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Biological Monitoring of Low Level Occupational Xylene Exposure and the Role of Recent Exposure

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The correlation between low level time-weighted average (TWA) atmospheric xylene exposure (p.p.m.) and urinary methylhippuric acid (MHA) expressed per gram of creatinine was examined. Subjects were recruited from workplaces that utilized xylene. Ambient monitoring of *o*-, *m*- and *p*-xylene isomers was carried out using passive diffusion vapour monitors. Adjusted (post-shift minus pre-shift) and post-shift urinary levels of xylene metabolites (2-, 3- and 4-MHA) were determined by GC–MS. Twenty subjects were recruited into the study. Total xylene TWA exposures were 3.36 ± 3.63 p.p.m. (mean ± SD) with a range of 0.03–14.44 p.p.m. The r^2 values for the regression equations between xylene exposure and individual and total adjusted MHA isomers were 0.390, 0.709, 0.677 and 0.631 for *o*-, *m*-, *p*- and total xylenes, respectively, which was greater than the respective correlations between non-adjusted samples. In conclusion, biological monitoring of occupational xylene exposure at levels <15 p.p.m. using urinary MHA showed a good correlation with atmospheric levels and is a valid complement to ambient monitoring. Even though occupational xylene exposure in the workplaces studied was generally low, MHA was found in the pre-shift urine of all workers and the use of adjusted values showed modest improvements in correlations. Recent exposure prior to sampling, either from occupational or non-occupational sources, should be considered when biological monitoring of xylene is undertaken. Extrapolation of data from this study predicted a MHA concentration in post-shift urine of 1.3 g/g creatinine after exposure to a TWA of 100 p.p.m. xylene.

Keywords: biological exposure index; biological monitoring; methylhippuric acid; xylene

INTRODUCTION

Commercial xylene is a colourless liquid produced from petroleum or coal tar and is one of the most commonly used solvents in industry. Xylene is commonly used as a motor and aviation fuel additive, a solvent in the paint, printing, rubber and leather industries, a starting material in the plastics and textile industries, a carrier in the production of epoxy resins and a constituent of paint, lacquers, varnishes, inks, dyes, adhesives and cleaning fluids (Low *et al.*, 1989). Xylene can exist in three isomeric forms with each isomer possessing similar properties. Commercial xylene is a mixture of the three isomeric forms, ortho (*o*-), meta (*m*-) and para (*p*-), with *m*-xylene usually being the principle component making up 45–70% of the total (National Occupational Health and Safety Commission, 2002).

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Xylene vapour is absorbed rapidly from the lungs and xylene liquid and vapour are absorbed slowly through the skin (Riihimaki and Pfaffli, 1978). In humans, ~64% of the inspired dose reaches the systemic circulation with little variation in absorption between the three isomers (Sedivec and Flek, 1976). Of the xylene absorbed, ~95% is metabolized in the liver to methylhippuric acid (MHA) and 70–80% of metabolites are excreted in the urine within 24 h (Langman, 1994). Chronic occupational exposure has been associated with neuropsychological and neurophysiological dysfunction, anaemia, thrombocytopaenia, leukopaenia, chest pain with ECG abnormalities, dyspnoea and cyanosis (Langman, 1994).

Occupational health authorities in Australia recommend a xylene time-weighted average (TWA) atmospheric exposure standard of 80 p.p.m. in the workplace (National Occupational Health and Safety Commission, 2002). The major metabolite of xylene, MHA has been proposed as a means of biologically monitoring atmospheric exposure to xylene in the

workplace (Lowry, 1986). Biological monitoring of solvent exposure complements ambient atmospheric monitoring since it takes into account all routes of exposure and differences between workers such as size, fitness and work practices. For example, for the same concentration of xylene in ambient air, absorption may be greater if there is skin contact (Daniell *et al.*, 1992) or physical exertion and increased respiratory rate reflected in a greater concentration of xylene in alveolar air (Lapare *et al.*, 1993).

The American Conference of Governmental Industrial Hygienists (ACGIH, 1989) has recommended a biological exposure index for MHA of 1.5 g MHA/g creatinine measured from an end of shift urine sample after a TWA exposure to 100 p.p.m.. The results are expressed per gram of creatinine to allow for spot urine sampling and partially standardize for urinary dilution. There have been several relatively large field studies in which correlations between occupational xylene exposure and urinary MHA excretion have been observed (Kawai *et al.*, 1991; Inoue *et al.*, 1993; Huang *et al.*, 1994).

