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Trans, trans muconic acid as the biological expossure indicator in occupational exposure to hydrocarbons

Kwas trans, trans mukonowy jako marker biologiczny ekspozycji zawodowej na węglowodory aromatyczne

INTRODUCTION

Petrochemical industry and petrochemical cistern service plants are known as sources of aromatic and polycyclic hydrocarbons as well as many other chemicals [7]. Cistern cleaning and cistern mechanical repair processes emit a complex mixture of vapor and gases. The exposure to chemicals is usually assessed by environmental monitoring. Therefore there are plenty of occupational activities where the biological monitoring seems to be more useful and reliable. Determination of volatile solvents and their metabolites in biological materials such as expired air, blood or urine, makes it possible to estimate the exposure levels to specific organic compounds that contaminate the environment. Biological monitoring was used to assess the exposure via both skin absorption and the gastrointestinal system. Biological monitoring of the mixed and short time vs. prolonged exposure is a growing field of interest in many occupational and toxicological investigative laboratories [4,6,13,23]. Mixed exposure to aromatic hydrocarbons had been previously investigated by many authors [4,13,16,21].

The aim of the present study was to assess the mixed exposure of cistern service plant workers to benzene and homologues with air and biological monitoring. Biological monitoring was conducted by the analysis of urinary trans, trans-muconic acid (tt-MA).

METHODS AND MATERIALS

In this study, analyses were carried out during three years period. The investigated group comprised 149 workers (120 smokers and 29 nonsmokers), mean age 44 years (range 26-64) exposed to aromatic hydrocarbons in a cistern service plant, a part of the DEC Company (recently PKN Orlen branch) situated in northern Poland. Workers were engaged as operators and servicemen in the cistern cleaning department (group A) and as operators in the cistern mechanical repair department (group B). The survey was performed for six daysand especially at the end of working week, when the metabolite excretion should have reached a plateau. The air and urine samples were collected each day three times during the day shift (6:00-7:30 AM; 11:00-12:30PM; 2:00-3:30PM). To determine the physiological levels of urine metabolites the group of 35 workers (29 smokers and 6 nonsmokers) not occupationally exposed to aromatic hydrocarbons, mean age 40 years (range 22-60) were investigated as a control.

Sampling and analysis of aromatic hydrocarbons

Air samples were collected in the breathing zone of the workers during the work shift. The sampling systems included a sorbent tube (SKC 226-01 charcoal tube, 100 mg/50 mg, SKC Inc., Eighty-Four, PA) and a low-flow sampling pump (type AFC 122 Casella Ltd, London, UK). The sampling flow rate was 0.5 l/min for 6 h (180 l). After sample collection, the analytes were released from the charcoal by treatment with 1 ml of carbon disulfide-methanol (60:1, v/v) in an ultrasonic bath for 15 min. The amount of the compound in the back-up showed no more than 10% of the compound in the sampling section. Desorption efficiency for each target volatile compound was determined by analyzing three sorbent tubes. Desorption efficiencies for benzene, toluene and xylene isomers were in the range 95-98%. The analysis of the charcoal extracts was performed with a Hewlett Packard, Model 5890 Series II gas chromatograph equipped with a flame ionization detector, an autosampler/autoinjector (HP7673) and an integrator (HP3396II). Ten duplicate analyses were performed at the lowest concentration of the calibrating curve, for all hydrocarbons, and the detection limit of the hydrocarbons was determined as three times the magnitude of the resultant standard deviation.

