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A SELECTION OF BIOLOGICAL INDICATORS IN OCCUPATIONAL EXPOSURE TO TOLUENE AND XYLENE

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Results of a comparative study of occupational exposure to toluene and xylene are presented. The study was carried out in a group of 24 workers from a paint factory and aimed to determine the most appropriate exposure indicators for use in biological monitoring. Measurements included toluene and xylene in blood and their metabolites in urine. Toluene in blood proved to be the best indicator of toluene exposure, and both xylene in blood and its only major metabolite methylhippuric acid in urine to be reliable indicators of xylene exposure. The validity of hippuric acid and o-cresol as toluene exposure indicators has not been confirmed.

Key terms: hippuric acid, methylhippuric acid, o-cresol, toluene in blood, xylene in blood.

Solvent neurotoxicity is among major issues in occupational health. Damage to the central nervous system caused by solvents is sometimes irreversible and before an adequate programme of prevention is developed it is essential to define the early effects of solvents on the target organ. At the same time, it is as important to establish a relationship between early clinical signs of exposure and the levels of solvents and their metabolites in biological samples. Until now such integral investigations have not been carried out in this country. The risk of chronic damage to the central nervous system from occupational exposure to toluene and the comparatively small number of papers on chronic xylene toxicity have brought to light the need for establishing the most reliable exposure indicators for use in biological monitoring. Our objective was to perform a comparative study of toluene and xylene absorption in actual conditions of occupational exposure by determining toluene and xylene in blood and their metabolites in urine. It was anticipated that the results obtained would allow to select and recommend the best indicator for biological monitoring of occupational exposure to toluene and xylene.

SUBJECTS AND METHODS

The subjects in the study were 24 male workers employed in a paint factory. According to the factory manager all workers were exposed to both toluene and xylene. Biological monitoring, however, showed that only five workers were exposed to toluene and 19 to xylene. The occupational and health histories of all workers were recorded as were data on medical treatment, chronic diseases, smoking habit and alcohol consumption. The workers' characteristics are presented in Table 1. Toluene and xylene in the working atmosphere were measured twice a year. Their concentrations showed a very wide range (toluene 6–350 mg m^{-3}; xylene 16–185 mg m^{-3}). The biological indicators being the first aim of this study ambiental monitoring was considered to be less important.

Table 1 *Characteristics of the exposed workers*

	Workers exposed to toluene (n=5)	Workers exposed to xylene (n=19)
Age (years)	36.8 ($\bar{X}$) R=31–49	37.4 ($\bar{X}$) R=33–49
Duration of exposure to solvents (years)	13.0 ($\bar{X}$) R=10–18	12.6 ($\bar{X}$) R=2–20
Alcohol consumption	3 – moderate 2 – none	16 – moderate 3 – none
Smoking status (smokers/nonsmokers)	3/2	9/10
Medication	none	none

The exposed workers were examined three times: on Monday morning before work, and on Wednesday before work shift and at the end of it. Each time venous blood and urine samples were taken in the factory health unit. Blood was analysed on the same day and urine samples were frozen at –20 °C before analysis. Toluene and xylene in blood were determined by own modification of the head-space method described by *Angerer and co-workers* (1), hippuric and m-methylhippuric acids in urine by the extraction method of *Buchet and Lauwerys* (2), and o-cresol in urine by Sherwood's and Carter's extraction method (3). All applied methods were gas chromatographical and were in accordance with the accepted criteria for reliability. Having no standards of o- and p--isomers we analysed only m-xylene in blood and m-methylhippuric acid in urine. This did not affect the study objective as m-xylene is the predominant isomer in a typical xylene mixture.

The results for toluene, m-xylene, hippuric and m-methylhippuric acids and o-cresol are presented as median and range values since the distribution of results is skewed. The significance of the differences between the three sampling times for all examined parameters within the group was tested by paired Student's t-test. The correlation analyses were done by a standard statistical method.

RESULTS AND DISCUSSION

Analysis of blood and urine samples from five workers exposed to toluene showed no traces of m-xylene or m-methylhippuric acid thus confirming that the workers were not exposed to xylene. Table 2 therefore shows only the data for toluene, hippuric acid and o-cresol.

