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EXCRETION OF METHYLBENZOIC ACID IN URINE AS A RESULT OF SINGLE AND COMBINED EXPOSURE TO M-XYLENE

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Abstract. Influence of combined exposure to solvent vapours on the metabolism of m-xylene and the kinetics of excretion of methylbenzoic acid (MBA) in urine was investigated.

The volunteers were exposed in groups of four or five. Each experiment consisted of two parts. During the first part, the same test group was exposed to m-xylene for a period of 4 hours, whereas during the second part, the group was exposed to m-xylene in combination with other solvents.

No significant differences in the excretion of MBA in urine were observed after exposure to m-xylene in concentrations of about 45 and 70 ppm and exposure to m-xylene together with n-hexane, toluene, methyl isobutyl ketone or n-butyl alcohol in combined concentrations of about 90 and 140 ppm. Only in the case of combined exposure to m-xylene and n-butyl acetate was a significant increase in the excretion of MBA in urine found.

For both the single and combined exposure to kinetics of excretion of MBA was similar with $t_{1/2}$ values in the first phase of excretion ranging between 1 — 1.3 h.

INTRODUCTION

The majority of methods for the biological monitoring of exposure to organic solvents have been developed for particular chemicals under experimental conditions with only one substance present in the atmosphere. Their application in industrial conditions, where workers are exposed to mixtures rather than single chemicals, has been based on the assumption that the probability of toxicokinetic interactions at concentrations of solvents in the air approximating MAC-values is rather low. However, little is known about both the pharmacokinetics of inhaled solvents in relation to atmospheric exposure concentrations, and the possible interactions of industrial solvents. According to David et al. (2), the human capacity to biotransform m-xylene amounts to the dose ab-

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sorbed at about 800 mg/m³, and according to Riihimäki (8) to 1300 mg/m³. Excretion of mandelic acid in urine increases linearly with the increase of styrene concentrations in the air up to 600 mg/m³ (3).

Combined exposure of m-xylene and ethylbenzene in concentrations of 655 mg/m³ both lowered the amounts of metabolites, as compared to separate exposures, and delayed the excretion of most metabolites (4). Coexposure to m-xylene and methyl ethyl ketone (MEK) in concentrations of 100 ppm (435 mg/m³) and 200 ppm (590 mg/m³) resulted in inhibited xylene metabolism (6). All these data suggest that toxicokinetic interactions between inhaled solvents may occur at rather high levels of exposure approaching the metabolic capacity of the human liver.

It has also been suggested, however, that the efficiency of the metabolism of xylene to methylhippuric acid can decrease as a result of exposure to a mixture of solvents at much lower concentrations (about 100—150 mg/m³) (10).

The aim of this study was to evaluate the excretion methylhippuric acid and its kinetics after combined experimental exposure to m-xylene and other solvents at concentrations of about 100 and 150 ppm, respectively. Confirmation of toxicokinetic interactions within this range of concentrations could be of real importance as regards the possibility of applying biological monitoring for the purpose of evaluating industrial solvent exposure.

MATERIAL AND METHOD

Subjects

The subjects of this study were six male volunteers aged 32—40 who showed no abnormalities in routine clinical examinations.

Exposure conditions

The exposures were carried out in an exposure chamber with a volume of 11.7 m³ and ventilation adjusted to about 200 m³/h. Solvent vapours were generated in a saturator immersed in a thermostated water bath and diluted with carrier air to produce the desired environmental concentration. The concentration of solvents in the chamber was monitored by gas chromatography and infrared spectrophotometry. The GC determinations were carried out with a GCHF-18.3 gas chromatograph equipped with a FID detector. A metal column (2 m × 4 mm id.) packed with 15% SE-30 on Varaport 30 (100—120 mesh) was used. The temperatures were as follows: oven-120°C, injector-150°, detector-180°C.