Urine metabolites

Urine samples were collected from the exposed workers three times during the work shift (6:00-7:30 AM; 11:00-12:30PM; 2:00-3:30PM) into sterile containers (Merck), taken to the laboratory in an ice cooler at 6° to 7°C, separated in 10-ml aliquots (for t,t-MA and creatinine determination, respectively) in glass centrifuge tubes, and frozen at -20 °C until the time of purification and analysis. Urine samples were subjected to centrifugation and dilution with phosphate buffered saline, after which the urine was cleaned by solid-phase extraction with an Isolute (Mid Glamorgan, United Kingdom]) SAX cartridge. High-performance liquid chromatography was carried out with a [C.sub.18] column (250 mm x 4.6 mm, 3-μm film thickness). A signal was acquired at 259 nm with a photodiode array detector. A calibration curve for quantization of t,t-MA was constructed with a spiked human urine sample (linearity range of t,t-MA standards: 50-500 μg/l. The limit of detection, as defined by a 3:1 signal-to-noise ratio, was 5 μg/l. Clean up of samples and chromatographic analysis were performed according to the method described previously by Maestri et al.[14]. Creatinine analysis was performed according to the method of Jaffe.

Statistical methods

Statistical analyses were conducted with STATISTICA version 8.1 software. The tt-MA in urine was compared using t-test unpaired 1 way procedure. Differences in air and urine levels among the exposure categories were tested separately using one way analysis of variance (ANOVA) procedures, with adjustment for multiple comparisons, available with the GLM procedure. Regression was restricted to measurements with values above the limit of quantitation that decreased with time, using a mixed effects model with a random intercept and common slope among subjects. Multiple linear regression analysis was performed, to investigate effects of covariates on benzene concentrations in air and on tt-MA concentrations in urine of workers.

RESULTS

The time weighted average concentrations of benzene, toluene, xylene isomers, and ethylbenzene, detected in the breathing zone air of the exposed workers are summarized in Table 1. It is apparent, that the workers are exposed to relatively high concentrations of aromatic hydrocarbons. For A-group of workers, concentrations of benzene, toluene, and xylenes in air are significantly higher ($p<0.001$) than for B-group. Table 2 summarizes the results for urinary tt-Ma for exposed workers (groups A and B) and non-exposed subjects (Control). Urinary metabolite levels were corrected against the creatinine concentration [14].

Table 1. Median and quartiles (unit ppb) of the hydrocarbons in air.

Breathing zone air									
Hydrocarbons	Group A (n=39)				Group B (n=100)				p
	Cistern cleaning department				Mechanical repair department				
	n	M	quartile		n	M	quartile		
			lower	upper			lower	upper	
Benzene	38	1072.0	478.2	2976.1	87	229.4	27.3	695.1	p<0.001
Toluene	38	417.5	104.9	820.0	94	78.1	7.9	289.1	p<0.001
Xylenes	38	245.9	10.8	435.3	101	69.9	9.1	226.1	p<0.01
Ethylbenzene	38	9.4	1.6	17.3	97	3.5	0.9	8.9	p<0.05

In Table 2, significant differences ($p<0.001$) between average concentrations of urinary metabolite of non-exposed and exposed workers, can be seen. In the exposed group A urinary concentrations of tt-MA are significantly higher than in group B ($p<0.001$. The correlation between the time-weighted average benzene exposure and the urinary tt-MA level, during the shift, were examined by regression analysis. Figure 1 shows the correlation between the concentration of benzene in air and the tt-MA concentrations in shift-end urine of operators of the cistern mechanical repair department. The correlation between the summary concentration of tt-MA in urine and the benzene concentrations in the breathing zone air are statistically significant at $p<0.001$. No significant association was found between urinary tt-MA and toluene, xylenes and ethylbenzene in air.

Table 2. Results of biological monitoring of workers

Metabolites in urine	Control (n=35)				Group A (n=39) Cistern cleaning department				Group B (n=100) Cistern mechanical repair department				p
	n	M	quartile low	quartile up	n	M	quartile low	quartile up	n	M	quartile low	quartile up	
		mg/g creat.				mg/g creat.				mg/g creat.			
tt-MA	34	0.16	0.08	0.23	38	7.3	2.9	10.5	100	4.7	0.97	5.7	< 0,001

M – median, creat. – creatinine, low – lower, up-upper, n – Number of subjects in whom the tt-MA excreted were above the analytical detection limits, p-value for group A vs B