In many modern workplaces, exposure of workers to solvents such as xylene has been minimized by changes and improvements in work practices. The objective of this study was to investigate the correlation between low level TWA atmospheric xylene exposure (p.p.m.) and urinary MHA expressed per gram of creatinine. A comparison was made between adjusted (post-shift minus pre-shift) and post-shift samples to investigate the role of recent exposure prior to the sampled shift at low occupational levels. A biological indicator value equivalent to 100 p.p.m. TWA exposure was determined by extrapolation.

METHODS

Subject recruitment and questionnaire data

Subjects were recruited from local businesses that utilized xylene in their work environment. A total of 11 manufacturing firms, 14 tile and floor covering companies, two painters and two car bodywork repair companies were contacted by phone, and if interest was expressed, an appointment was made to visit. One laboratory worker was also recruited from our laboratory. The employer benefits of free atmospheric monitoring were stressed at the visit.

Basic demographic details of subjects were obtained by questionnaire, which was completed by the subject by the completion of the work shift. The following details were collected: age, sex, weight, smoking history and estimated hours per week associated with solvent exposure. Details of concomitant medications were also collected. This study was approved by the University Human Experimentation Ethics Committee and all subjects gave informed consent prior to inclusion in the study.

Ambient monitoring and assay

Ambient monitoring of xylene isomers was carried out using passive diffusion samplers (3520 Organic Vapor Monitors; 3M Australia Pty Ltd, NSW). The use of the monitors was explained to the workers on a visit to the workplace the day before the monitoring was due to take place. Subjects were instructed to wear the monitors near their breathing zone and to appropriately identify the monitors, including the start and finish sampling times. It was stressed that the monitors should be placed back in the original containers, the containers sealed with the plastic lid provided and the used monitors stored away from the solvents until collection after the working shift, when they were then refrigerated at 4°C until analysis. Only subjects not wearing personal respiratory equipment were included in the study.

The desorption of trapped solvent was carried out using carbon disulphide (BDH Chemicals Australia Pty Ltd, Victoria) according to the manufacturer's directions (3M, 1982). A glass 2 ml syringe was used to deliver 1.5 ml of carbon disulphide into the vapour monitor injection port which was spiked with 1 mg/ml of anisole (BDH Chemicals Australia Pty Ltd). The desorption of contaminants by the carbon disulphide was carried out by agitation in an orbital shaker at room temperature (17 ± 3°C) for 30 min. The desorbed solution was then decanted from the monitor using the rim port into a 6 ml glass vial and stored at 4°C until analysis.

Analysis was performed within 1 week of the monitor exposure using a Hewlett Packard model 5700 gas chromatograph with a flame ionization detector and a 25 m × 0.32 mm i.d. × 0.3 μm film thickness Hewlett Packard HP-20M Carbowax column (Agilent Technologies Australia Pty Ltd, Victoria). Standard solutions of *o*-, *m*- and *p*-xylene were prepared in carbon disulphide over the calibration range 10–400 ng/ml, each containing anisole internal standard, and analysed by GC using a 1 μl split injection. A linear regression equation was fitted to the *o*-, *m*- and *p*-xylene calibration curves and used to calculate the analyte concentrations in the samples.

The atmospheric levels of each solvent trapped in the monitor was calculated according to the manufacturer's instructions (3M, 1982). The amount of solvent in the exposed monitors was calculated by subtracting the amounts of solvent present in a desorbed blank monitor. The calculations were performed for a sampling temperature of 25°C and 760 mmHg atmospheric pressure.

Urinary metabolite analysis

Individual stock standards of 2-MHA, 3-MHA and 4-MHA (Sigma Aldrich Pty Ltd, NSW) were accurately prepared in methanol at concentrations of ~1 mg/ml. Working standards were prepared by dilution with distilled water and used to spike 1 ml of

urine to give calibration concentrations over a range of 0.5–300 µg/ml in urine, to which internal standard was added (50 µl of 20 µg/ml 2,3,5-trimethyl phenol; Sigma Aldrich Pty Ltd). Calibration standards were treated with 100 µl of extract of *Helix pomatia* (β-glucuronidase plus aryl sulphatase; Roche Diagnostics Pty Ltd, NSW) and 200 µl of acetate buffer, pH 5.2, heated at 37°C and slowly agitated in an orbital shaking bath for 8 h, then acidified with 100 µl of 5 N HCl and 500 mg of ammonium chloride added. Enzymatic hydrolysis was performed on the urine samples as the analytical techniques were adapted from routine assays used for animal studies where phenolic metabolites were examined.

