

Field Study

Occupational Exposure to Phenolic Compounds at Coke Plants—Urinary Excretion of Methoxyphenols as an Indicator of Exposure to Methoxyphenols

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Abstract: Occupational Exposure to Phenolic Compounds at Coke Plants—Urinary Excretion of Methoxyphenols as an Indicator of Exposure to Methoxyphenols: Grażyna BIENIEK, et al. Department of Instrumental Analysis, Faculty of Pharmacy, Medical University of Silesia, Poland—Objectives:

This study describes the exposure of coke plant workers to methoxyphenols. The relationship between exposure to methoxyphenols and urinary excretion of metabolites was examined. **Methods:** We determined concentrations of 2-methoxyphenol, 2-methoxy-4-methylphenol and 1-(4-hydroxy-3-methoxyphenyl)ethanone in the breathing-zone air and in the urine of workers, collected after the workshift. Urine metabolites were extracted after enzymatic hydrolysis by solid-phase extraction. Concentrations of methoxyphenols in air and urine were determined by gas chromatography with flame-ionization. **Results:** The time-weighted average concentrations (median) of methoxyphenols in the breathing zone air were as follows: 9.9 ng/m³, 15.4 ng/m³ and 92.5 ng/m³ for 2-methoxyphenol, 2-methoxy-4-methylphenol and 1-(4-hydroxy-3-methoxyphenyl)ethanone, respectively. The median values of urinary concentrations were as follows: 582.5, 190.1, 235.0 and 21.8 μmol/mol creatinine for 2-methoxyphenol, 2-methoxy-4-methylphenol, 1-(4-hydroxy-3-methoxyphenyl)ethanone and 2,6-dimethoxyphenol, respectively. A statistically significant correlation between the exposure level and the urinary level was found for 2-methoxyphenol ($r=0.573$, $p<0.01$). **Conclusion:** We found that the presence of 2-methoxyphenol in urine can be used as a biomarker for 2-methoxyphenol exposure. The analysis performed at the coke plant showed that the workers were exposed

to relatively low concentrations of methoxyphenols. (J Occup Health 2011; 53: 110–114)

Key words: Biological monitoring, Biomarker, Coke plant, Methoxyphenols, Urine

Coke plants are a source of aromatic hydrocarbons, polycyclic aromatic hydrocarbons^{1, 2)} and phenolic compounds^{3, 4)}. In the current literature, woodsmoke exposure is well documented^{5–8)}, whereas there are very few reports on exposure to methoxyphenols at coke plants. Biomass smoke is a complex mixture of gases and particles including methoxyphenols. Volatile methoxybenzene compounds can be found in biomass smoke generated by burning sorghum, soybean⁹⁾, grass, heather and birch wood¹⁰⁾. For instance, large amounts of the 2,6-dimethoxyphenol are produced by eucalyptus firewood combustion¹¹⁾, however most samples containing methoxybenzenes are odorless. Methoxyphenols are derived from the pyrolysis of the wood polymer, lignin, and have been used as specific tracers of woodburning^{12–14)}. Exposure to methoxyphenols may occur in the juice processing¹⁵⁾ and when burning forest plant materials¹⁰⁾.

Methoxyphenols are used as atmospheric markers to determine the contribution of wood smoke to ambient fine particulate matter^{7, 8, 16)}. According to Hawthorne *et al.*¹⁷⁾, the total methoxyphenol exposure levels measured in ambient samples in wintertime 1988–1989 in Minneapolis were in the range of 354–3,510 ng/m³ (median 907 ng/m³). Schauer *et al.*¹⁸⁾ reported methoxyphenol levels ranging from 0.4 to 876 ng/m³ (median 97 ng/m³) in ambient samples collected at three locations in the USA. Bari *et al.*¹⁶⁾ found that the total methoxyphenol levels in ambient air of a residential area near Stuttgart, Germany, were in the range of 2–451 ng/m³.

