

EFFECTS OF ACUTE COMBINED INHALATION EXPOSURE TO n-BUTYL ALCOHOL AND n-BUTYL ACETATE IN EXPERIMENTAL ANIMALS

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Abstract. The effects of combined exposure to n-butyl alcohol and n-butyl acetate on rotarod performance and hot plate behaviour in rats and respiratory rate in mice were investigated in the condition of an acute inhalation experiment. Rotarod performance and hot-plate behaviour were tested in rats exposed to various concentrations of n-butyl alcohol, n-butyl acetate and their mixture consisting of 50 Vol-% n-butyl alcohol and 50 Vol-% n-butyl acetate immediately after termination of a 4-hour exposure period. The respiratory rate in mice was recorded continuously before the exposure to solvents, during 6 min of exposure and 6 min after termination of exposure using whole body plethysmographic method. Mice were exposed to vapours of single solvents and their 50:50 Vol-% mixture.

Both solvents and their mixture caused concentration-dependent disturbances of rotarod performance in rat. The medial effective concentration (EC50) for the effect amounted to 7559 ppm, 8339 ppm and 10672 ppm for n-butyl alcohol, n-butyl acetate and their mixture, respectively. Both solvents and their mixture decreased sensitivity to the pain and changes were concentration-dependent. In condition of combined exposure the results obtained in rotarod and hot-plate behaviour test indicate the summation of individual solvent effects. The tested solvents resulted in concentration-dependent decrease in respiratory rate in mice. n-Butyl alcohol produced maximal decrease in respiratory rate in the first minute of exposure whereas n-butyl acetate in the sixth minute. The concentrations depressing the respiratory rate in mice to 50% (RD50) was 4300 ppm (1 min) and 1755 ppm (6 min) for n-butyl alcohol and n-butyl acetate respectively.

In the animal tests neurotoxic effect of combined exposure to n-butyl alcohol and n-butyl acetate was additive whereas irritating effect was independent.

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 (9) however, this assumption is not sufficiently validated (8, 15, 18). Our previous observations indicate the more than additive toxic effects of combined exposure to m-xylene and toluene (11, 12) and less than additive toxic effects of combined exposure to m-xylene and n-butyl alcohol (13, 14). It is well known that both n-butyl alcohol and n-butyl acetate, like other solvents, 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 n-butyl alcohol and n-butyl acetate in condition of acute inhalation study.

MATERIALS AND METHODS

Chemicals

n-Butyl alcohol and n-butyl acetate were supplied by the Polish Chemical Reagent Company.

Male Wistar rats of Imp: DAK stock outbred, body weight 250–300 g, were exposed to vapours of n-butyl alcohol, n-butyl acetate and their mixture consisting of 50 Vol-% n-butyl alcohol and 50 Vol-% n-butyl acetate in a dynamic inhalation chamber (volume of 1.3 m³) for 4 hours. Vapours of n-butyl alcohol and n-butyl acetate were generated by heating of 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 type Varian 1400 with flame-ionization detector using 2 m glass column with 20% SE-30 on chromosorb WHP (100–120 mesh) as a stationary phase at column temperature of 150°C.

Rotarod performance was tested according to the principle described by Kaplan and Murphy (10). The rotarod apparatus used consisted of a 8-cm diameter wooden rod rotarod 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 n-butyl alcohol, n-butyl acetate and their mixture and in sham exposed control animals for four hour. Each group consisted of 10 rats.

Hot plate behaviour was tested immediately after termination of exposure. The hotplate test was used to measure the level of analgesia (3). The rat was placed on the hot-plate within the plastic enclosure and after occurrence of the expected response – licking the foot, or after 60 sec, animal was removed. The latency of the paw-lick response was measured at plate temperature of 54.5°C. Each group consisted of 10 rats.