Extraction was carried out by adding 2 ml of ethyl acetate, and the samples were mixed, centrifuged at 1200 r.p.m. for 5 min and the ethyl acetate layer transferred to tapered test tubes by Pasteur pipette. Samples were then evaporated to dryness using a gentle stream of nitrogen in a heating block at 45°C before reconstitution with 100 µl of ethyl acetate, then vortex mixed for 2 min. Aliquots of 20 µl of sample were added to a 100 µl vial insert and reacted with 20 µl of BSTFA (Alltech Associates Australia Pty Ltd, Victoria) at 60°C for 15 min to form trimethylsilyl (TMS) derivatives of MHA.

Samples of 1 µl volume were injected in split-less mode and analysed by GC–MS using a Hewlett Packard model 5890 gas chromatograph with a 25 m × 0.32 mm i.d. × 0.52 µm film Hewlett Packard HP-5 column linked to a Hewlett Packard model 5970 mass selective detector. Selective ion monitoring was used for MHA-TMS ions at *m/z* 105, 119, 206, 220, 250, 308 and 322. Study samples were treated in the same manner as the spiked urine calibration standards. Calibration curves were constructed from MHA-TMS derivative peaks attributable to respective metabolites and were used for the determination of metabolite levels in urine.

Urinary creatinine analysis

The method for determining urinary creatinine was derived from a previously reported method (Clark and Thompson, 1949) and was used to correct the urinary metabolite concentration for urinary dilution by expressing the results as the amount of metabolite excreted per gram of creatinine.

A freshly prepared creatinine in picric acid solution of 13.60 g/l and an accurately prepared creatinine solution (2 mg/ml in 0.1 N HCl) were freshly prepared. The creatinine solution was diluted 1:50 with distilled water and aliquots of this solution (25 µl–1 ml) were added to test tubes and made to 2.0 ml with distilled water. To this was added 1 ml of picric acid solution (13.60 g/l) and the solution mixed thoroughly by vortexing. Sodium hydroxide (0.5 ml of 1.4 N NaOH) was added to each of the samples and exactly 15 min later the absorbance was measured at 500 nm using a spectrophotometer against an absorbance blank. A linear regression line was constructed from the calibration data. Urine samples were diluted 1:200 with distilled water and analysed in the same manner as the creatinine calibration solutions.

Relationship between atmospheric levels and urinary metabolites

The relationship between atmospheric TWA xylene levels and urinary post-shift MHA was examined using linear regression. This relationship was explored using individual and total xylene isomer and metabolites. In addition, adjusted MHA isomers and totals (which were calculated as the difference between post- and pre-shift levels) were investigated. All statistical tests were performed by Statview 5.0.1 (SAS Institute Inc., NSW).

RESULTS

Subject recruitment and questionnaire data

A total of three lacquerers from three different woodworking firms agreed to participate in the study. A total of 11 print workers (including two screen printers) from five different firms agreed to participate along with three automotive bodywork repair spray painters, two painters and one laboratory worker. Of the 14 tile and floor covering companies, none agreed to participate. Only two subjects were using medication at the time of the study (sulphasalazine and an oral contraceptive). To be eligible, only workers who did not wear respiratory protective equipment in the course of their normal shift were included. The details obtained from the subject questionnaire are summarized in Table 1.

