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UNMETABOLIZED VOCs IN URINE AS BIOMARKERS OF LOW LEVEL OCCUPATIONAL EXPOSURE

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Abstract
Objectives: To compare the usefulness of determining unchanged forms of volatile organic compounds (VOCs), namely toluene (TOL), ethylbenzene (EB) and xylene (XYL), in urine with the effectiveness of the already used biomarkers of occupational exposure. **Materials and Methods:** Surveys were conducted in two workplaces (paint factory and footwear factory). In total, 65 subjects participated in the study. Air samples were collected using individual samplers during work shift. Urine and blood samples were collected at the end of work shift. Urine samples were analyzed for unchanged compounds and selected metabolites, while blood samples were tested for unchanged compounds. VOCs in blood and urine were determined by solid phase microextraction gas chromatography (SPME-GC-MS). **Results:** In the paint factory, the geometric mean (GM) concentrations of VOCs in the air ranged as follows: 0.2–4.7 mg/m^3 for TOL, 0.4–40.9 mg/m^3 for EB and 0.1–122.6 mg/m^3 for XYL. In the footwear factory, the GM concentration of TOL in the air amounted to 105.4 mg/m^3. A significant correlation ($p < 0.05$) was observed between VOCs in blood, urine and air. The regression analyses performed for paint factory workers showed that TOL-U and TOL-B were better biomarkers of exposure ($r = 0.72$ and $r = 0.81$) than benzoic acid ($r = 0.12$) or o-cresol ($r = 0.55$). **Conclusion:** The findings of the study point out that the concentration of unchanged VOCs in urine can be a reliable biological indicator of low level occupational exposure to these compounds.

Key words:
Volatile organic compounds, Urinanalysis, Blood analysis, Biological monitoring, Occupational exposure

INTRODUCTION

Volatile organic compounds such as toluene (TOL), ethylbenzene (EB) or xylene (XYL) are the popular components of organic solvents that are widely used in industries. For many years, biological monitoring of occupational exposure to volatile organic compounds was based on the determination of their specific metabolites in urine. However, in some cases, the normal physiological processes or digestion of food additives may modify urinary excretion of the metabolites and thereby affect the specificity and sensitivity of this method. Furthermore, the determination of urinary metabolites is reliable in the cases involving exposure to a single compound, and VOCs are almost as a rule present as mixtures in the occupational setting. The method of simultaneous determination of different VOCs in blood or exhaled air, which was proposed in the past, has not gained wide acceptance because it was invasive and the sampling was difficult. Attempts to apply the determination of unchanged VOCs in urine to assess occupational exposure began over twenty years ago, but this approach was rather limited due to the low rates of urinary excretion of VOCs and the low sensitivity of the determination methods. With the improvement of analytical methods, the determination of unmetabolized VOCs in urine has gained interest anew. Moreover, it has been assumed that the influence of the kinetics of VOCs elimination in urine on the results will be much lower than for VOCs determinations in blood, where the half-life during phase I of elimination is as short as 3–5 min [1]. The kinetics of urinary VOCs elimination complies with an open two-compartment model. The half-time values

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for toluene, ethylbenzene, xylene and mesitylene varied from 0.45 h to 0.88 h for phase I and from 6.7 h to 19.2 h for phase II [2].

The data reported recently for exposure in occupational setting show that the determination of unmetabolized solvents in urine provides a highly sensitive and specific index of exposure to VOCs [3–11]. The purpose of the present study was to compare the validity of various biomarkers of exposure to toluene, ethylbenzene and xylene at low levels of occupational exposure and to find out whether the determination of unchanged VOCs in urine would be useful for the assessment of exposure around and below the current occupational exposure limit.

MATERIALS AND METHODS

Ethical issues

The local Bioethical Committee approved the study protocol. Each of the participants agreed to join the survey.

Study population and air sampling

Paint factory: Twenty eight workers (20 males and 8 females) exposed to volatile organic compounds participated in the study.

Footwear factory**:** Thirty three workers (9 males and 26 females) were recruited for the study.

In both workplaces, airborne VOCs were sampled in the workers´ breathing zone, using a passive diffusive personal sampler (SKC, Gilian Air-350). The sampling was performed during the work shift.

Collection of biological material

Urine samples were collected at the end of work shift. Blood samples were collected 15 min after the work shift was over. About 5 ml of venous blood from the cubical vein was drawn using the venoject system.

Urine samples were collected into glass bottles. Immediately after collection, 2 ml of urine was transferred into 10 ml headspace vials containing 1 g NaCl. The vials were sealed using caps with teflon membrane and stored at 4–8°C until analysis.