The urinary assay for methoxyphenols was developed for the biological monitoring of wood smoke exposure⁶⁾. It was found that methoxyphenols are rapidly and efficiently eliminated by urine. According to Dills *et al.*⁷⁾

Received Feb 26, 2010; Accepted Nov 15, 2010

Published online in J-STAGE Jan 17, 2011

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and Clark *et al.*⁸), urinary methoxyphenols were proposed as biomarkers for woodsmoke exposure. Human and animals studies show that 2-methylphenol (guaiacol) and 2,6-dimethoxyphenol (syringol) do not undergo phase I metabolism and are rapidly excreted in urine as glucuronide and sulfate conjugates^{19,20}. Urinary elimination half-lives are generally shorter for 2,6-dimethoxyphenol (3 h) than for 2-methylphenol (5 h)⁷.

In our previous investigation²¹, methoxyphenols were detected in the urine of coke-plant workers, however a detailed analysis regarding the relationship between exposure and metabolite levels was not performed. The methoxyphenols in urine of coke plant workers were identified by GC/mass spectrometry after enzymatic hydrolysis and solid phase extraction on a styrene divinyl benzene copolymer²².

The aim of the present study was to determine the methoxyphenols in the breathing zone air and their metabolites in the urine of coke plant workers. The urinary excretion of methoxyphenols was also evaluated as a biomarker for personal exposure to methoxyphenols.

Material and Methods

Subjects

The study population consisted of 39 male workers of a coke plant situated in the industrial area of Poland. The workers, aged 23–63, were mainly exposed to aromatic hydrocarbons and phenolic compounds (group A). The survey was performed on the 4th day of the workweek. The air and urine samples were collected on the same day during the day shift (6:00 AM–2:00 PM). About seventy two (71.8%) percent of workers were current smokers (15.5 ± 4.6 cigarettes/day). The reference group (group B) comprised 19 men and 10 women aged 19–55 who were not occupationally exposed. They were investigated as a control group. Sixty-two percent of the subjects were smokers and the number of cigarettes consumed per day was 9.6 ± 3.6 .

Air and urine sampling was approved by the Human Experimentation Committee of the Medical University of Silesia, Poland.

Air sampling

Air samples were collected in the workers' breathing zones during the shift. Personal sampling was performed using a battery-operated pump (type AFC 123 Casella Ltd, London) connected to a filter holder attached to the shoulder of the subject. Air was aspirated at a flow rate of 0.5 l/min through silica gel (Kieselgel 40, 0.2–0.5 mm; Fluka). The methoxyphenols were released from the silica gel by treating them with 1 ml of methanol, and then the methanol solution (1 μ l) was injected into the gas chromatography (GC) column. Precision was in the range of 3.2–10.0%, and the limit of detection varied in the range of 0.06–0.21 ng/m³.

Urine sampling and analysis

The exposed workers and the unexposed reference group were asked to collect urine over the last 4 h of the 8-hour shift. The samples were transferred to the analytical laboratory and kept frozen (–20°C) until the analysis.

The GC/FID analyses of the 2-methoxyphenol (guaiacol), 2-methoxy-4-methylphenol (methylguaiacol), 1-(4-hydroxy-3-methoxyphenyl)ethanone (acetovanillone), and 2,6-dimethoxyphenol (syringol) content in urine were carried out. A slight modification was introduced to the procedure²¹ we described previously. First, the conjugated metabolites in a 10 ml urine sample were hydrolyzed with β -glucuronidase/arylsulfatase and then the metabolites were extracted by solid phase extraction on octyl cartridges. The methoxyphenols were separated using a capillary column Ultra 2 (cross-linked 5/95% diphenylpoly(dimethylsiloxane) 25 m \times 0.32 mm i.d., 0.52 μ m film thickness). The methanol extract (1 μ l) was subjected to GC analysis for quantification of methoxyphenols according to our previously described method²¹. Precision was in the range of 2.8–16.2%, and the limit of detection varied in the range of 0.04–0.12 μ g/ml.

Urinary methoxyphenol concentrations were normalized to creatinine to account for diuresis. As the WHO²⁰ recommends an optimal creatinine range of 30–300 mg/dl for analysis of urine samples, the two urine samples with creatinine values outside this range were excluded from all data analyses. The creatinine content in each sample was analyzed by a method based on the Jaffe reaction and determined spectrophotometrically.

Statistical analysis

Statistical analysis was conducted with STATISTICA 6.0 software. The parameters were characterized as median, the 5th percentile and the 95th percentile because assumptions about the shape of the distribution could not be made. Therefore, we decided to use the nonparametric Mann-Whitney U test for comparisons between the groups. Correlations were evaluated with Spearman's test.