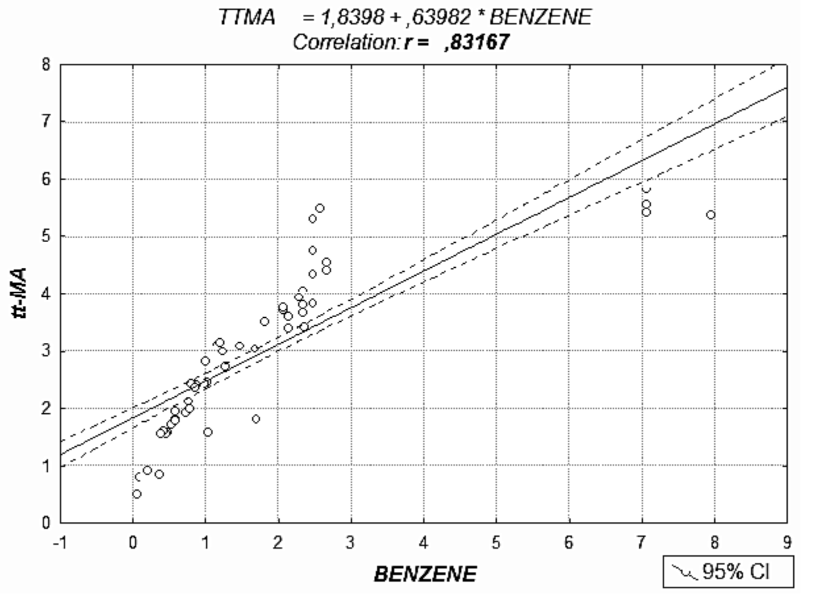

Figure 1. Relation between benzene in breathing-zone air and the tt-MA in shift-end urine of workers of the cistern mechanical repair department

The final multiple regression model for tt-MA in post-exposure urine (*TTMA*) (R^2 = 0.809). The following significant predictors were observed: benzene air concentration (BENZENE), work net energetic cost (W_EN_C), self reported alcohol consumption (ALCOHOL), age of workers (AGE) and time of exposure to chemicals during the shift (T_EXPO). Self reported tobacco smoking (SMOKING) has been found as not significant.

Table 3. Regression Summary for dependent variable TTMA

N = 149	R= ,89956919 R²= ,80922473 Adjusted. R²= ,80273577 F(5,147)=124,71 p<0,0000 Std Error of estimate: ,07190					
	BETA	Std. Err. of Beta	B	Std. Err. of B	t(146)	*p*
Intercept			-.499496	0.109135	-4.57686	0.000010
BENZENE	0.675103	0.051525	0.120373	0.009187	13.10247	0.000000
AGE	0.184421	0.040169	0.003690	0.000804	4.59116	0.000009
ALCOHOL	0.120081	0.044458	0.000167	0.000062	2.70103	0.007730
SMOKING	*0.036559*	*0.014497*	*0.000672*	*0.000282*	*0.81280*	*0.417655*
T_EXPO	0.189949	0.040023	0.002069	0.000436	4.74594	0.000005
W_EN_C	0.197298	0.048216	0.000314	0.000077	4.09198	0.000070