The respiratory rate was measured in Balb/C male mice weighing 25–30 g by the use of plethysmographic method (17). Each animal was placed in a body plathysmograph attached to a small dynamic inhalation chamber (volume of 2.3 l). 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 to solvents, during 6 min of exposure and 6 min after termination of exposure. Mice were exposed to vapours of single solvents and their mixture at various concentrations. Each exposure group consisted of 8–10 mice.

Exposure concentration of n-butyl alcohol and n-butyl acetate are expressed in ppm; 1 ppm n-butyl alcohol = 3.08 mg, 1 ppm n-butyl acetate = 4.83 mg/m³.

Probit analysis was applied to determine the medial effective concentration (EC₅₀, RD₅₀) values (7). The obtained data were analysed with ANOVA and Kruskal-Wallis test (2).

RESULTS

All rats exposed for 4 hours to the tested concentrations of n-butyl alcohol, n-butyl acetate and their mixture survived the exposure. Both solvents and their

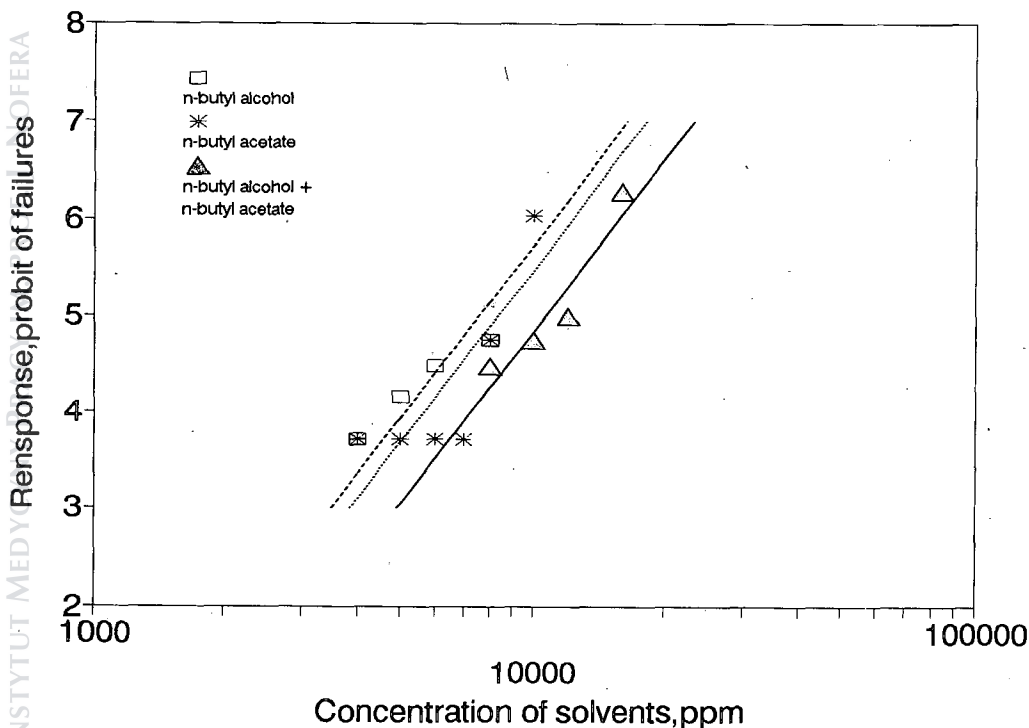


Fig. 1. Rotarod performance of rats exposed to n-butyl alcohol, n-butyl acetate and their mixture containing 50 Vol-% n-butyl alcohol and 50 Vol-% n-butyl acetate. Rats were exposed to vapours of solvents for 4 hours. Rotarod performance was tested immediately after termination of exposure. Each point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

mixture caused concentration-dependent disturbance in the rotarod performance of rats (Fig. 1). All control animals performed correctly in the test throughout the experiment.