Relationship between atmospheric levels and urinary metabolites

The TWA concentration expressed as p.p.m. and total MHA isomer urinary concentration expressed as mg/g creatinine are listed in Table 2. All the TWA exposures were calculated over a normal shift, corres-

Table 1. Study participant details obtained from questionnaire

	Mean ± SD	Range	Number
Age (yr)	32.3 ± 7.5	22–50	
Weight (kg)	78.5 ± 9.7	64–92	
Average hours/week associated with occupational solvent exposure	28.2 ± 16.4	2–65	
Male/female			18/2
Non-smokers			15
Smokers (cigarettes per day)			
1–10			0
11–24			3
>25			2

ponding to periods of between 435 and 510 min, except for four subjects with a TWA calculated from periods between 115 and 120 min. There were no differences in mean xylene TWA values (2.7 versus 6.2 p.p.m.) between the long and short exposure groups, respectively ($t = -1.8$, $P = 0.08$, df = 18). All subjects had detectable 3-MHA isomers in pre-shift urine with a total MHA level of 14.3 ± 13.4 mg/g creatinine (mean ± SD) and a range of 1.0–57.8 mg/g creatinine. Pre-shift isomer levels were up to 12.5, 30.8 and 14.9 mg/g creatinine for 2-MHA, 3-MHA and 4-MHA, respectively. In two subjects, adjusted levels were less than 0 with pre-shift levels higher than post-shift levels, indicative of recent exposure prior to the sampled work shift. Precision data were satisfactory with intra-day relative standard deviations of <5, 10 and 15% at the lowest calibration levels for atmospheric sampling, urinary creatinine and urinary MHA metabolites, respectively.

Table 2. TWA xylene concentration and pre-shift, post-shift and adjusted MHA concentration ($n = 20$)

	Mean ± SD	Range
TWA (p.p.m.)		
o-xylene TWA	0.9 ± 1.0	0.0–4.3
m-xylene TWA	1.7 ± 1.8	0.0– 6.9
p-xylene TWA	0.8 ± 0.8	0.0–3.3
Total xylenes TWA	3.4 ± 3.6	0.0–14.4
Creatinine (mg/g)		
Pre-shift 2-MHA	4.2 ± 4.1	0.0–12.5
Pre-shift 3-MHA	7.5 ± 6.7	1.0–30.8
Pre-shift 4-MHA	2.5 ± 3.3	0.0–14.9
Total pre-shift MHA	14.3 ± 13.4	1.0–57.8
Post-shift 2-MHA	13.4 ± 14.4	0.0–59.6
Post-shift 3-MHA	30.5 ± 31.7	0.2–128.4
Post-shift 4-MHA	13.4 ± 15.5	0.0–60.9
Total post-shift MHA	57.2 ± 60.7	0.2–248.9
Adjusted 2-MHA	9.1 ± 13.1	–0.5–53.9
Adjusted 3-MHA	23.0 ± 31.2	–1.2–118.1
Adjusted 4-MHA	10.8 ± 15.3	0.0–58.0
Total adjusted MHA	43.0 ± 58.5	–1.6–229.9

The correlations between individual post-shift MHA isomers and TWA xylene isomer exposure and total post-shift MHA and total TWA xylene exposure are shown in Fig. 1. The correlations between individual adjusted MHA isomers and TWA xylene isomer exposure and total adjusted MHA and total TWA xylene exposure are shown in Fig. 2.

The r^2 values for the regression model between respective MHA acid isomers and total metabolites obtained from the post-shift urine and atmospheric TWA xylene exposure were 0.322, 0.668, 0.657 and 0.579 for *o*-, *m*-, *p*- and total xylenes, respectively. The r^2 values were greater when the adjusted (post-shift urine less pre-shift) values for MHA were used (r^2 values of 0.390, 0.709, 0.677 and 0.631 for *o*-, *m*-, *p*- and total xylenes, respectively).