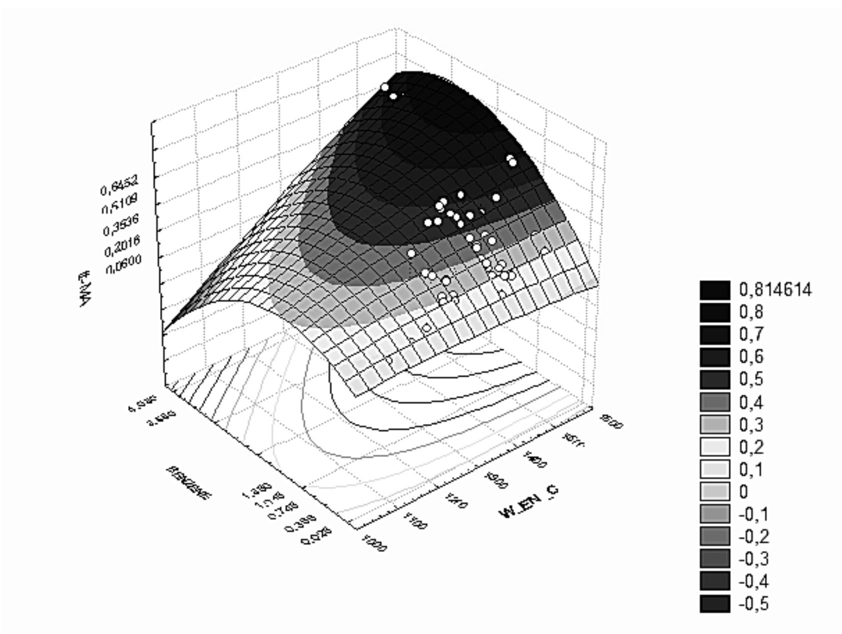

Figure 2. Relation between benzene in breathing-zone air and the work energetic cost W_EN_C effecting on tt-MA level in urine of workers of the cistern mechanical repair department

DISCUSSION

During the cistern cleaning and cistern mechanical repair processes many chemicals are produced, which can contaminate the environment. According to the current standards of the occupational risk assessment, selected compounds, e.g.: benzene, have been assigned to study the levels of exposure to different toxic chemicals [4, 20]. Our results show that the average concentrations of benzene, toluene, xylenes, and ethylbenzene in the breathing zone air (Table 1) are relatively high in comparison to the exposure limits given by ACGIH [1,2] The cistern cleaning process brings higher exposure to benzene and its homologues. Occupational risk from benzene of the cistern service plant workers has been also estimated by the determination of tt-MA in urine. As can be seen in Table 2, concentrations of the tt-MA in urine are also estimated to be high. Nevertheless, significant differences ($p<0.001$) are found between the concentrations of urinary metabolites detected in the urine of exposed workers (group A and B) and the reference Control group. The data have shown that for the biomarker studied, significant differences are found between the non-exposed subjects and those occupationally exposed to variable concentrations of hydrocarbons. Biological monitoring of hydrocarbons provides better estimation of the absorbed substances than measurements of the corresponding contaminants in air, because the skin can be an alternative route of exposure. Multivariable analyses yielded considerable model of tt-MA in urine of exposed workers. As expected, significant predictors of tt-MA in urine included the concentrations of benzene in air. Other significant covariates included work energetic cost, workers' age and the time of exposure, as well as alcohol consumption customs. All of these effects are logical. Physical activity, which increases both urineing rate and cardiac output, increased levels of tt-MA in urine [26]. Also, time of exposure (during the shift) was associated with higher levels of benzene in urine. Alcohol consumption (especially the concentrated beverages – more popular among workers in Poland) in the range up to 140g/day (estimated pure alcohol) may affect the metabolite processes and increase tt-MA excretion. In the analyzed model – cigarettes smoking has not been found as significant predictor. It may result from the comparatively high concentration of benzene in the air and the differences in the smokers group.

CONCLUSIONS

We conclude that benzene exposure was significant among subjects having regular contact with hydrocarbons vapor mixture emitted from the cistern during its cleaning. Indeed, more than 25% of benzene air measurements were above the TLV among workers in the moderate exposure categories (Group B). Trans,trans muconic acid levels in urine of exposed workers were highly correlated with benzene concentration in the breathing air. By modeling urine levels of tt-MA and benzene we infer that benzene was predominantly absorbed by inhalation, it was also absorbed by dermal contact. Thus, biomonitoring of tt-MA in urine should reflect both respiratory and dermal uptake of benzene in the presence of other hydrocarbons and aldehydes vapor mixture emitted from the cistern during its cleaning and might be preferred to air monitoring for exposure assessment.