Results

Table 1 presents the results describing the occupational exposure to methoxyphenols of the coke plant workers. Values of the statistical parameters, median and the 5th and 95th percentiles, indicate that the exposure levels to 2-methoxyphenol, 2-methoxy-4-methylphenol and 1-(4-hydroxy-3-methoxyphenyl) ethanone were rather low. 2,6-Dimethoxyphenol was not found in the air samples.

Table 2 shows the results of statistical analysis of the methoxyphenols which were identified in the urine of exposed workers and non-exposed subjects. All compounds under investigation appeared simultaneously in the urine of only 27 workers (69.23%). Concentrations

Table 1. Results of personal air monitoring of coke plant workers (group A)

Compounds	Personal exposure level [ng/m ³]			
	N ^b	Median	5 th percentile	95 th percentile
2-methoxyphenol	32	9.9	3.1	22.9
2-methoxy-4-methylphenol	29	15.4	4.2	35.2
1-(4-hydroxy-3-methoxyphenyl) ethanone	35	92.5	33.73	209.2

^b Number of subjects in whom the methoxyphenols in air were excreted within the analytical detection limits.

Table 2. Results of biological monitoring of coke plant workers (group A), and the control group B. Median, 5th and 95th percentile

Metabolites	Urinary metabolites [$\mu\text{mol/mol creat.}$]								<i>p</i>
	Workers				Control				
	Group A (n=39)				Group B (n=29)				
	N ^b	Median	5 th percentile	95 th percentile	N ^b	Median	5 th percentile	95 th percentile	
2-methoxyphenol	32	582.5	278.6	892.3	28	223.3	119.2	531.9	<0.05
2-methoxy-4-methylphenol	29	190.1	57.2	311.0	27	21.5	88.7	97.1	<0.05
1-(4-hydroxy-3-methoxyphenyl) ethanone	35	235.0	46.5	641.5	23	16.3	3.2	54.3	<0.05
2,6-dimethoxyphenol	28	21.8	7.5	37.1	14	9.5	2.5	24.2	<0.05

^b Number of subjects in whom the methoxyphenols were excreted within the analytical detection limits; *p*-value for group A vs. B.

of the urinary methoxyphenols were adjusted for the urinary creatinine concentration ($\mu\text{mol/mol creatinine}$) to control for the variability in urine dilution. The creatinine content in urine samples varied, ranging from 35.4 to 312.8 mg/dl (mean \pm SE, 143.4 \pm 61.6).

The upper limits of urinary concentrations of 2-methoxyphenol, 2-methoxy-4-methylphenol, 1-(4-hydroxy-3-methoxyphenyl)ethanone and 2,6-dimethoxyphenol were found to be equal to 1101.5, 417.6, 728.0 and 42.6 $\mu\text{mol/mol creatinine}$, respectively.

As seen in Table 2, methoxylated phenols were also present in the urine of non-exposed subjects (group B). Although the exposure levels were low, statistically significant differences ($p < 0.05$) between methoxyphenol concentrations in the urine of coke plant workers (group A) and non-exposed subjects (group B) were found.

The correlation between the time-weighted average methoxyphenols exposure and the creatinine-adjusted concentrations of urinary methoxyphenols was examined by regression analysis. Figure 1 shows the linear regression graph for 2-methoxyphenol corrected for creatinine content in urine. A significant correlation between the concentrations of 2-methoxyphenol in air and in urine was found ($r = 0.573$, $p < 0.01$). Correlation coefficients for 2-methoxy-4-methylphenol and 1-(4-hydroxy-3-methoxyphenyl) ethanone were low, i.e. 0.373 and 0.49, which indicating a statistically insignificant result.

