Polish Journal of Occupational Medicine and Environmental Health, Vol. 6, No 1, 35-41, 1993

EFFECTS OF ACUTE COMBINED EXPOSURE TO N-BUTYL ALCOHOL AND M-XYLENE

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Key words: m-Xylene, n-Butyl alcohol, Combined exposure, Nervous system, Respiratory system

Abstract. The effects of combined exposure to m-xylene and n-butyl alcohol on rotarod performance and motor activity in rats and respiratory rate in mice were investigated in the condition of an acute inhalation experiment. Rotarod performance and motor activity were tested in rats exposed to various concentrations of m-xylene, n-butyl alcohol and their mixture consisting of 50 Vol-% m-xylene and 50 Vol-% n-butyl alcohol immediately after termination of a 4-hour exposure period. The respiratory rate in mice was recorded in short 6 min duration exposures to individual solvents and their 50:50 Vol-% mixture.

Both solvents and mixtures caused concentration-dependent disturbances of rotarod performance in rats. The medial effective concentration (EC_{50}) for the effect amounted to 6530 ppm, 1980 ppm and 3080 ppm for n-butyl alcohol, m-xylene and their mixture, respectively. Both solvents and their mixture changed the spontaneous motor activity in the rat. Because of a two-phase effect, the concentration-dependance of the observed changes could not be defined. The evaluation of the combined effect in motor activity test was carried out by comparing experimental values with expected ones assuming the summation of individual solvent effects.

The tested solvents resulted in a concentration-dependent decrease in respiratory rate in mice. The concentration which decreased the respiratory rate to 50% (RD_{50}) was 3010 ppm, 1360 ppm and 3140 ppm for n-butyl alcohol, m-xylene and their mixture, respectively.

In the animal tests the effect of the combined exposure to m-xylene and n-butyl alcohol was less than additive suggesting that a similar phenomenon might occur in the conditions of combined occupational exposure to m-xylene and n-butyl alcohol.

INTRODUCTION

A combined exposure to various mixtures of organic solvents frequently occurs under industrial conditions, whereas exposure limits are set separately 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 (6), however this assumption is not sufficiently validated (14, 5, 11). Our previous observations indicate the more than additive toxic effects of combined exposure to

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m-xylene and toluene (9, 10). It is well known that both m-xylene and n-butyl alcohol primarily depress the control nervous system and irritate the respiratory tract.

The objective of the present study was to evaluate the neurotoxic and irritating effects of combined exposure to m-xylene and n-butyl alcohol in conditions of acute inhalation study.

MATERIALS AND METHODS

Chemicals

m-Xylene and n-butyl alcohol were supplied by Reachim and the Polish Chemical Reagent Company.

In experiments the rotarod performance and motor activity were tested in rats and respiratory rate was measured in mice. Male Wistar rats of Imp: DAK stock outbred, body weight 250–300 g, were exposed to vapours of m-xylene, n-butyl alcohol and their mixture consisting of 50 Vol-% m-xylene and 50 Vol-% n-butyl alcohol in a dynamic inhalation chamber (1.3 m^3 volume). Vapours of m-xylene and n-butyl alcohol were generated by heating liquid solvents in washers. The desired concentrations of vapours were obtained by diluting them in the air. Concentrations of solvents vapour in the exposure chamber were measured every 30 min with a gas chromatograph with a flame ionization detector using 1.5 m metal column with 10% OV–17 on chromosorb WHP (80–100 mesh) as a stationary phase at column temperature of 100°C.

Rotarod performance

Rotarod performance was tested according to the principle described by Kaplan and Murphy (8). The rotarod apparatus used consisted of a 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 a 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 the experiment, the animals were trained and only those rats which could perform normally on the rotarod for at least 10 consecutive days were used in the experiment. Rotarod performance was tested before exposure and immediately after termination of exposure to several concentrations of m-xylene, n-butyl alcohol and their mixture and in sham exposed control animals for one hour.

Spontaneous motor activity was measured during one hour immediately after the termination of 4 hours of exposure by using of UMA-2-10 actometer for small laboratory animals.

The respiratory rate was measured in Balb/C male mice weighing 25–30 g by the use of plethysmographic method (13). 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. The respiratory rate was recorded continuously before the exposure solvents, during 6 min of exposure and 6 min after termination of exposure. Mice were exposed to vapours of single solvents and their mixtures at various concentrations. Each exposure group consisted of 8–10 mice.

Exposure concentrations of m-xylene and n-butyl alcohol were expressed in ppm; 1 ppm m-xylene = 4.35 mg/m^3, 1 ppm n-butyl alcohol = 3.08 mg/m^3.

Probit analysis was applied to determine the medial effective concentraion (EC_{50}, RD_{50}) values (4). Frequency data were also compared using the Chi – square test (2).