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SUMMARY

The objective of the study is to assess the external and internal exposures to aromatic hydrocarbons in the cistern cleaning and cistern mechanic repair processes at a coke plant. One hundred and forty nince (149) workers engaged as operators in cistern cleaning and cistern mechanic repair processes and 35 non-exposed subjects were examined. Personal analyses of the benzene, toluene, xylene isomers, ethylbenzene, PAH's and aldehydes in the breathing zone air allowed us to determine the time weighted average exposure levels to the aromatic hydrocarbons listed above.The internal exposure to benzene was investigated by measurement of the urinary excretion of tt-MA by means of high pressure liquid chromatography (HPLC). Time of workers exposure to chemicals, work energetic cost and dietary customs of workers including also smoking and alcoholic beverages consumption were monitored during the experiment. The time-weighted average concentrations of the hydrocarbons detected in the breathing zone air shows that the exposure levels of the workers are relatively high in comparison to the exposure limits. Statistically significant correlations of tt-MA concentration in urine and benzene concentration in the breathing zone air (Pearson $r = 0.832$, $n = 141$, $p < 0.001$) determined at the workplaces in the cistern cleaning department and cistern mechanic repair department have been found. Concentrations of the benzene, toluene and ethylbenzene detected in workers from the cistern mechanical repair department are higher that those from the cistern cleaning department. Concentrations trans,trans-muconic acid (t,t-Ma) in the urine of occupationally exposed workers were significantly higher than those of non-exposed subjects. Concentrations of tt-MA in urine were significantly higher for the cistern cleaning workers. Operators at the cistern cleaning and cistern mechanical repair processes are simultaneously exposed to a mixture of different hydrocarbons, and aldehydes.

Keywords: occupational exposure, BTX, BEI's, metabolites

STRESZCZENIE

Celem badania jest ocena zewnętrznych i wewnętrznych ekspozycji pracowników na działanie węglowodorów aromatycznych podczas czyszczenia cystern i procesu naprawy cysterny oraz w otaczarni. Badano 149 pracowników podczas czyszczenia cystern i zbadano proces czyszczenia cystern oraz 35 pracowników nie wystawionych na działanie węglowodorów. Analizowano stężenia benzenu, toluenu, izomerów ksylenu, etylobenzenu, WWA i aldehydów we wdychanym powietrzu, co pozwoliło określić średni czas i ważony poziom ekspozycji. Ekspozycję na benzen badano poprzez pomiar wydalania z moczem tt-MA za pomocą wysokociśnieniowej chromatografii cieczowej (HPLC). W trakcie eksperymentu monitorowano czas narażenia pracowników na działanie substancji chemicznych, wydatek energetyczny i zwyczaje żywieniowe pracowników, w tym także palenie tytoniu i spożycie napojów alkoholowych. Wykryte w tym czasie w powietrzu w obrębie strefy oddychania średnie stężenie węglowodorów pokazuje, że poziomy narażenia pracowników są stosunkowo wysokie w porównaniu do NDS. Znaleziono statystycznie istotne korelacje stężenia tt-MA w moczu oraz stężenia benzenu w strefie oddechowej (Pearson $r = 0.832$, $n = 141$, $p < 0{,}001$), które określono w miejscach pracy: na stanowisku w dziale czyszczenia cystern oraz dziale napraw mechanicznych. Stężenia benzenu, toluenu i etylobenzenu wykryte u pracowników z działu napraw mechanicznych są wyższe niż u tych z działu czyszczenia. Stężenie kwasu (tt-Ma) w moczu pracowników narażonych zawodowo było znacznie wyższe niż u osób nienarażonych. Stężenie tt-MA w moczu było znacząco wyższe w przypadku pracowników zajmujących się czyszczeniem cystern. Operatorzy czyszczący cysterny i wykonujący mechaniczne naprawy są jednocześnie narażeni na mieszankę różnych węglowodorów i aldehydów.

Słowa kluczowe: ekspozycja zawodowa, BTX, markery ekspozycji, metabolity