Table 2 *Median (M) and range (R) values of toluene in blood and hippuric acid and o-cresol in urine of workers exposed to toluene on Monday before work (a) and on Wednesday before (b) and after work (c)*

Biological indicator	Statistical parameter	a	b	c
Toluene (mg L^{-1})	M	0.11	0.081	0.660
	R	0.005–0.026	0.017–0.121	0.092–1.004
	n	5	5	5
		t=3.291; P<0.05		
			t=3.546; P<0.05	
		t=3.683; P<0.05		
Hippuric acid (mg g^{-1} creat.)	M	340	250	610
	R	180–450	230–440	390–1890
	n	5	5	5
		t=1.143; P>0.10		
			t=1.808; P>0.10	
		t=1.784; P>0.10		
o–Cresol (mg g^{-1} creat.)	M	0.57	1.17	1.26
	R	0.09–2.53	0.45–4.90	0.68–1.53
	n	5	5	5
		t=1.431; P>0.10		
			t=1.027; P>0.10	
		t=0.876; P>0.10		

All blood samples taken on Monday before work showed the presence of toluene; its concentration increased significantly during the working week. In a very detailed and well elaborated study on toluene *Nise and Orbaek* (4) established a relationship between toluene in the workroom air and toluene in blood; exposure to 300 mg m^{-3} corresponded to a post-shift toluene in blood of 0.75 mg L^{-1}, which is comparable with the biological limit value set by the *American Conference of Governmental Industrial Hygienists (ACGIH)* of 1.0 mg toluene per litre of blood at 375 mg m^{-3} toluene in air (5). Only one among our workers had such a high toluene concentration in blood (1.004 mg L^{-1}). Hippuric acid in urine was higher on Wednesday after work in comparison with the two concen-

trations before work but the difference was not significant. It is generally accepted that hippuric acid in urine is used as exposure index for toluene only when toluene in the working area exceeds 50 ppm, because there are large interindividual differences in similar toluene exposures (5). It should be emphasized that hippuric acid is a normal urinary constituent originating from a dietary source. Although pre-shift urine samples are considered to reflect body burden of hippuric acid arising from a supposedly constant dietary source, *Jonai and Sato* (6) revealed the existence of a wide range of intraindividual variations. Our investigations produced similar results. Median values of o-cresol were very similar on Wednesday before and after work although both were higher than the median value on Monday. Such a result is difficult to explain and similar investigations are planned to comprise a greater number of workers exposed to toluene. Because of the small number of workers the correlation analyses between indicators were not done.

Most investigations show large interindividual variations of both hippuric acid and o-cresol (6, 7). Conclusions about the reliability of these two metabolites are not consistent. While *Baelum and co-workers* (7) state that o-cresol is a more specific metabolite than hippuric acid, *De Rosa and co-workers* (8) and *Hasegawa and co-workers* (9) claim that o-cresol is a less reliable indicator of toluene exposure. *Jonai and Sato* (6) indicate that hippuric acid in urine is not a suitable index of exposure to toluene and *De Rosa and co-workers* (10) conclude that hippuric acid is a valid test for evaluating even low exposure to toluene.

Table 3 *Median (M) and range (R) values of m-xylene in blood and m-methylhippuric acid in urine of workers exposed to xylene on Monday before work (a) and on Wednesday before (b) and after work (c)*

Biological indicator	Statistical parameter	a	b	c
m-Xylene ($mg\ L^{-1}$)	M	0.48*	0.77**	0.388***
	R	0.020–0.236	0.022–0.313	0.138–1.544
	n	19	19	18
		t=1.847; P<0.05		
			t=5.129; P<0.0001	
		t=5.148; P<0.0001		
m-Methylhippuric acid ($mg\ g^{-1}$ creat.)	M	36*	40**	364***
	R	14–129	12–121	26–1233
	n	17	17	19
		t=0.498; P>0.50		
			t=4.891; P<0.001	
		t=4.934; P<0.001		

* r = 0.602; P<0.02
** r = 0.690; P<0.01
*** r = 0.530; P<0.05

Table 3 presents the values of m-xylene in blood and of m-methylhippuric acid in urine for workers exposed to xylene. Measured before work the values of both parameters were comparable. After work they increased and the difference between the two morning concentrations and the afternoon one was highly significant for both parameters. The biological tolerance value for xylene in blood after work is 1.5 mg L^{-1}. In this investigation only one subject had such a high m-xylene concentration (1.544 mg L^{-1}). m-Methylhippuric acid did not achieve biological tolerance value of 1.5 g g^{-1} creatinine set by *ACGIH* (5).