Analytical methods

After desorption from coconut shell charcoal, airborne VOCs were determined by GC-MS analysis. The analysis was performed with 6890N gas chromatograph (Agilent Technologies) equipped with HP 5973 mass detector, split-splitless injector and HP-PONA column (50 m length, 0.2 mm ID, 0.5 μm film thickness).

VOCs in blood and urine were determined by solid phase microextraction gas chromatography (SPME-GC-MS). The analyses were carried out on HP 6890 gas chromatograph with HP 5973 mass detector, split injector, and capillary column (HP-INNOWAX) using the method previously described by Fustinioni et al. [12].

Metabolites in urine were determined by gas chromatography (Hewlett-Packard 5890 Series II Plus, GC column HP-5, 50 m × 0.32 mm × 1.05 μm) using the method described by Janasik et al. [2].

Statistical analysis

Statistical analysis was performed using Statistica StatSoft®Polska software package. Linear regression analysis was used to estimate the slope and intercepts of the relationship between variables. The P value of 0.05 was considered statistically significant. Air and urinary concentrations below the level of quantification were replaced with the values equal to the level of quantification divided by two.

RESULTS

The geometric mean concentration for toluene, xylene and ethylbenzene in the air in the paint and footwear factories are presented in Table 1. The concentrations in occupational setting were generally low, with the exception of toluene concentrations in the footwear factory that were close to the value of occupational exposure limit valid currently in Poland (100 mg/m^3). The results of determinations of unchanged VOCs in urine and blood and their metabolites in urine collected from workers at both workplaces are summarized in Table 2. The data on the relationship between VOC concentrations in the air and concentrations of unchanged compounds in blood and

Table 1. VOC concentrations in the air

Workplace	VOC	No. of subjects	Exposure concentrations (mg/m^3) GM±GSD	Min.	Max.
Paint factory	toluene	19	1.1±2.23	0.2	4.7
	ethylbenzene	23	3.1±3.85	0.4	40.9
	m,p-xylene	24	9.7±4.66	0.6	122.6
	o-xylene	22	1.9±4.09	0.1	20.9
Footwear factory	toluene	35	105.4±1.76	31.9	349.4

Table 2. Concentrations of biomarkers in blood and urine

Workplace	VOC	No. of subjects	Blood (µg/l) GM±GSD	Urine (µg/l)" GM±GSD	Urinary metabolites GM±GSD
Paint factory	toluene	19	5.36±2.03	2.01±1.73	benzoic acid 10.65±2.58 (mg/h)
	ethylbenzene	23	16.47±3.54	12.38±2.52	mandelic acid 1.77±5.31 (mg/h)
	m,p-xylene	24	35.14±5.26	2.04±2.77	m,p-methylhippuric acid 0.053±5.45(g/l)
	o-xylene	22	8.05±4.73	1.98±2.43	o-methylhippuric acid 0.01±3.71 (g/l)
Footwear factory	toluene	35	363.1±1.51	228.1±1.68	benzoic acid 35.5±1.86 (mg/h)
					o-cresol 1.1±2.4 (mg/l)

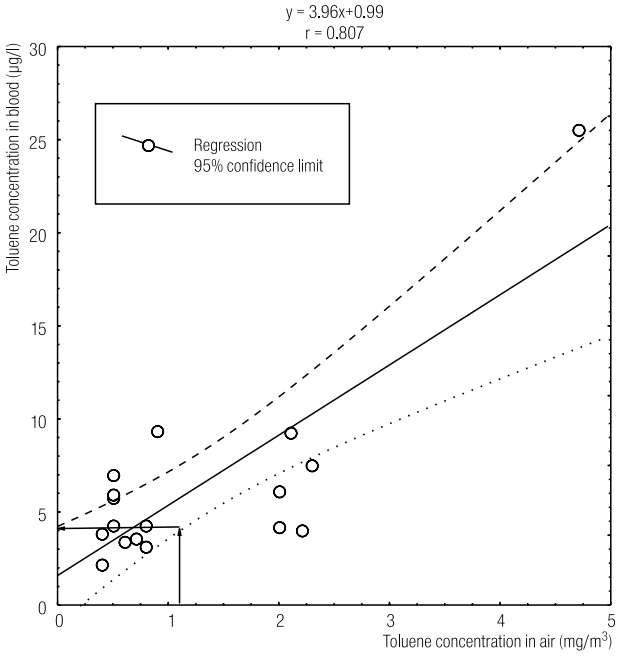

Fig. 1. Relationship between toluene concentration in the air and in blood samples collected from paint factory workers.