EC50 values with their 95% confidence intervals amounted to 7559 ppm (6327–9500 ppm), 8339 ppm (7399–9622 ppm) and 10672 ppm (9200–12224 ppm) for n-butyl alcohol, n-butyl acetate and their mixture, respectively. The evaluation of relative potency shows that in the rotarod performance test the effects of n-butyl alcohol and n-butyl acetate were similar whereas the effects of their mixture was slightly lower but not statistically different in relation to the individual solvents groups (Table 1).

Table 1. The relative potency of n-butyl alcohol, n-butyl acetate in rotarod performance test in rats

Reference compound	Reference potency	Compared compound	Relative potency	95% confidence intervals
n-butyl alcohol	1	n-butyl acetate	0.9065	0.7305–1.15558
n-butyl alcohol	1	n-butyl alcohol	0.7083	0.5669–0.9428
		+ n-butyl acetate		
n-butyl acetate	1	n-butyl alcohol	0.7814	0.6532–0.9692
		+ n-butyl acetate		

The mixture of n-butyl alcohol and n-butyl acetate consisted of 50 Vol-% n-butyl alcohol and 50 Vol-% n-butyl acetate. Effect of mixture was not statistically different in relation to the individual solvents groups.

EC50 values with their 95% confidence intervals amounted to 7559 ppm (6327–9500 ppm), 8339 ppm (7399–9622 ppm) and 10672 ppm (9200–12224 ppm) for n-butyl alcohol, n-butyl acetate 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 n-butyl acetate.

The pain sensitivity measured as latency of the paw-lick response was changed in rats exposed to n-butyl alcohol, n-butyl acetate and their (1:1) mixture. The changes were concentration-dependent (Fig. 2).

Both solvents and their mixture decrease sensitivity to the pain. Effect of n-butyl acetate was more pronounced than that of n-butyl alcohol and solvents (1:1) mixture. EC50 values with their 95% confidence intervals amounted to 5901 ppm (4841–7232 ppm), 4491 ppm (3578–5643 ppm) and 5766 ppm (4701–7041 ppm) for n-butyl alcohol, n-butyl acetate and their mixture, respectively. The effect of combined exposure to solvents was not statistically different than that of single solvents. Results obtained in hot-plate behaviour test indicate that effect on n-butyl and n-butyl acetate mixture follows the additivity principle.

Both n-butyl alcohol and n-butyl acetate caused concentration-dependent decrease in respiratory rate in mice, but dynamics of the observed changes was different. n-Butyl alcohol produced maximal decrease in respiratory rate in the first minute whereas the maximal respiratory rate decrease caused by n-butyl acetate was observed in the sixth minute of exposure. The concentration depressing the respiratory rate in mice to 50% (RD50) was 4300 pmm (1 min) and 1755 (6 min) for n-butyl alcohol and n-butyl acetate, respectively. The effect of mixture of solvents in the first minute of exposure was similar to that of n-butyl alcohol and in the sixth minute similar to the effect of n-butyl acetate (Fig. 3).