Table 3 also shows the results of correlation analyses. The correlation between m-xylene and m-methylhippuric acid could have been better had all three xylene isomer relations to all three methylhippuric acids been determined. The best correlation between m-xylene and m-methylhippuric acid on Wednesday before work justifies the most often recommended sampling time at 16 hours after the end of work. Generally, as methylhippuric acids are not normally present in the urine of subjects not exposed to xylene, urinary levels of methylhippuric acids reliably reflect xylene exposure.

Table 4 *Median (M) and range (R) values of toluene in blood, hippuric acid and o-cresol in urine of workers exposed to xylene on Monday before work (a) and on Wednesday before (b) and after work (c)*

Biological indicator	Statistical parameter	a	b	c
Toluene (mg L^{-1})	M	0.005	0.006	0.015
	R	0.004–0.014	0.0–0.013	0.006–0.238
	n	19	19	18
		t=0.724; P>0.10		
			t=2.130; P<0.05	
		t=2.121; P<0.05		
Hippuric acid (mg g^{-1} creat.)	M	450	530	490
	R	40–1320	70–1670	80–1580
	n	17	17	19
		t=0.412; P>0.50		
			t=0.559; P>0.50	
		t=0.180; P>0.50		
o-Cresol (mg g^{-1} creat.)	M	0.29	0.44	1.18
	R	0.09–0.75	0.14–1.37	0.26–2.05
	n	17	17	19
		t=0.802; P>0.10		
			t=7.244; P<0.001	
		t=5.535; P<0.001		

In workers exposed to xylene toluene was also detected in all blood samples at all three sampling times except in one subject on Wednesday before work when toluene concentration was below the detection limit i.e. < 0.003 mg L^{-1}). The concentrations of toluene in blood and of hippuric acid and o-cresol in urine are presented in Table 4. These blood toluene concentrations were much lower in comparison with the concentrations in workers exposed only to toluene. However, statistically significant differences were found between the two morning concentrations and the toluene concentration after work. Our earlier assumption that toluene was present as a contaminant of the xylene intended for industrial use was not confirmed because the correlation between toluene and xylene was insignificant. Hippuric acid concentrations in the morning urine samples were even higher than in the urine samples of workers exposed only to toluene. This could be a sign of unreliability of hippuric acid as indicator of toluene exposure.

The o-cresol concentrations increased during the working week and the difference between the two morning concentrations and the afternoon one was highly significant. However, the correlation between toluene and o-cresol at all sampling times was insignificant.

The results of the study, which was performed in conditions of actual occupational exposure, allow to conclude that

- exposure to toluene and simultaneous exposure to xylene and toluene can be determined by biological monitoring provided adequate exposure indicators are used,
- toluene in blood is the best indicator of toluene exposure,
- the validity of hippuric acid and o-cresol as toluene exposure indicators remains to be proved,
- xylene in blood and its only major metabolite, methylhippuric acid, which are not normally present in urine, are good indicators of xylene exposure.

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Sažetak

ODABIR BIOLOŠKIH POKAZATELJA PRI PROFESIONALNOJ IZLOŽENOSTI TOLUENU I KSILENU

Provedeno je komparativno istraživanje profesionalne izloženosti toluenu i ksilenu radi odabira najpogodnijih bioloških pokazatelja u biološkom monitoringu. Određeni su toluen i ksilen u krvi i njihovi metaboliti u urinu 24-ju radnika profesionalno izloženih toluenu i ksilenu. Toluen u krvi pokazao se najboljim pokazateljem izloženosti toluenu, dok su i ksilen u krvi i njegov glavni metabolit metilhipurna kiselina pouzdani pokazatelji izloženosti ksilenu. Vrijednosti hipurne kiseline odnosno o-krezola nisu jednoznačni pokazatelji izloženosti toluenu što je poznato iz ranijih istraživanja a potvrđeno je i našim mjerenjima.

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Ključne riječi: hipurna kiselina, ksilen u krvi, metilhipurna kiselina, o-krezol, toluen u krvi