TABLE 1. Excretion of Methylbenzoic Acid in Urine

Experiment No		No of subjects exposed	Solvent	Conc. in chamber in mg/m³ (ppm)	Methylbenzoic acid excreted in urine (mg)			
					During 4 h exposure		Within 22 h from the beginning of exposure	
					$\bar{x}\pm SD$	p	$\bar{x}\pm SD$	p
I		4	m-xylene	290 (67)	492±138		765±144	
	a	4	m-xylene + toulene	298 (68) 167 (45)	441±117	NS	645±125	NS
		4	m-xylene	302 (69)	465±165		664±184	
	b	4	m-xylene + toulene	310 (71) 260 (69)	392±132	NS	597±130	NS
II		5	m-xylene	193 (45)	251±69		360±82	
	a	5	m-xylene + n-butyl acetate	217 (49) 220 (46)	346±69	$p<0.01$	495±66	$p<0.01$
		5	m-xylene	320 (74)	356±105		509±131	
	b	5	m-xylene + n-butyl acetate	300 (69) 340 (72)	443±116	NS	597±123	NS
III		4	m-xylene	214 (49)	307±82		464±92	
	a	4	m-xylene + n-hexane	212 (49) 157 (44)	259±63	NS	376±106	NS
		4	m-xylene	303 (70)	466±226		690±188	
	b	4	m-xylene + n-hexane	300 (69) 240 (68)	468±123	NS	656±166	NS
IV		4	m-xylene	202 (46)	370±122		601±133	
	a	4	m-xylene + n-butyl alcohol	195 (45) 121 (40)	268±22	NS	412±25	NS
		5	m-xylene	300 (69)	368±40		571±45	
	b	5	m-xylene + n-butyl alcohol	302 (69) 210 (69)	407±126	NS	611±144	NS
V		5	m-xylene	200 (46)	226±85		373±93	
	a	5	m-xylene + methyl isobutyl ketone	200 (46) 183 (45)	233±52	NS	395±101	NS

Nitrogen was used as a carrier gas, at the flow rate of 30 ml/min, 1 ml air samples from the chamber were injected using directly into the gas chromatograph every 15 minutes a gastight Hamilton syringe.

The IR determinations of m-xylene were carried out continuously with a Beckman model Acculab 6 spectrophotometer with a 20 metre gas cell (Foxboro Analytical).

The volunteers were exposed in groups of four or five (Table 1) to m-xylene only and to m-xylene in combination with toluene, n-butyl acetate, n-hexane and n-butyl alcohol and to methyl isobutyl ketone in the first series of experiments. Each of the nine experiments consisted of two parts. During the first part, the same test group was exposed to m-xylene for a period of 4 hours, whereas during the second part it was exposed to m-xylene in combination with other solvents. This procedure was applied in order to eliminate the possible influence on the results of experiments of individual differences in metabolic efficiency.

Collection of urine samples

Urine samples were collected before the onset of exposure and then after a period of 2, 4, 6, 8, 10, 12, 14 and 22 hours. The samples were kept at −18°C until determination. Urine samples from both parts of each experiment were analysed at the same time to avoid the possibility of systematic analytical errors affecting the results.

Determination of methylbenzoic acid in urine

Urinary methylbenzoic acid was measured according to Wieczorek et al. (11). 0.5 ml internal standard solution (β-naphthol in ethyl acetate) was added to a glass test tube and evaporated to dryness under nitrogen. Then 2 ml urine was added and hydrolyzed with 2 ml 11 mol NaOH at 100°C (water bath) for 1 hour. After hydrolysis, 5 ml 3 mol H_2SO_4 was added and saturated with NaCl and extracted with 10 ml ethyl ether for 10 minutes. Then, after centrifugation, the organic layer was evaporated to dryness. The residue was silylated for 5 min with 0.1 ml of the solution Silyl 8-pyridine (1:1). One microlitre of aliquot was used for each injection. The concentrations of methylbenzoic acid were calculated from the ratio between the peak height of methylbenzoic acid silyl ester and the internal standard after calibration had been made.

The laboratory has participated in the Quality Control Programme for Toxic Metals and Organic Compounds in Urine coordinated by the Institute of Occupational Health in Helsinki (the mean values found in all participating laboratories amounted to 1.26 ± 1.34 and 0.86 ± 1.09 mmol/l; found: 1.58 and 0.61 mmol/l).

Statistical analyses

The differences between the excretion of methylbenzoic acid during the first and second part of each experiment were tested using the t-test for paired observations.

RESULTS

The data concerning urinary excretion of methylbenzoic acid within 4 h and 22 h after the beginning of exposure are presented in Table 1.

To eliminate the influence of differences in m-xylene concentrations in the air in the individual experiments on the excretion of methylbenzoic acid in urine, the results presented in Table 1 were recalculated according to the following equations:

$$E_2 = E_1 \cdot \frac{200}{C} \quad \text{or} \quad E_2 = E_1 \cdot \frac{300}{C},$$

where:

E_1 = excretion of methylbenzoic acid within 4 or 22 h after the beginning of exposure (in mg),

E_2 = corrected excretion,

C = concentration of m-xylene vapours in the chamber during a given experiment (in mg/m^3).

The recalculated results are presented in Table 2. No significant differences in the excretion of methylbenzoic acid in urine could be observed after exposure to m-xylene in concentrations of about 45 and 70 ppm and exposure to m-xylene together with n-hexane, methyl isobutyl ketone or n-butyl alcohol in combined concentrations of about 90 and 140 ppm (Table 1 and 2). In the case of exposure to m-xylene and toluene, in both the experiments the same trend of a decrease in the excretion of methyl benzoic acid after combined exposure was observed. The differences, amounting to about 10—15% decrease in methyl benzoic acid excretion, were, however, statistically insignificant. A significant increase in the excretion of methyl benzoic acid was noted after combined exposure to m-xylene and n-butyl acetate (Table 1 and 2).