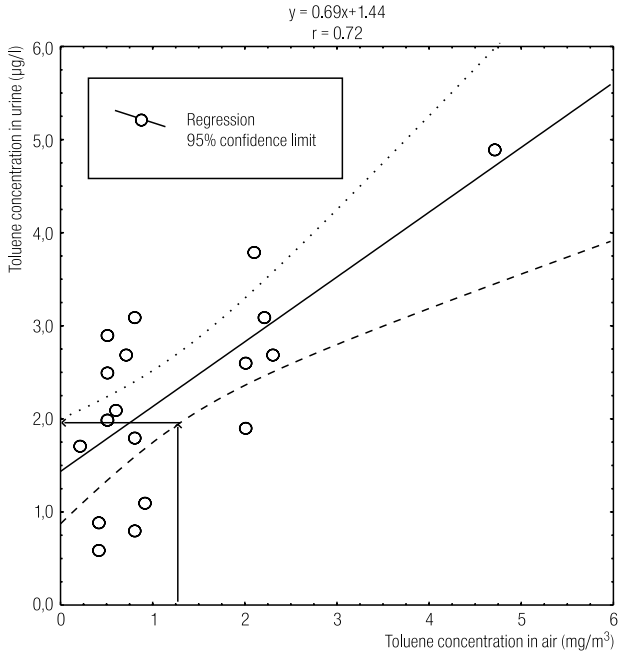

Fig. 2. Relationship between toluene concentration in the urine samples collected from paint factory workers.

Table 3. Relationship between VOCs concentrations in the air, blood and urine and VOCs metabolites in urine

VOCs in air (mg/m^3) GM±GSD	Biomarkers	Regression equation	Correlation coefficient	P values
Toluene[1]	toluene in blood (μg/l)	Y = 3.96x+0.99	0.81	p < 0.05
N=19	toluene in urine (μg/l)	Y = 0.69x+1.44	0.72	
1.1±2.2	benzoic acid in urine (mg/h)	Y = 0.59x+15.23	0.12	
Toluene[2]	toluene in blood (μg/l)	Y = 2.24x+120.5	0.87	p < 0.05
N = 35	toluene in urine (μg/l)	Y = 0.71x–5.5	0.91	
105.4±1.76	benzoic acid in urine (mg/h)	Y = 0.33x+2.34	0.83	
	o-cresol in urine (mg/l)	Y = 0.01x+0.30	0.52	
Ethylbenzene N = 24	ethylbenzene in blood (μg/l)	Y = 2.84x+10.7	0.72	p < 0.05
3.5±4.2	ethylbenzene in urine (μg/l)	Y = 0.13x+1.02	0.71	
	mandelic acid in urine (mg/h)	Y = 0.23x+0.17	0.88	
o-Xylene	o-xylene in blood (μg/l)	Y = 3.50x+6.19	0.75	p < 0.05
N = 24	o-xylene in urine (μg/l)	Y = 0.799x–0.65	0.88	
1.9±3.9	o-methylhippuric acid in urine (g/l)	Y = 0.0046x+0.0062	0.91	
m,p-Xylene	m,p-xylene in blood (μg/l)	Y = 2.84x+33.4	0.72	p < 0.05
N = 24	m,p-xylene in urine (μg/l)	Y = 0.592x–2.506	0.93	
9.8±4.7	m,p-methylhippuric acid in urine (g/l)	Y = 0.006x+0.026	0.92	

[1] Paint factory.
[2] Footwear factory.

urine or of selected metabolites in urine samples collected from workers at the paint and footwear factories are presented in Table 3.