Discussion

The coal-tar industry is a major source of small amounts

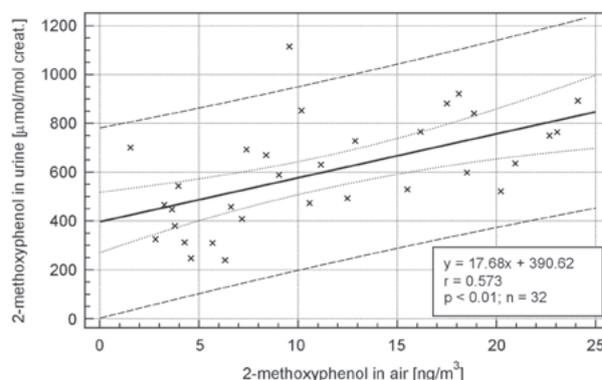


Fig. 1. Relationship between breathing-zone air and urinary 2-methoxyphenol. (—: calculated regression line, ----: 95% CI,: range of individual samples)

of phenolic compounds which are directly emitted to the environment. In an earlier investigation, the occupational exposure to phenolic compounds (phenol, methylphenol and dimethylphenol isomers) was analysed by the determination of these compounds in the breathing zone air and urine of workers³. In the present study, we found that the time-weighted average concentrations of 2-methoxyphenol, 2-methoxy-4-methylphenol and 1-(4-hydroxy-3-methoxyphenyl) ethanone in the vapor phase were relatively low in comparison to the concentrations of methylphenol and dimethylphenol isomers in the breathing zone air of workers engaged as

operators in the tar distillation process.

On the basis of GC analysis of the breathing zone air, we showed that the mean concentrations of the 2-methoxyphenol, 2-methoxy-4-methylphenol and 1-(4-hydroxy-3-methoxyphenyl)ethanone were equal to 9.9 ng/m³, 15.4 ng/m³ and 92.5 ng/m³, respectively. Concentrations of 2-methoxyphenol detected in our experiment were lower than target values. OSHA²³⁾ recommends a level of 5 mg/m³ as the TWA for 4-methoxyphenol. Despite these low concentrations, methoxyphenols are a health risk for coke workers' skin, eyes and respiratory system²³⁾.

It is difficult to estimate the dose of methoxyphenols absorbed by the respiratory tract of workers because their content in the air has not been determined. In the highly industrialized and urbanized Upper Silesia, only the phenol content is monitored. According to the data collected in 2004 by the Sanitary and Epidemiological Station in Katowice (Poland), the phenol levels in ambient air ranged from 2.9 to 5.6 µg/m³²⁴⁾. The highest values were recorded in the areas of the greatest industrial concentration. However, methoxyphenol pollution levels have been determined by other researchers. According to the data presented by Simpson *et al.*⁵⁾, the median PM_{2.5} concentrations for ambient PM samples of 2-methoxyphenol, 2-methoxy-4-methylphenol and 1-(4-hydroxy-3-methoxyphenyl) ethanone were equal to 0.64 ng/m³, 0.06 ng/m³, and 0.22 ng/m³, respectively.

We found that the 2-methoxyphenol, 2-methoxy-4-methylphenol, 2,6-dimethoxyphenol and 1-(4-hydroxy-3-methoxyphenyl) ethanone concentrations in the urine of workers (group A) were higher than those in the non-exposed subjects (group B). This result shows rather low occupational exposure to methoxyphenols. The median values for 2,6-dimethoxyphenol in the urine of workers and non-exposed subjects were 21.8 and 9.5 µmol/mol creatinine, respectively (Table 2). Probably, 2,6-dimethoxyphenol appeared in urine as a metabolite of aromatic hydrocarbons because 2,6-methoxyphenol was not found in the air samples.

In our study the concentration of 1-(4-hydroxy-3-methoxyphenyl) ethanone (acetovanillone) in air was significantly higher than the concentration of the other methoxyphenols. Further research is needed to explain this result. According to Dills *et al.*⁷⁾, the concentration of acetovanillone was significantly higher than the 2-methoxyphenol, 2-methoxy-4-methylphenol, and 2,6-dimethoxyphenol content in the personal air of subjects exposed to woodsmoke.

In the present study, a low positive correlation ($r=0.573$) was found between 2-methoxyphenol detected in the breathing zone air and urinary 2-methoxyphenol of coke plant workers. Dills *et al.*⁷⁾ found a significant correlation between urinary methoxyphenols for PM_{2.5} ($r^2=0.738$) and levoglucosan ($r^2=0.775$). Yingratanasuk *et al.*²⁵⁾ reported

significant relationships for methoxyphenol ($r^2=0.5838$) and dimethoxyphenol ($r^2=0.5618$) concentrations with levoglucosan. According to Neitzel *et al.*²⁶⁾, the creatinine-adjusted summed methoxyphenols were highly associated with CO exposure levels ($r=0.83$).