RESULTS

All rats exposed for 4 hours to the tested concetrations of m-xylene, n-butyl alcohol and their mixture survived the exposure. Both solvents and their mixture caused concentration-dependent disturbances in the rotarod performance of rats (Fig. 1). All control animals performed normally in the test throughout the experiment. In the rotarod performance test the effect of m-xylene was more pronounced than that of n-butyl alcohol. EC_{50} values with 95% confidence intervals amounted to 6531 ppm (4950 – 10370 ppm), 1982 (1530 – 2565 ppm) and 3088 ppm (2440 – 4240 ppm) for n-butyl alcohol, m-xylene and their mixture, respectively. The results obtained in the rotarod performance test suggest the additive toxic effect of combined exposure to n-butyl alcohol and m-xylene.

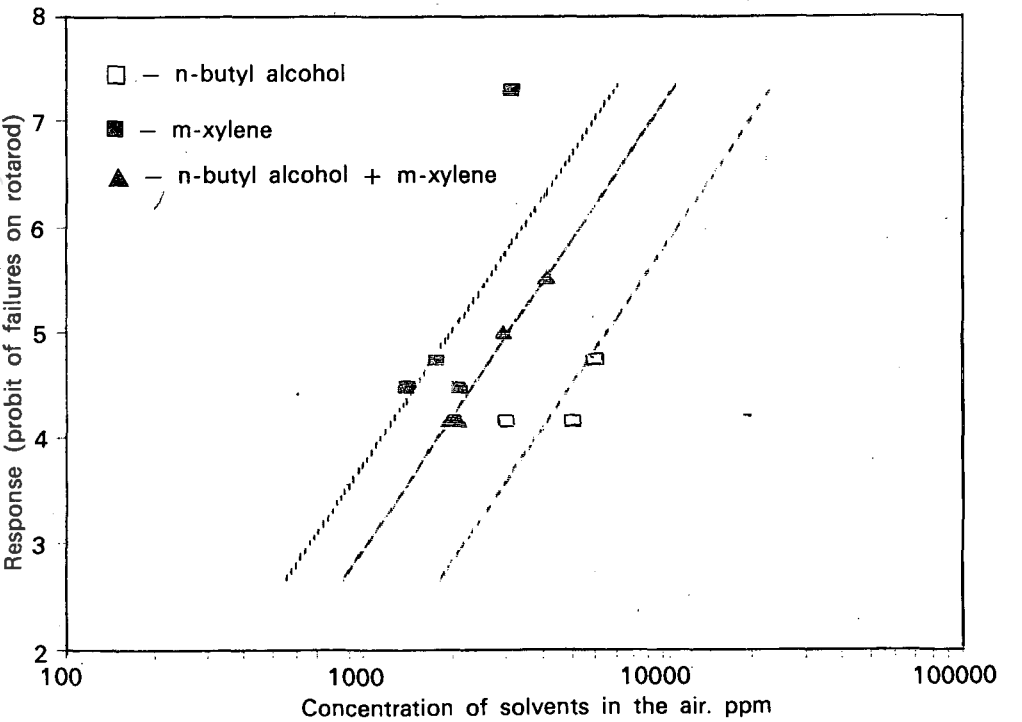

Fig. 1. Rotarod performance of rats exposed to n-butyl alcohol, m-xylene and their mixture containing 50 Vol-% n-butyl alcohol and 50 Vol-% m-xylene.
Rats were exposed to vapours of solvents for 4 hours. Rotarod performance was tested immediately after termination of exposure. Each point represents probit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