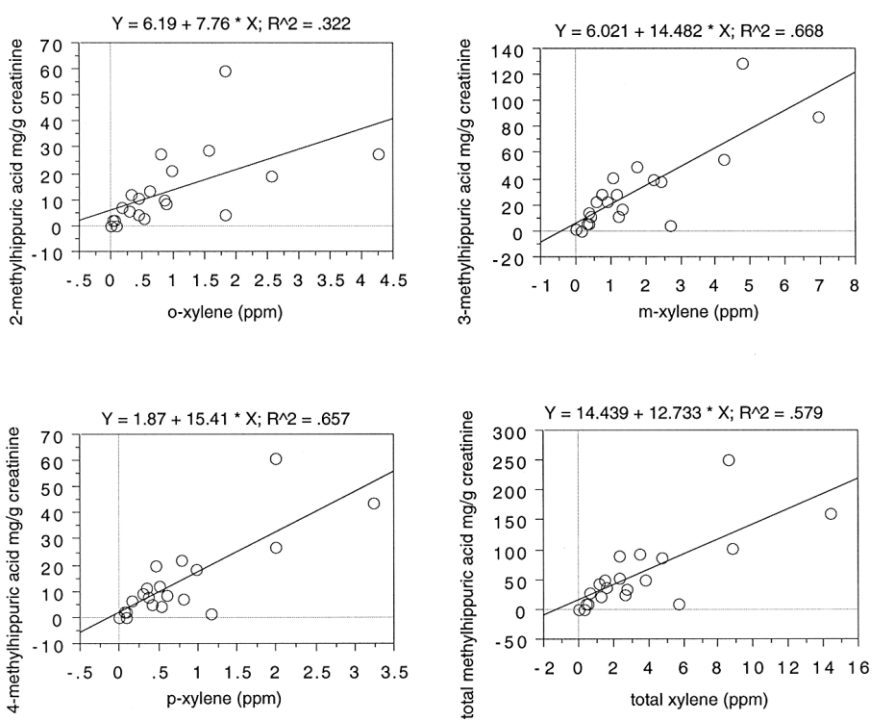

Fig. 1. Correlation between atmospheric xylene levels and urinary MHA metabolites from a post-shift urine sample.

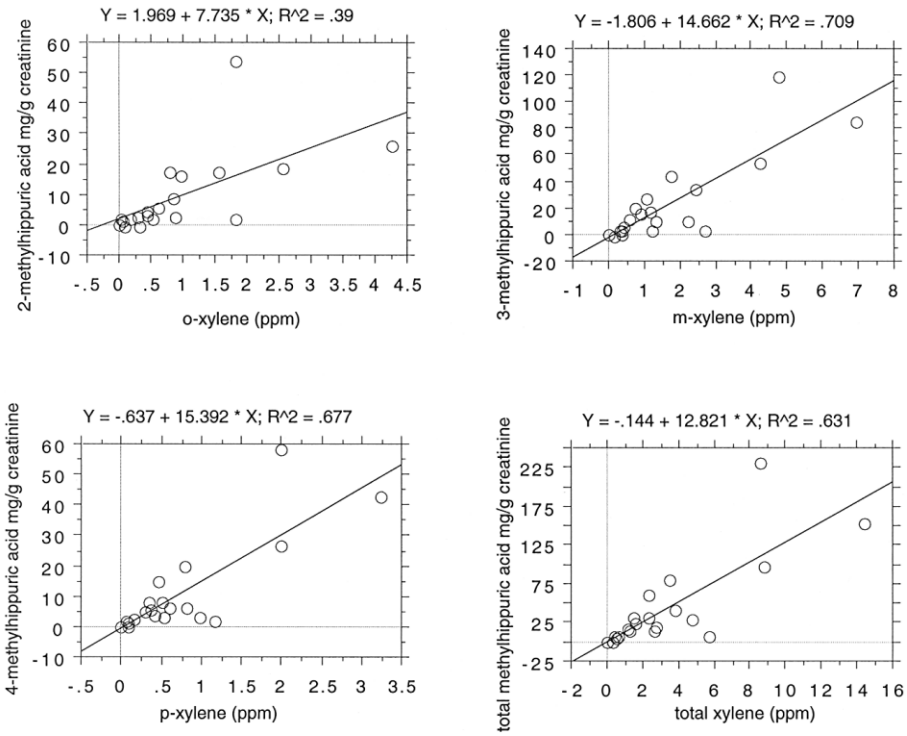

Fig. 2. Correlation between atmospheric xylene levels and adjusted urinary MHA metabolites (difference between post-shift and pre-shift samples).

Based on the regression model equations derived for both the post-shift and adjusted MHA levels, a biological indicator value equivalent to a TWA of 80 p.p.m. was found to be 1.0 g/g creatinine. Using a 100 p.p.m. TWA, this was equivalent to 1.27 and 1.28 g/g creatinine for the post-shift and adjusted MHA level models, respectively.

DISCUSSION

The linear correlation between TWA atmospheric levels and MHA metabolites was comparable with that found in other studies (Kawai *et al.*, 1991; Inoue *et al.*, 1993). The results suggest that biological monitoring of xylene exposure using MHA metabolites is a valid means of assessing low level xylene exposure. It must be remembered that the goal is not to achieve perfect correlation between TWA atmospheric levels and MHA metabolites, as the proposed advantage of biological monitoring is that differences between workers can be taken into account when assessing exposure. For example, with an r^2 of 0.631, 63% of the variation between the MHA levels in urine is accounted for by the TWA xylene atmospheric levels. The remaining 37% of the variation can be attributed to factors such as anatomical and physiological differences between workers, individual work practices, differences between inhaled xylene concentration and that determined from the passive vapour monitors, urine dilution corrections reliant on urinary creatinine and assay precision and accuracy.