The unsatisfactory correlation found between urinary 2-methoxy-4-methylphenol and 1-(4-hydroxy-3-methoxyphenyl) ethanone in air may result from the uncontrolled occurrence of these compounds in the environment.

The urinary methoxyphenols found in the control group may reflect the uptake of small amounts of methoxyphenols from environmental sources or food¹⁵⁾ and tobacco smoke^{27, 28)}. Other studies have shown that urinary methoxyphenols^{12, 14)} are also derived from the consumption of smoked foods or smoke flavoring. The reference group (B) comprised subjects who lived in a highly industrialized region, but who were not occupationally exposed. According to our results, concentrations of urinary 2-methoxyphenol, 2-methoxy-4-methylphenol, 2,6-dimethylphenol and 1-(4-hydroxy-3-methoxyphenyl) ethanone were higher than those measured by Dills *et al.*⁷⁾ but lower than the values reported by Clark *et al.*⁸⁾.

In conclusions, we found that the coke plant workers were exposed to relatively low concentrations of methoxyphenols in comparison to exposure limits. The statistically significant correlation between the exposure levels and the urinary levels of 2-methoxyphenol show that this compound can be used as an effective biomarker of methoxyphenol exposure.

Acknowledgments: This work was supported by the Medical University of Silesia in Katowice, Poland, grant no. KNW-1-079/07. The authors gratefully acknowledge the Managing Board of the Zabrze Coke Plant, Poland, for their kind permission allowing us to pursue our research.

References

- 1) International Agency for Research on Cancer: IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Polynuclear Aromatic Compounds, Part 3, Industrial Exposure in Aluminium Production, Coal Gasification Coke Production, and Iron and Steel Founding. Lyon: IARC, 1984; 65–131.
- 2) Romundstad PR, Ronneberg A, Leira HL, Bye T. Health survey of former workers in a Norwegian coke plant: part 1. Estimation of historical exposure. *Occup Environ Med* 1998; 55: 616–21.
- 3) Bieniek G. Urinary excretion of phenols as an indicator of occupational exposure in the coke plant industry. *Int Arch Occup Environ Health* 1997; 70: 334–40.
- 4) WHO. Environmental health criterion 161: phenol. Geneva: WHO; 1984.
- 5) Simpson ChD, Paulsen M, Dills RL, Liu LJ, Kalman DA. Determination of methoxyphenols in ambient