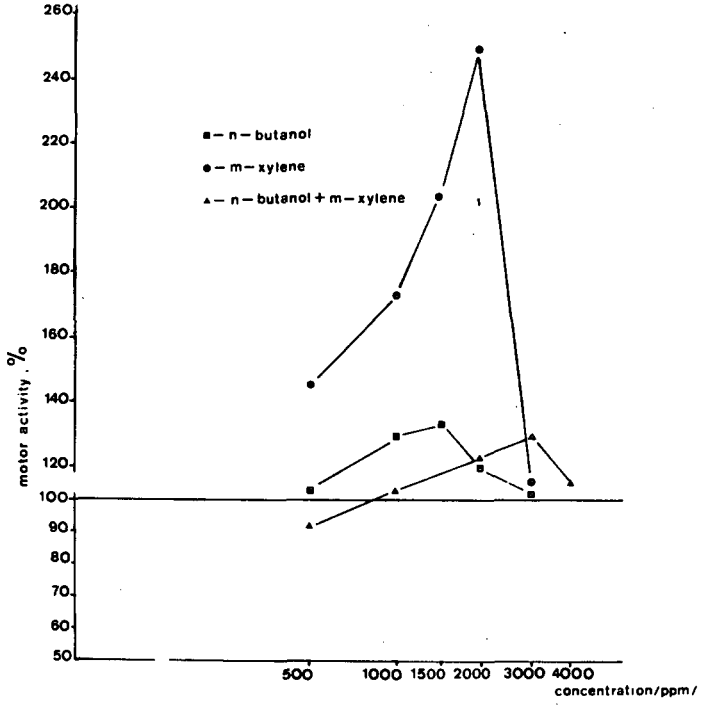

Fig. 2. Effect of n-butanol, m-xylene and their mixture on spontaneous motor activity in rat.
Rat were exposed to the vapour of solvents for 4 hours. Spontaneous motor activity was measured during 1 hour immediately after termination of exposure. Each point represents no. of movements (%) in comparison to control. ± SD values omitted.

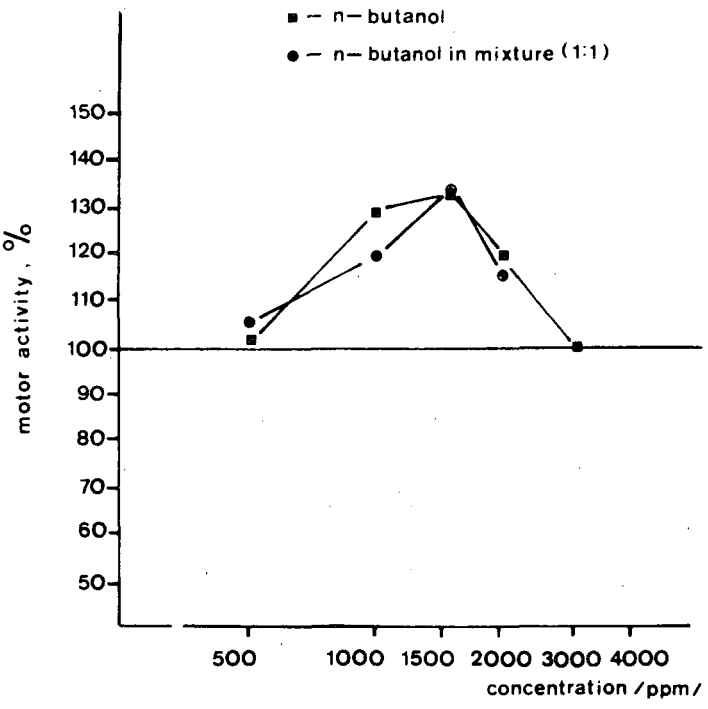

Fig. 3. Effect of n-butanol applied separately and in mixture (1:1) with m-xylene on spontaneous motor activity in rat.
Each point represents no. of movements (%) in comparison to control. ± SD values omitted.

Both solvents and their mixture changed the spontaneous motor activity in the rat (Fig. 2). At lower concentrations of solvents the animals were excited and increased motor activity was observed. Further increases in solvent concentration caused marked decreases of spontaneous motor activity. Because of a two-phase effect, the concentration-dependance of changes in spontaneous motor activity could not be defined. Evaluation of combined exposure to n-butyl alcohol and m-xylene in spontaneous motor activity test was carried out by comparing the experimental values with those expected, assuming the summation of individual solvent effects (Table 1). Comparison indicates that the solvents' mixture's effect on spontaneous motor activity is characteristic for antagonistic effects. The dependence between

Table 1. Spontaneous motor activity in rats exposed to mixture of n-butyl alcohol and m-xylene (1:1). Comparison of experimental values with those expected, assuming the summation of effects of individual solvents

n-butanol + m-xylene (1:1), ppm	Spontaneous motor activity	
	experimental	expected
968	+ 5%	+ 49%
1976	+ 20%	+ 104%
3041	+ 34%	+ 136%
3761	+ 16%	+ 172%

the motor activity changes and concentrations of n-butyl alcohol applied individually and in mixture with m-xylene is shown in Fig. 3. Both curves are almost identical which indicates that the effect of m-xylene in conditions of combined exposure with n-butyl alcohol was not added to the effect of n-butyl alcohol. The results obtained in spontaneous motor activity test suggest less than additive effect of the combined exposure to n-butyl alcohol and-m-xylene.

Both m-xylene and n-butyl alcohol caused a concentration- dependent decrease in the respiratory rate of mice (Fig. 4). The maximum decrease of respiratory rate was always observed in the 1st min of exposure. The effect of m-xylene was clearly more pronounced than that of n-butyl alcohol.

The effect of mixture of m-xylene and n-butyl alcohol was similar to that of n-butyl alcohol and thus it was lower 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 1361 ppm, 3008 ppm and 3143 ppm for m-xylene, n-butyl alcohol and their mixture, respectively.

