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Rise of Inhaled Toluene, Ethyl Benzene, m-Xylene, or Mesitylene in Rat Blood After Treatment with Ethanol

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Toluene, ethyl benzene, m-xylene, and mesitylene (1,3,5-methyl benzene) are widespread as solvents in industries and laboratories or in the manufacture and application of glues, paints, printing inks etc. These aromatics may be absorbed by employees during exposure at the workplace. Alcoholic beverages may be consumed during occupational inhalation or after shift's end at times. Toxicokinetic interactions between the aromatics and ethanol must be assumed because of the common pathway of biotransformation (Freundt 1980). The blood levels of toluene (Waldron et al. 1983) and m-xylene (Riihimäki et al. 1982) after inhalation increased significantly in volunteers dosed simultaneously with ethanol. In this view the present experiments in rats should elucidate whether the blood concentrations of inhaled ethyl benzene and mesitylene (both structurally related to toluene and m-xylene) can rise under the influence of ethanol, and whether quantitative differences of this effect due to the structure of these aromatics can occur. From the results informations important for the assessment of occupational health risk are to be expected.

MATERIALS AND METHODS

Adult female SPF Sprague-Dawley rats, weighing 200 - 220 g, were obtained from the Central Breeding Station of the University of Heidelberg / FRG. The animals were housed under conditions as described previously (Römer et al. 1985). The following chemicals were used: ethanol, toluene, and m-xylene from Merck, Darmstadt / FRG, mesitylene from Baker, Deventer / Holland.

Groups of 3 rats each - mixed randomly - were exposed in a 20 l glass chamber under dynamic conditions (air flow 1.25 l/min) for 2 h to various concentrations of the aromatics in air. Atmospheres containing the aromatics fluctuated no more than $\pm$ 5 percent and were delivered by means of a specially constructed device (evaporator). One of two corresponding animal groups received 20 mmol ethanol/kg b. w. in physiological saline (0.9 % NaCl, w/v) intraperitoneally before exposure. The other animals were sham-treated with the corresponding volume (5 ml/kg) of physiological saline. During the exposures food and water were withdrawn. Exposure concentrations during the inhalations were monitored repeatedly by GC analyses using air samples (100 µl) collected in gas-tight syringes. Blood (0.02 ml) was collected repeatedly for analysis from the retro-orbital

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plexus of the rats using disposable pipettes. The blood concentrations of ethanol and the aromatics were determined simultaneously by gas chromatography (Sigma 1 with HS-6 head space sampler, Perkin-Elmer, Ueberlingen / FRG) using a method described elsewere (Römer et al. 1985). The following analytical conditions were changed: Operating temperatures: oven, 85° C; head space HS-6, 60° C. The pipettes containing the blood samples were transferred into autosampler vials containing 0.5 ml aqueous NaCl (25 %, w/v) to enhance the volatility of the substances to be determined. Calibration curves were determined on every experimental day. The coefficients of variation indicating the reproducibility of the method were found to be maximally 8 % (n = 6 within the daily series). Aromatics containing air samples collected in syringes were transferred into empty autosampler vials and monitored using GC. The coefficients of variation for the air analysis were observed to be lower than 1 %.

The means $\pm$ SEM were calculated from the corresponding individual values determined. The treatment groups were compared with controls using Student's t-test. A p value below 0.05 was considered as significant.

RESULTS AND DISCUSSION

The 2-h exposure concentrations applied in the present study are realistic, because the theshold limit values (TLVs 1985 - 86; MAK values 1985) amount to 100 ppm for toluene, ethyl benzene, and m-xylene referring to an 8-h shift, but no hygienic limit value is assigned to mesitylene. The applied ethanol produced in the rats blood concentrations (Table 1) which are comparable with those achieved in humans after alcohol intake (e.g. approximately 5 mmol/l = 0.023 %). Ethanol enhanced significantly the blood levels of inhaled toluene, ethyl benzene, or m-xylene (Table 1). The blood concentration of toluene appeared more augmented than that of xylene within the same design (220 ppm of the aromatics, 20 mmol ethanol/kg; Table 1). In spite of a lowered exposure concentration (180 ppm) the blood level of ethyl benzene rose markedly more than those of toluene or m-xylene (Table 1). After exposure to a low concentration (110 ppm) the blood level of mesitylene showed a tendency to increase that was, however, not significant even when the animals had been exposed to as much as 580 ppm (Table 1). The cause of the enhanced blood concentrations of the aromatics observed all over is a displacement of the primary alcohols of toluene, ethyl benzene, m-xylene, and mesitylene - produced by the catalysis of hepatic microsomal oxygenases (first step of degradation) - from the liver alcohol dehydrogenase by ethanol because of a lower affinity to this enzyme system (Riihimäki et al. 1982). It is to assume that the degradation pathway of mesitylene to 3,5-dimethylhippuric acid is less affected because ethanol may not be able to effectively displace the mesitylene metabolite 3,5-dimethylbenzyl alcohol from the alcohol dehydrogenase. Assuming that results from well designed animal experiments can help to assess the human health risk, it may be concluded from the present findings that alcohol intake is more critical during or after occupational exposure to ethyl benzene than to the other aromatics. In the case of co-exposure with alcohol mesitylene seems to be the least dangerous compared with the other aromatics. But toluene showed no advantage over m-xylene. A corresponding increase in central nervous disturbances (e.g. depression) may be expected in the occupational area after co-exposure to ethanol and the investigated aromatics.

Table 1. Blood concentrations (means ± SEM from 3 rats per group) of toluene, ethyl benzene, m-xylene, mesitylene, or ethanol after 2-h inhalation of these aromatics and ip. application of ethanol at the beginning of the exposure.

Solvent	Exposure concentration (ppm)	Blood concentration: Solvent (10^{-6} mol/l)			Blood concentration: Ethanol (10^{-3} mol/l)
toluene	220 (a)	56.8 ± 2.1			
	220 (b)	91.5 ± 3.5	(+ 61%)	(c)	6.41 ± 0.63
ethyl benzene	180 (a)	25.3 ± 2.6			
	180 (b)	60.5 ± 6.1	(+ 139%)	(c)	3.81 ± 0.48
m-xylene	220 (a)	67.6 ± 7.8			
	220 (b)	92.7 ± 5.1	(+ 37%)	(c)	5.10 ± 1.00
mesitylene	110 (a)	10.5 ± 2.0			
	110 (b)	11.3 ± 0.3	(+ 8%)		4.92 ± 0.96
	580 (a)	113.8 ± 25.8			
	580 (b)	137.0 ± 9.3	(+ 20%)		5.03 ± 0.73

Co-administration before start of exposure: (a) physiological saline (5ml/kg b.w.) ip. - (b) ethanol (20 mmol/kg b.w.) in physiological saline (final volume: 5 ml/kg b.w.) ip.
Enhancement (in percent) in parenthesis. - (c) significant: p less than 0.05

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