Biological indicator values, equivalent to 100 p.p.m. TWA exposure and determined by extrapolation for post-shift and adjusted MHA level models, were 1.27 and 1.28 g/g creatinine, respectively, which is slightly less than the 1.5 g MHA/g creatinine measured from an end of shift urine sample by the ACGIH (1989). A study by Inoue *et al.* (1993) at atmospheric levels similar to this study also found urinary MHA levels after hypothetical exposure to xylenes at 100 p.p.m. was less than the proposed biological exposure index. The biological indicator value derived from this study, based on the Australian 80 p.p.m. National Occupational Health and Safety Commission (2002) exposure standards for xylene, was 1.0 g/g creatinine. Caution should be exercised when interpreting large extrapolations such as these as extrapolation is reliant on both a good fit for the regression model and linearity of the biological response.

The pre-shift findings presented here would suggest that recent xylene exposure, either occupational from a previous work shift or from non-occupational exposure outside the workplace, needs to be considered in biological monitoring of xylene and warrants further investigation.

Adjusted MHA levels appeared to be a slightly better measure than non-adjusted post-shift MHA levels with improved r^2 values, however, the practical advantages are limited as the improvements

were modest, especially when considering the additional costs involved. All subjects had occupational exposure (determined by questionnaire) on the day previous to sampling and this was reflected in the pre-shift urine MHA levels of 14.3 ± 13.4 mg/g creatinine (mean ± SD) with a range of 1.0–57.8 mg/g creatinine. All samples were taken on at least a subject's second shift of the working week given that a site visit was arranged to the workplace on the day before sampling. It was found that two subjects had negative accumulation of MHA since the pre-shift sample was greater than the post-shift sample. This is entirely plausible given the possibility of a high exposure on the previous days shift, however, this cannot be confirmed as sampling was only carried out on one day.

Reference ranges of xylene in whole blood have been described in non-occupationally exposed populations (Ashley *et al.*, 1994; Buratti *et al.*, 1999) and given the reported half-life of xylene it was expected that pre-shift MHA would be observed for some subjects with occupational exposure from the previous days shift. Approximately 3.7–8.0% of the total body uptake of xylene is distributed to the adipose tissue with a median elimination half-time of *m*-xylene from subcutaneous fat of 58 h (range 25–128 h; Engstrom and Riihimaki, 1979).

Data on different slopes for individual MHA isomers remains ambiguous. It has been suggested previously that *m*-xylene isomers undergo preferential metabolism compared with *o*- and *p*-xylene (Miller and Edwards, 1999). It was found in the study by Miller and Edwards (1999) that the slopes of the regression lines (mg MHA/g creatinine/p.p.m. xylene) were 3.2 for *o*-isomers, 14.4 for *m*-isomers and 7.0 for *p*-isomers and that 3-MHA appeared in urine in a greater proportion than would be predicted from the proportion of *m*-xylene detected in air. These results are comparable with slopes found in this current study (Figs 1 and 2) with gradients of 7.7, 14.7 and 15.4, respectively, for the adjusted MHA values. This is in contrast to the gradients reported by Kawai *et al.* (1991), where *o*- and *m*-xylene were similar and *p*-xylene was larger. Because of the small sample size, the effect of smoking could not be examined in this current study.

CONCLUSION

Biological monitoring of occupational xylene exposure at levels <15 p.p.m. using urinary MHA showed a good correlation with atmospheric levels and is a valid complement to ambient monitoring. Even though occupational xylene exposure in the workplaces studied was generally low, MHA was found in the pre-shift urine of most workers and the use of adjusted values showed modest improvements in the correlation. Recent exposure prior to sampling, either from occupational or non-occupational sources, should be considered when biological monitoring of xylene is undertaken. Extrapolation of data from this study predicted a MHA concentration in post-shift urine of 1.3 g/g creatinine after exposure to a TWA of 100 p.p.m. xylene.

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