Table 3 displays the data on the excretion of methyl benzoic acid in urine after single and combined exposure of the individuals. After a 4 hour exposure to m-xylene at a concentration of about 45 ppm, mean excretion varied from 237 to 338 mg within the first four hours of exposure and from 303 to 498 mg within 22 h after the beginning of exposure. For each individual, the relative standard deviation of the mean values of excretion amounted to from ±18% to ±33% and from ±23 to ±27% within the 4 hour and 22 hour period after the beginning

TABLE 2. Excretion of Methylbenzoic Acid in Urine. Values Recalculated to Eliminate the Influence of Differences in M-xylene Concentrations in the Air

Experiment No		No. of subjects exposed	Solvent	Methylbenzoic acid excretion in urine (mg)			
				During 4 h exposure		Within 22 h from the beginning of exposure	
				$\bar{x}\pm SD$	p	$\bar{x}\pm SD$	p
I	a	4	m-xylene	335±94	N.S.	520±106	N.S.
		4	m-xylene + toluene	295±70		431±91	
	b	4	m-xylene	450±164	N.S.	647±180	N.S.
		4	m-xylene + toluene	379±127		578±125	
II	a	5	m-xylehe	247±68	$p < 0.01$	355±81	$p < 0.01$
		5	m-xylene + n-butyl acetate	319±64		470±62	
	b	5	m-xylene	334±99	$p < 0.01$	477±123	$p < 0.01$
		5	m-xylene + n-butyl acetate	443±116		597±123	
III	a	4	m-xylene	292±78	N.S.	441±87	N.S.
		4	m-xylene + n-hexane	252±61		365±103	
	b	4	m-xylene	461±224	N.S.	683±186	N.S.
		4	m-xylene + n-hexane	468±123		656±166	
IV	a	4	m-xylene	374±124	N.S.	601±133	N.S.
		4	m-xylene + n-butyl alcohol	319±27		491±30	
	b	5	m-xylene	368±40	N.S.	571±45	N.S.
		5	m-xylene + n-butyl alcohol	404±126		606±142	
V	a	5	m-xylene	226±85	N.S.	373±93	N.S.
		5	m-xylene + methyl isobutyl ketone	233±52		395±101	

NS — non significant

TABLE 3. Stability of Methylbenzoic Acid Excretion in Urine After Single and Combined Exposure to M-xylene in Human Volunteers

Person exposed	Series of experiments **)	No. of experiments	Methylbenzoic acid excreted in urine (mg/*)			
			During 4 h exposure		Within 22 h from the beginning of exposure	
			x±RSD	% ***)	x±RSD	% ***)
P.K.	a	10	313±21%	102	456±26%	
	b	8	496±13%		679±14%	96
G.P.	a	8	338±28%		479±27%	
	b	6	563±18%	107	761±15%	98
R.R.	a	10	237±31%		303±23%	
	b	6	331±25%	91	490±25%	96
J.F.	a	8	251±33%		411±24%	
	b	6	289±20%	74	484±15%	76
A.S.	a	6	316±18%		498±23%	
	b	2	491±23%	100	652±24%	84
T.G.	b	8	349±23%		546±12%	
All volunteers	a	44	286±29%		440±25%	
	b	36	414±30%	95	598±23%	90

*) after recalculation made according to equation 1

**) m-xylene concentration in the chamber: series a — about 200 mg/m³; series b — about 300 mg/m³

***) percentage of the value expected from results of experiment a).

of exposure. Similar results were obtained during the second series of experiments, with the air concentrations of m-xylene amounting to about 70 ppm.

The diagram of excretion kinetics for methyl benzoic acid in urine after single or combined exposure to m-xylene at a concentration of about 70 ppm is presented in Fig. 1.

DISCUSSION

The results, obtained from the experiments, presented in Table 1 and 2 do not definitely indicate that combined exposure affected the metabolism of m-xylene to methylbenzoic acid excreted in urine. It was only in the case of combined exposure to m-xylene and n-butyl acetate that a significant increase in the excretion of methylbenzoic acid in urine was found; but the finding cannot possibly be attributed to increased metabolic efficiency. The more probable explanation is increased lung ventilation during the exposure to n-butyl acetate.