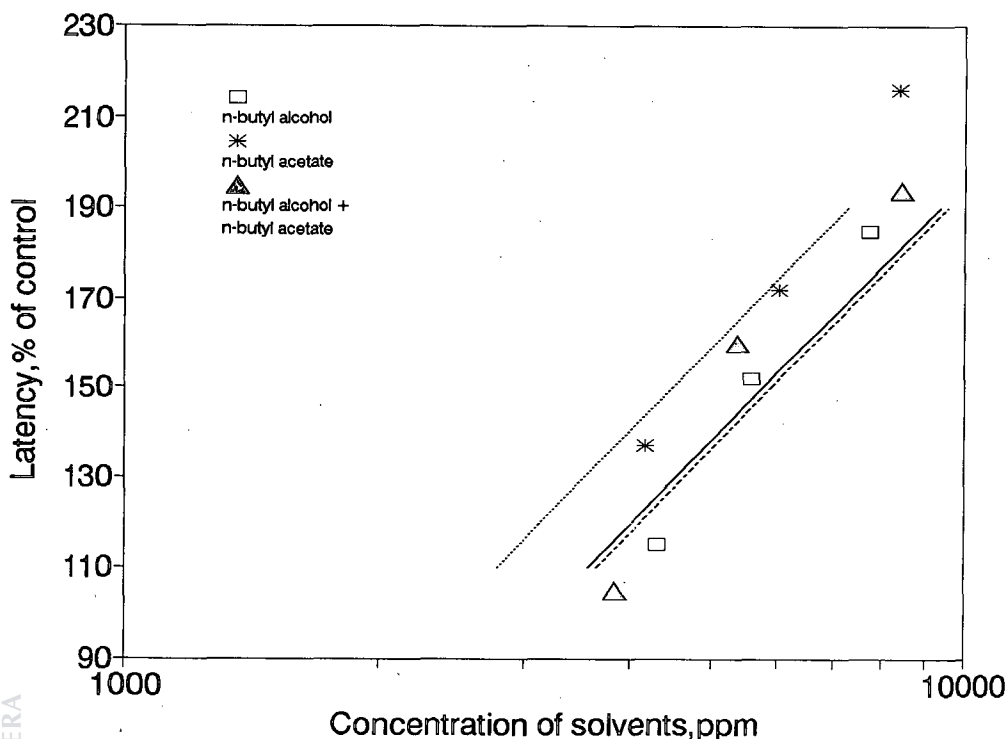


Fig. 2. Hot-plate behaviour in rats exposed to n-butyl alcohol, n-butyl acetate and their mixture containing 50 Vol-% n-butyl alcohol and 50 Vol-% n-butyl acetate. Rats were exposed to vapours of solvents for 4 hours. Hot-plate behaviour was tested immediately after termination of exposure. Each point represents the mean value of separate measurements in 10 rats.

In the first minute of exposure to 1:1 mixture of solvents at concentration of 7986 ppm, the decrease of respiratory rate amounted 55.4% and was similar to that observed at exposure to n-butyl alcohol at concentration of 3899 ppm (58.7%) (Fig. 3). In the sixth minute of exposure to mixture of solvents the decrease of respiratory rate amounted 82% and was similar to that observed during exposure to n-butyl acetate at concentration of 3933 ppm (87%) (Fig. 3).

DISCUSSION

The toxic effects of acute inhalation exposure to n-butyl alcohol and n-butyl acetate include mainly: 1) depression of central nervous system 2) irritation of eyes and upper respiratory tract (5, 6). In our animal inhalation study neurotoxicity of these solvents was assessed on the basis of the rotarod and hot-plate behaviour tests, 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 (1, 16).