- atmospheric particulate matter: tracers for wood combustion. *Environ Sci Technol* 2005; 39: 631–7.
- 6) Dills RL, Zhu X, Kalman DA. Measurement of urinary methoxyphenols and their use for biological monitoring of wood smoke exposure. *Environ Res* 2001; 85: 145–58.
 - 7) Dills RL, Paulsen M, Ahmad J, Kalman DA, Elias FN, Simpson CD. Evaluation of urinary methoxyphenols as biomarkers of woodsmoke exposure. *Environ Sci Technol* 2006; 40: 2163–70.
 - 8) Clark M, Paulsen M, Shmith KR, Canuz E, Simpson CD. Urinary methoxyphenol biomarkers and woodsmoke exposure: comparisons in rural Guatemala with personal CO and kitchen CO, levoglucosan and PM_{2.5}. *Environ Sci Technol* 2007; 41: 3481–7.
 - 9) Seitz LM, Ram MS. Volatile methoxybenzene compounds in grains with off-odors. *J Agric Food Chem* 2000; 48: 4279–89.
 - 10) Kjallstrand J, Ramnas O, Petersson G. Methoxyphenols from burning of Scandinavian forest plant materials. *Chemosphere* 2000; 1: 735–41.
 - 11) Schauer JJ, Kleeman MJ, Cass GR, Simoneit BR. Measurement of emissions from air pollution sources. 3. C1–C29 Organic compounds from fireplace combustion of wood. *Environ Sci Technol* 2001; 35: 1716–28.
 - 12) Simoneit BRT, Rogge WF, Mazurek MA, et al. Lignin pyrolysis products, lignans, and resin acids as specific tracers of plant classes in emissions from biomass combustion. *Environ Sci Technol* 1993; 27: 2533–41.
 - 13) Hawthorne SB, Miller DJ, Barkley RM, Krieger MS. Identification of methoxylated phenols as candidate tracers for atmospheric wood smoke pollution. *Environ Sci Technol* 1988; 22: 1191–6.
 - 14) Hawthorne SB, Krieger MS, Miller DJ, Mathiason MB. Collection and quantitation of methoxylated phenol tracers for atmospheric pollution from residential wood stoves. *Environ Sci Technol* 1989; 23: 470–5.
 - 15) Orr RV, Shewfelt RL, Huang CJ, Tefera S, Beuchat LR. Detection of guaiacol produced by *Alicyclobacillus acidoterrestris* in apple juice by sensory and chromatographic analyses and comparison with spore and vegetative cell populations. *J Food Prot* 2000; 63: 1517–22.
 - 16) Bari MA, Baumbach G, Scheffknecht G, Kuch B. Characterisation of particle-phase wood smoke tracer compounds in ambient air of a residential area. European Aerosol Conference 2009. Karlsruhe.
 - 17) Hawthorne SB, Miller DJ, Langenfeld JJ, Krieger MS. PM-10 high-volume collection and quantitation of semi- and nonvolatile phenols, methoxylated phenols, alkanes, and polycyclic aromatic hydrocarbons from winter urban air and their relationship to wood smoke emissions. *Environ Sci Technol* 1992; 26: 2251–62.
 - 18) Schauer JJ, Cass GR. Source Apportionment of Wintertime Gas-Phase and Particle-Phase Air Pollutants Using Organic Compounds as Tracers. *Environ Sci Technol* 2000; 34: 1821–32.
 - 19) Miller JJ, Powell GM, Olavesen AH, Curtis CG. The toxicity of dimethoxyphenol and related compounds in the cat. *Toxicol Appl Pharmacol* 1976; 38: 47–57.
 - 20) WHO ed. Biological Monitoring of Chemical Exposure in the Workplace; World Health Organization. vol. 1. Geneva; 1996.
 - 21) Bieniek G. Simultaneous determination of 2-methoxyphenol, 2-methoxy-4-methylphenol, 2,6-dimethoxyphenol and 4'-hydroxy-3'-methoxyacetophenone in urine by capillary gas chromatography. *J Chromatogr B* 2003; 795: 389–94.
 - 22) Bieniek G, Kurkiewicz S, Wilczok T. Occupational exposure to aromatic hydrocarbons at a coke plant: part I. Identification of hydrocarbons in air and their metabolites in urine by a gas chromatography-mass spectrometry method. *J Occup Health* 2004; 46: 175–80.
 - 23) Occupational Safety and Health Guidelines for 4-Methoxyphenol. Salt Lake City: OSHA Occupational Safety and Health Administration, United States Department of Labor; 1992.
 - 24) Sanitary Epidemiological Station, Katowice. Air pollution in the Katowice voivodeship in 2004. Katowice (Poland): Regional Sanitary Epidemiological Station; 2004.
 - 25) Yingratanasuk T, Cheevaporn V, Saowakontha S, et al. Exposure assessment for biomass smoke among “Rice in the Bamboo” producing workers: comparison between PM_{2.5}, levoglucosan and methoxyphenols. *EnvironmentAsia* 2009; 2: 36–44.
 - 26) Neitzel R, Naeher LP, Paulsen M, Dunn K, Stock A, Simpson CD. Biological monitoring of smoke exposure among wildland firefighters: a pilot study comparing urinary methoxyphenols with personal exposures to carbon monoxide, particulate matter, and levoglucosan. *Journal of Exposure Science & Environmental Epidemiology* 2009; 19: 349–58.
 - 27) Arnarp J, Bielawski J, Dahlin B-M, Dahlman O, Enzell CR, Pettersson T. Tobacco smoke chemistry. 2. Alkyl and alkenyl substituted guaiacols found in cigarette smoke condensate. *Acta Chem Scand* 1989; 43: 44–50.
 - 28) Arrendale RF, Severson RF, Chortyk OT, Snook M. Analyses of mono- and dihydroxybenzenes in tobacco smoke and pyrolyzates by glass capillary gas chromatography. *J Chromatogr Sci* 1982; 20: 136–43.