The study results seem not to confirm the suggestion regarding possible impact that a mixture of aliphatic hydrocarbons, n-butanol, methyl isobutyl ketone and n-butyl acetate may exert on the metabolism of m-xylene at concentrations approximating Polish MAC values (100 mg/m^3 for m-xylene) (10). Our findings are rather consistent with other data from literature which indicate that, during exposure to organic solvent vapours, human biotransformation capacity is sufficient to

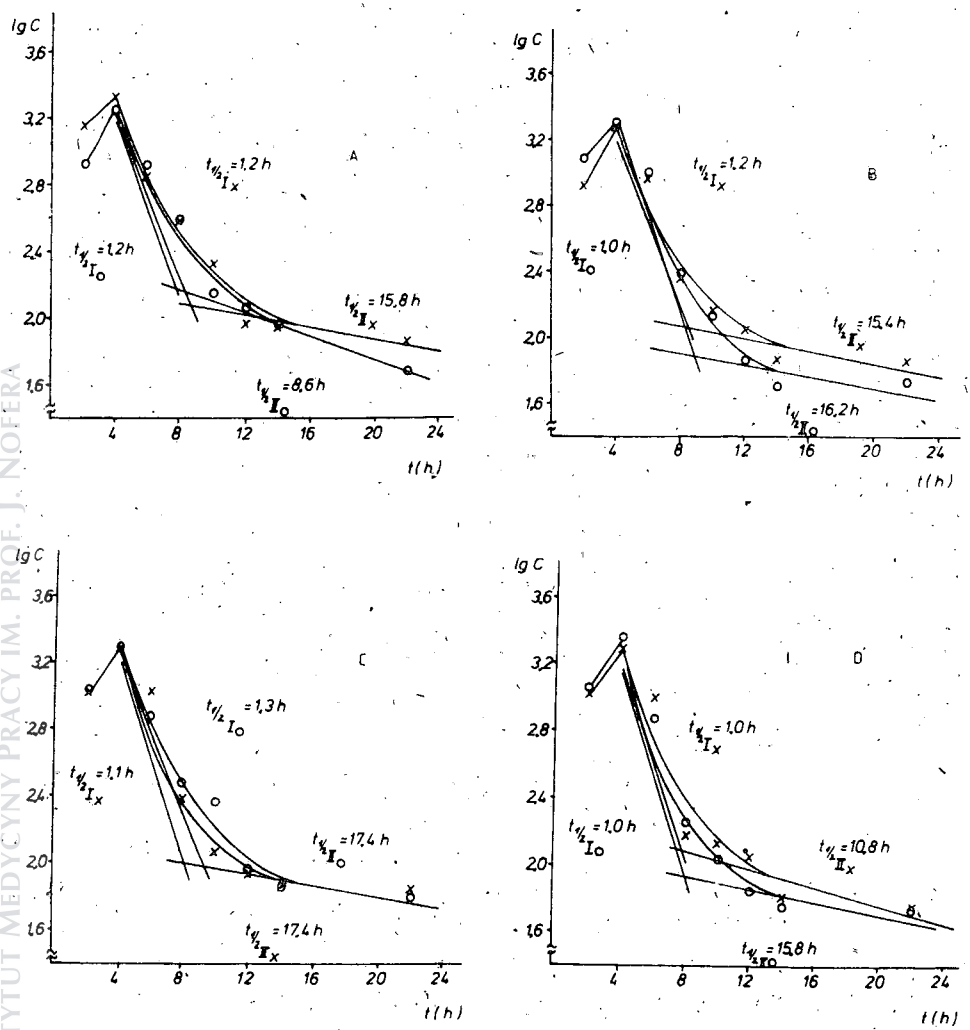

Fig. 1. Kinetics of methylbenzoic acid excretion in urine after single or combined exposure to m-xylene. x — x m-xylene (70 ppm); o — o combined exposure (140 ppm). A — m-xylene + toluene; B — m-xylene + n-butyl acetate; C — m-xylene + n-butyl alcohol; D — m-xylene + n-hexane.

metabolise the dose observed at concentrations of about 650—1300 mg/m^3 (4, 8, 13). Neither could any abnormal kinetics of excretion be found in the case of combined exposure. The excretion of methyl benzoic acid was similar in both the single and combined exposure and the excretion half-times were found to be similar to those reported in literature (7, 9).

Relative standard deviations of the mean urinary excretion of methyl benzoic acid after single and combined exposure (Table 3) fall within the range of values usually observed as a result of experimental exposures to single substances. This finding provides additional evidence in favour of the hypothesis that combined exposure to organic solvent vapours at the concentrations under study may not necessarily influence the metabolism of m-xylene. The data in Table 3 concerning the rates of excretion of methyl benzoic acid in series II of the experiments, as compared with those expected from the results of series I, indicate the very high stability of the metabolism rate between different individuals. Although the interval between the two series of experiments was 9 months, the rate of excretion which was 25% lower than that expected was found in only one subject.

The study results indicate that in combined exposure to solvent vapours at concentrations of up to 150 ppm, no toxicokinetic interaction should be expected. This finding is significant in view of the high applicability of biological monitoring to the examination of the absorption of m-xylene under industrial conditions (1, 5), where it is usually used in the form of mixtures with other solvents.

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