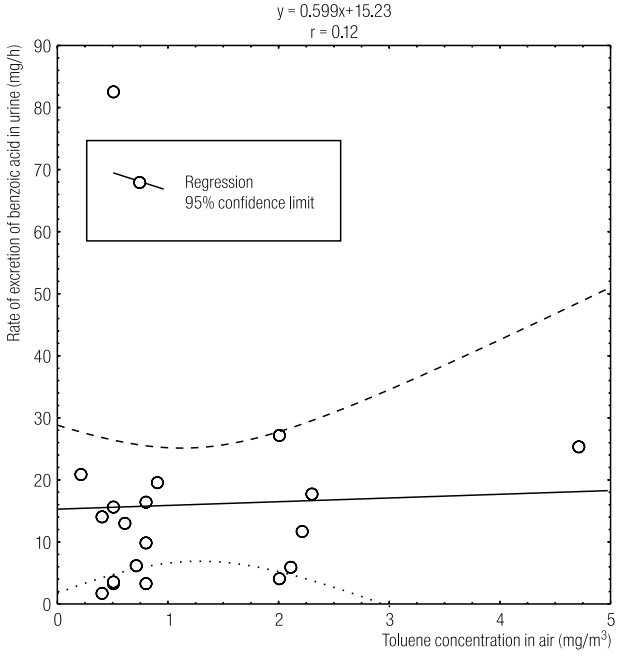

Fig. 3. Relationship between toluene concentration in the air and the rate of urinary excretion of benzoic acid in the samples collected from paint factory workers.

The correlation coefficients between the concentrations of TOL, EB, o-XYL, m,p-XYL in the air, urine and blood in the paint factory were high, r = 0.72, 0.71, 0.88, 0.93 and r = 0.81, 0.72, 0.75, 0.72, respectively. The results indicate that when the normal value of urinary metabolite is low, as in the case of methylhippuric acid, the correlation between the air concentration of a given VOCs and of urine concentration of its metabolite is high. However, in our study, this finding did not refer to toluene for which, under

Table 4. Comparative evaluation of blood analysis and urinanalysis as the means of biological monitoring

Compound	Blood LSC	Urine LSC	Urinary metabolites		
			compound	LSC_1	LSC_2
Toluene	1.2	1.2	benzoic acid	no regression	
Ethylbenzene	7.8	11.5	mandelic acid	2.0	25.00
Xylene	3.8	6.5	methylhippuric acids	2.5	1.75

LSC/LSC_1 — concentration of VOC or its metabolites at which the lower 95% confidence limit for the VOCs in blood or urine is equal to the upper 95% confidence limit at 0 mg/m^3. LSC_2 — normal level of metabolites in urine.

conditions of low level exposure, the correlation between air concentration of toluene and urinary concentration of benzoic acid was significantly lower (r = 0.12) than for TOL-U or TOL-B.

Using the method described by Kawai [13], trials were made to establish the lowest separation concentration (LSC) at which the exposed subject can be distinguished from the nonexposed one. The trials were performed with the use of the biological exposure index. A graphic analysis was carried out to determine the solvent concentration at which the lower 95% confidence limit for the solvent in blood or urine was equal to the upper 95% confidence limit at 0 mg/m^3. Examples of the graphic analysis for toluene are presented in Figures 1–3. The results are summarized in Table 4.

DISCUSSION

In our study, the relationship between VOCs concentrations in the air and urine samples was linear within the low study range of air VOCs concentrations. The high correlations between airborne and urinary VOCs are consistent with the previously reported data [14,4,6,7].

The comparative evaluation of toluene exposure showed that in the low range of exposure concentrations, TOL-U can be considered a better biomarker of exposure than BA-U and o-cresol in urine. These results are concordant with the findings reported by different authors [15,16].

The results of the LSC analysis conducted to distinguish between the exposed and non-exposed subjects showed that VOCs in urine and blood can be considered equivalent biomarkers. The determination of VOCs in urine is noninvasive and the excretion of unchanged VOCs integrates the first two rapid phases of elimination from blood, which simplifies the sampling strategy [2]. Moreover, the unchanged VOCs in urine are more specific biomarkers than their metabolites and the method enables simultaneous determinations of VOCs concentrations in mixtures. The determination of unmetabolized form of toluene as a biomarker of occupational exposure was for the first time proposed by ACGIH in the notice of intended changes [17,18].

The present study confirms that the concentration of unchanged volatile organic compounds in urine is a specific and sensitive biomarker for assessment of VOCs exposure.

The findings of the present study make us conclude that the unmetabolized forms of volatile organic compounds in urine can be regarded not only as biomarkers of occupational, but also of environmental exposure.

CONCLUSIONS

Biological monitoring of exposures to VOCs has been used for decades in the assessment of the internal exposure of workers. However, the progress in the application of biomonitoring in occupational health has been slow, mainly because monitoring has been based mainly on the separate determination of specific metabolites in urine. The arguments for the use of measurement of unchanged compounds in urine are: the non-invasive specimen collection, the simultaneous quantification of VOCs mixture in single urine sample, and the possibility of monitoring at low exposure concentrations. This is of particular importance as the biological monitoring has been increasingly used for assessment of VOCs exposure in the general population. For the practical implementation of the method both in occupational setting and indoor exposure, it would be necessary to conduct more detailed analyses of the findings, which could be the subject of further research on this topic.

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