benzene exposure. It also identifies a group of inner-city children at high risk for exposure to multiple toxicants. Further research is needed to determine the significance of the MA levels, particularly the extreme values. Validation of MA by correlation with personal air sampling in the environmental setting is necessary, as is follow-up of these children to determine variation in levels over time.

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