DISCUSSION

The toxic effects of acute inhalation exposure to m-xylene and n-butyl alcohol include mainly: 1) depression of central nervous system, 2) irritation of eyes and upper respiratory tract (7,3).

In our animal inhalation study neurotoxicity of these solvents was assessed on the basis of the rotarod and spontaneous motor activity test, and the irritation effect was quantified by measurements of respiratory rate in mice. It provided good

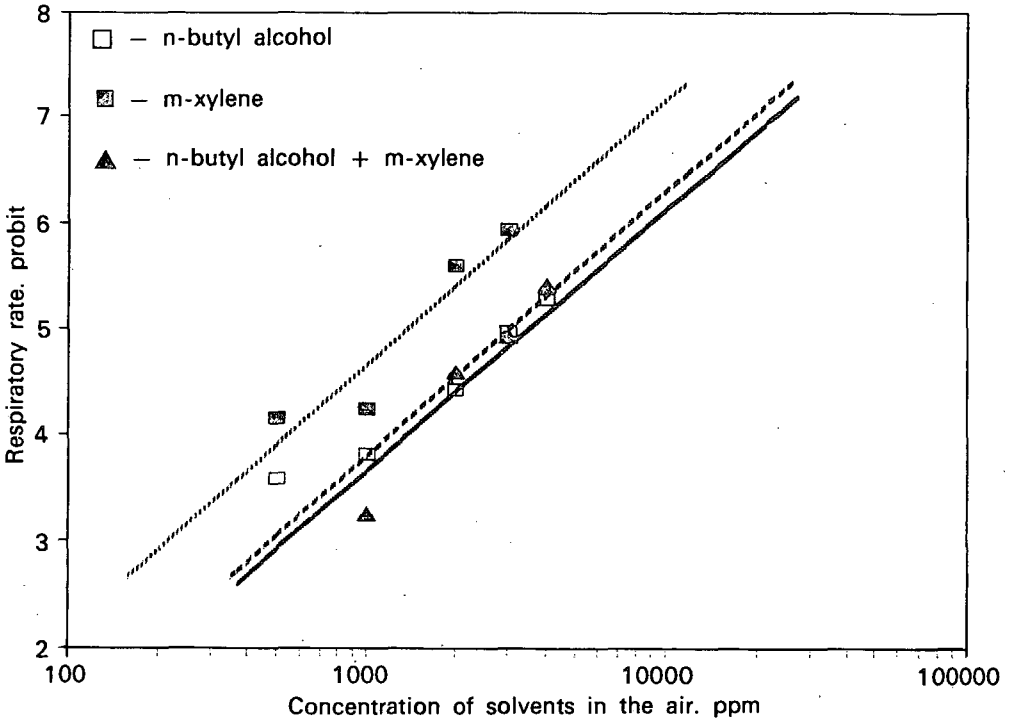

Fig. 4. Respiratory rate of mice exposed to n-butyl alcohol, m-xylene and their mixture containing 50 Vol-% n-butyl alcohol and 50 Vol-% m-xylene. Each point represents the mean value of separate measurements in 8–10 mice. The greatest decrease in respiratory rate observed in the 1st minute of exposure was taken for consideration. The regression lines were determined by the least squares procedure.

evidence that depression of respiratory rate in mice correlated well with the extent of eye and respiratory irritation in man (1, 12). At the concentrations studied in our experiment n-butyl alcohol, m-xylene and their mixture caused concentration--dependent disturbances in rotarod performance, two-phase manner changes in spontaneous motor activity in rats, and concentration-dependent decreases in respiratory rate of mice. The neurotoxic effect of the combined exposure to n-butyl alcohol and m-xylene mixture (1:1) seems to be less than additive and did not support the additiveness hypothesis which seemed to be justified for these solvents due to the similarity of their biological effects. The results of respiratory rate test in mice also suggest the less than additive irritation effect of n-butyl alcohol and m-xylene on the upper respiratory tract in condition of combined exposure.

The reasons for this phenomenon are not known. Nothing is known about toxicokinetic or toxicodynamic basis for these solvents' biological interaction. Also it would be difficult to explain the less than additive irritating effect of the solvents' mixture by metabolic interaction. In this case the interaction may rather be suspected at the receptor place.

The extrapolation of our animal data to occupational situations has to be tentative since exposure concentrations of n-butyl alcohol and m-xylene in animal experiments were one or two orders higher than occupational exposure limits of these solvents, and the mechanism of observed interactions is not known. However, the results obtained suggest that the less than additive effect of combined occupational exposure to n-butyl alcohol and m-xylene should be taken into consideration and studied further in conditions of subchronic animal exposition.

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Received for publication: October 5, 1992
Accepted for publication: December 12, 1992