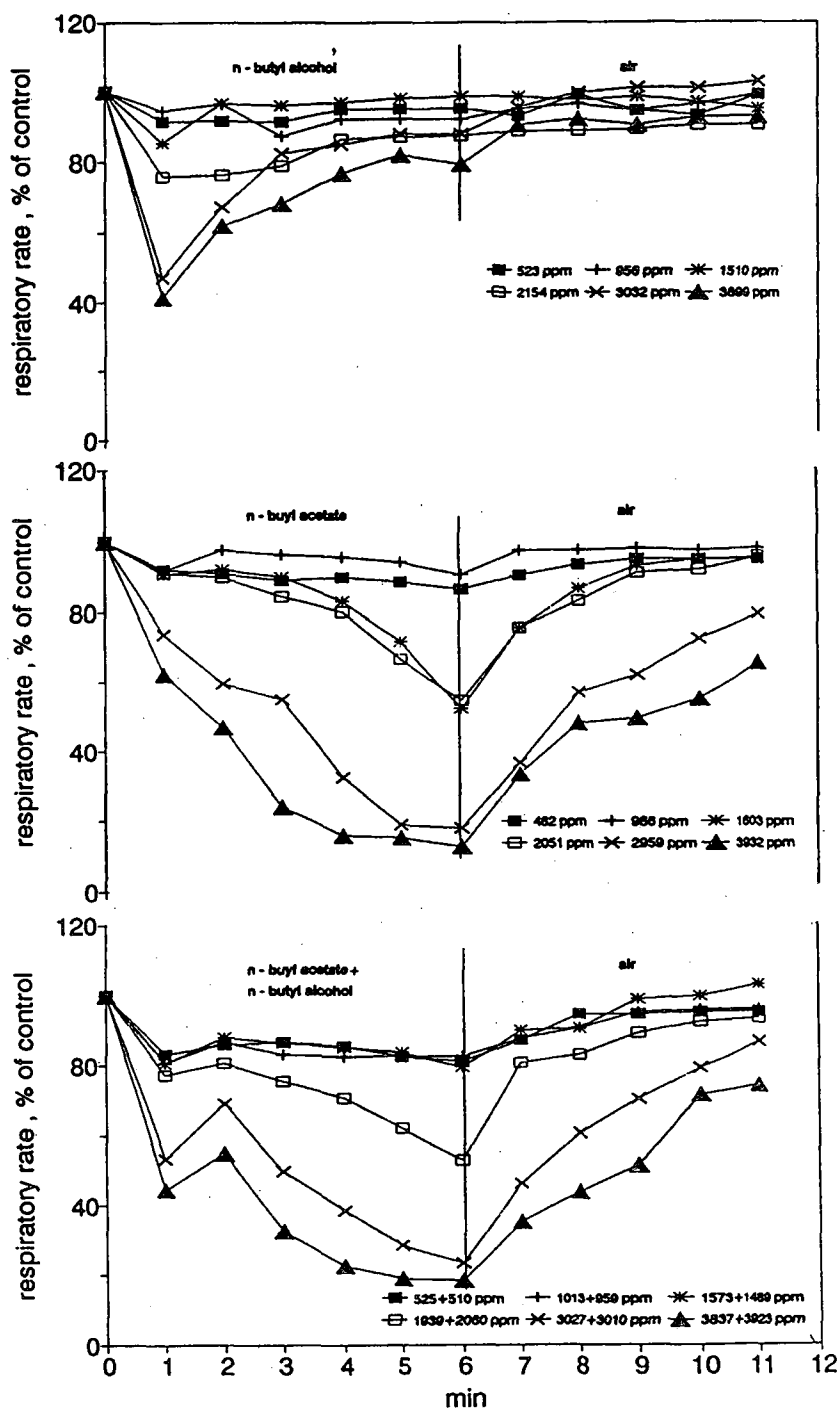


Fig. 3. Respiratory rate of mice exposed to n-butyl alcohol, n-butyl acetate and their mixture containing 50 Vol-% n-butyl alcohol and 50 Vol-% n-butyl acetate. Each point represents the mean value of separate measurements in 8–10 mice.

At concentrations studied in our experiment n-butyl alcohol, n-butyl acetate and their mixture caused concentration-dependent disturbances in rotarod performance and changes in pain sensitivity in rats and concentration-dependent decrease in respiratory rate in mice. In the rotarod and hot-plate behaviour test there were no significant differences in ED50 values for single solvents and their 1:1 mixture. Therefore neurotoxic effects of combined exposure to n-butyl alcohol and n-butyl acetate mixture (1:1) were additive. It supports the additiveness hypothesis which seems to be justified for these solvents due to the similarity of their chemical structure and biological effects.

The results of respiratory rate test in mice have shown the stronger n-butyl alcohol irritating effect on the upper respiratory tract than that of n-butyl acetate. The RD50 values defined for n-butyl alcohol and n-butyl acetate in our experiment, are similar to that reported by other authors (4).

n-Butyl alcohol and n-butyl acetate irritating effect has not been demonstrated at the same time therefore, these solvents do not satisfy a condition to reveal an interaction (19). The results obtained in respiratory rate test in mice indicate the independent effects of n-butyl alcohol and n-butyl acetate.

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