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TOXIC EFFECTS OF COMBINED EXPOSURE TO TOLUENE AND XYLENE IN ANIMALS. I. ACUTE INHALATION STUDY

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Abstract. Effect of combined exposure to toluene and xylene on the rotarod performance in rats and respiratory rate in mice was investigated in the conditions of acute inhalation experiment. Rotarod performance was tested in rats exposed to various concentrations of toluene, xylene and their mixture consisting of 50 Vol-% toluene and 50 Vol-% xylene immediately after termination of 4-hour exposure. Respiratory rate in mice was recorded in short 6 min duration exposure to individual solvents and their 50:50 Vol-% mixture.

Both solvents and their mixture caused concentration-dependent disturbances of rotarod performance in rats. The medial effective concentration (EC_{50}) for this effect amounted to 4050 ppm, 4520 ppm and 2770 ppm for toluene, xylene and their mixture, respectively.

The tested solvents resulted in concentration-dependent depression of respiratory rate in mice. The concentration depressing the respiratory rate to 50% (RD_{50}) was 4790 ppm, 2160 ppm and 1950 ppm for toluene, xylene and their mixture, respectively.

In both animal tests the effect of combined exposure to toluene and xylene was more than additive suggesting that similar phenomenon might occur in the conditions of combined occupational exposure to toluene and xylene.

INTRODUCTION

Occupational exposure to mixtures of organic solvents is common in many industrial situations. On the other hand, occupational exposure limits are set for single solvents. The assumption of additivity of health effects has often been used in industrial hygiene to cope with the problem of combined exposure to solvents. However, this assumption is not sufficiently validated by scientific data (1, 2).

To test the additiveness hypothesis for selected organic solvents of

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great practical importance, the animal studies have been undertaken in the conditions of acute and subchronic inhalation exposures. The objective of the present study was to evaluate the toxic effects of combined exposure to toluene and xylene in the conditions of acute inhalation exposure.

The results have not supported the additiveness hypothesis for these solvents.

MATERIAL AND METHOD

Toluene and o,m,p-xylene reagent grade were supplied by Polish Chemical Reagents Company.

In experiments rotarod performance was tested in rats and respiratory rate was measured in mice.

Male Wistar rats body weight 200—250 g were exposed to the vapours of toluene, xylene or their mixture consisting of 50 Vol-% toluene and 50 Vol-% xylene in a dynamic inhalation chamber (1,3 m^3 volume) for 4 hours. Vapours of toluene and xylene were generated by heating liquid solvents in washers. The desired concentrations of vapours were obtained by diluting them with the air. Rotarod performance was tested before exposure and immediately after termination of exposure to several concentrations of toluene (1030, 1980, 2950, 2970, 4120 and 4850 ppm), xylene (1030, 2010, 2930, 2870, 4150 and 4970 ppm) and their mixture (1050, 2030, 2710, 2610, 4130 and 4700 ppm) and in control animals. Each exposure group consisted of 10 rats and the parallel control group of 15 rats. Rotarod performance was tested according to the principle described by Kaplan and Murphy (3). The rotarod apparatus used consisted of 8-cm diameter wooden rod rotated at 12 rpm and suspended horizontally 20 cm above the floor which was constructed from metal bars connected to the power source of 80 V and 2 mA. The ability of rats to remain on the rotating rod for 2 min was taken as an index of normal neuromuscular function. Before experiment animals were trained and only those rats which could perform normally on the rotarod for at least three consecutive days were used in testing.

Respiratory rate was measured in Balb C male mice body weight 25—30 g by the use of plethysmographic method (4). Each animal was placed in a body plethysmograph attached to a small dynamic inhalation chamber (2.3 l volume). A Stattham pressure transducer was attached to each plethysmograph. The respiratory pattern was recorded by the use of a Beckman polyphysiograph. Respiratory rate was recorded continuously before the exposure to solvents, during 6 min of exposure and 2—3 min after termination of exposure. Mice were exposed to the va-

pours of toluene at concentrations 3030, 3850 and 4690 ppm, xylene at concentrations 2600, 4000, 4600 and 7000 ppm or to the mixture of these solvents (50 Vol-% toluene and 50 Vol-% xylene) at concentrations 1060, 2400 and 4400 ppm. Each exposure group consisted of 2—4 mice.

Concentrations of toluene and xylene in the air of inhalation chambers were measured by gas chromatography. Separation of solvents was performed using 3 m column with 10% FFAP (2-nitroterephtalate polyethylene glycol) on chromosorb WAW DMCS as a stationary phase, temperature of column 150°C. Measurements were made with the use of gas chromatograph type Varian 1400 with glame-ionization detector.

Probit analysis was applied for estimation of relative potency of toluene, xylene and their mixture in rotarod performance test and for determination of medial effective concentration (EC_{50}) value (5). Frequency data were also compared using the Chi-square test (6).

The concentration depressing the respiratory rate in mice to 50% (RD_{50}) was calculated from least squares regression lives of concentration-effect relationship (4).

Exposure concentrations of toluene and xylene were expressed in ppm; 1 ppm toluene = 3.75 mg/m^3; 1 ppm xylene = 4.35 mg/m^3.

RESULTS

All rats exposed for 4 hours to the tested concentrations of toluene, xylene and their mixture survived the exposure.

Both solvents and their mixture caused concentration-dependent disturbances in rotarod performance of rats (Fig. 1). At the concentration ±2000 ppm rotarod performance was disturbed only in rats exposed to the mixture of toluene and xylene. No effect was detected at concentrations ±1000 ppm. All control animals performed normally in the test throughout the experiment. In rotarod performance test effects of toluene or xylene were similar, whereas the effects of their mixture was about 50% higher than that of individual solvents (Table 1). EC_{50} values with their 95% confidence intervals amounted to 4050 ppm (3580—4580 ppm), 4520 ppm (3800—5390 ppm) and 2770 ppm (2560—2990 ppm) for toluene, xylene and their mixture, respectively. When frequencies of disturbances in rotarod performance were compared with the use of the Chi-square test, the differences between particular solvents and their mixture appeared also statistically significant ($p < 0.01$).

Both toluene and xylene caused a concentration-dependent decrease in respiratory rate in mice (Fig. 2). The maximum decrease of respiratory rate was always observed in the 1st min of exposure. The effect of xylene was clearly more pronounced than that of toluene. The effect of mixture

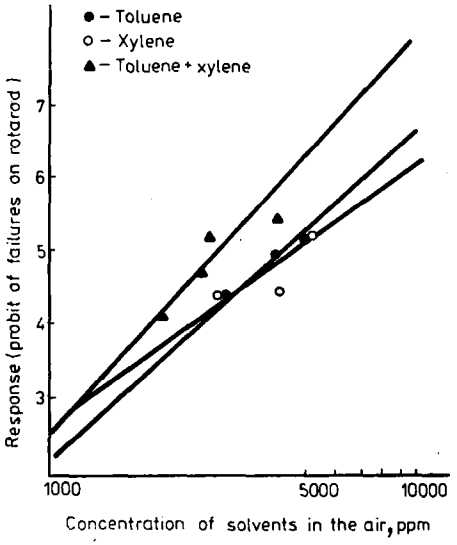

Fig. 1

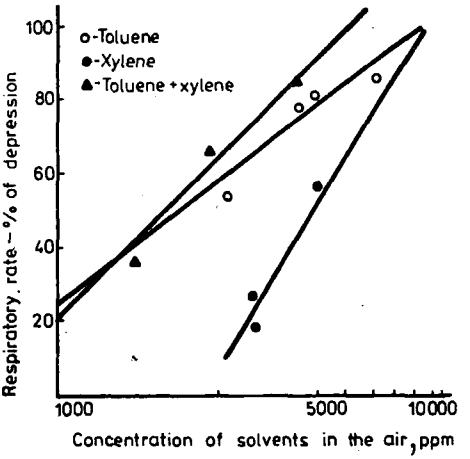

Fig. 2

Fig. 1. Rotarod performance of rats exposed to toluene, xylene and their mixture containing 50 Vol-% toluene and 50 Vol-% xylene.
Rats were exposed to the vapours of solvents for 4 hours. Rotarod performance was tested immediately after termination of exposure. Each point represents probit of failures on rotarod determined in a group of 10 rats.

Fig. 2. Respiratory rate of mice exposed to toluene, xylene and their mixture containing 50 Vol-% toluene and 50 Vol-% xylene.
Each point represents mean value of separate measurements in 2—4 mice. The greatest decrease of respiratory rate observed in the 1st minute of exposure was taken for consideration. The regression lines were determined by the least squares procedure.

TABLE 1. The Relative Potency of Toluene, Xylene and their Mixture in Rotarod Performance Test in Rats

Reference compound	Reference potency	Compared compound	Relative potency	95% confidence intervals
Toluene	1	Xylene	0.974	1.244—0.766
Toluene	1	Toluene+Xylene	1.489[a]	2.019—1.206
Xylene	1	Toluene	1.027	1.306—0.805
Xylene	1	Toluene+Xylene	1.529[a]	2.075—1.233

The mixture of toluene and xylene consisted of 50 Vol-% toluene and 50 Vol-% xylene.

[a] Statistically significant difference in relation to the individual solvents groups ($p < 0.01$).

of toluene and xylene was similar to that of xylene and thus it was higher than could be expected assuming the summation of effects of individual solvents.

The concentration depressing the respiratory rate in mice to 50% (RD_{50}) was 4750 ppm, 2440 ppm and 1900 ppm for toluene, xylene and their mixture, respectively.

DISCUSSION

Toxic effects of acute inhalation exposure to toluene and xylene include mainly depression of central nervous system and irritation of eyes and upper respiratory tract (7, 8). In our animal inhalation study neurotoxicity of these solvents was assessed on the basis of the rotarod test in rats and irritation effect was quantified by measurements of respiratory rate in mice. It provided good evidence that depression of respiratory rate in mice correlates well with the extent of eye and respiratory irritation in man (9, 10).

At the concentrations studied in our experiment toluene and xylene caused concentration-dependent disturbances of rotarod performance in rats. This effect was clearly more pronounced when animals were exposed to the mixture of 50 Vol-% toluene and 50 Vol-% xylene. Thus the results did not support the additiveness hypothesis which seemed to be justified for these solvents due to the similarity of their chemical structure and biological effects. The reasons for this phenomenon are not known. Enhanced neurotoxic effect of the mixture of toluene and xylene could have been related to the mutual suppression of the metabolism of these solvents reported by Ikeda (1), not confirmed, however, by the other authors (11). The relevant toxicokinetic data in rats are necessary to test this hypothesis.

The results of respiratory rate test in mice would also suggest the more than additive irritation effect of toluene and xylene on the upper respiratory tract in conditions of combined exposure.

The extrapolation of our animal data to occupational situations has to be tentative since exposure concentrations of toluene and xylene in animal experiments were one to two orders of magnitude higher than occupational exposure limits of these solvents and the mechanism of observed interactions is not known. However, the results obtained suggest that the more than additive effect of combined occupational exposure to toluene and xylene should be taken into consideration and studied further.

Subchronic animal experiment on the toxic effects of combined exposure to toluene and xylene at concentration levels of 1000 ppm and

100 ppm are under way in our laboratory. A human volunteers study is in preparation.

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