

TOXIC EFFECTS OF SUBCHRONIC COMBINED EXPOSURE TO N-BUTYL ALCOHOL AND M-XYLENE IN RATS

ZOFIA KORSAK, JUSTYNA WIŚNIEWSKA-KNYPL and RADOSŁAW ŚWIERCZ

Department of Toxicity Evaluation
The Nofer Institute of Occupational Medicine
Lodz Poland

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Abstract. Effects of combined exposure to m-xylene and n-butyl alcohol in the conditions of subchronic inhalation experiment in rats were examined. Rats were exposed to vapours of individual solvents at concentrations of 50 and 100 ppm and their 1:1 mixture at concentrations of 50 + 50 ppm and 100 + 100 ppm, 6 h/day, 5 days/week for 3 months. No significant changes in body weight gain, in absolute and relative organ weights and clinical biochemistry parameters were observed. The motor coordination disturbances caused by mixture of m-xylene and n-butyl alcohol at concentrations of 100 + 100 ppm were identical as those caused by n-butyl alcohol at concentration of 100 ppm. Thus, in condition of combined exposure, the toxic effect of m-xylene was not added to the effect of n-butyl alcohol. Significant increase in sensitivity to pain in animals exposed to m-xylene at both concentrations, and parallel lack of changes in the pain sensitivity in animals exposed to n-butyl alcohol and to the solvent mixtures was observed. n-Butyl alcohol provoked the increase of lipid peroxidation in hepatic microsomes without any induction of cytochrome P-450 monooxygenases. m-Xylene did not affect the activity of monooxygenase and lipid peroxidation rate in hepatic microsomes and moreover, in the case of combined exposure it abolished the effect on n-butyl alcohol. Results obtained in condition of acute and subchronic inhalation exposure interpreted jointly, indicate the less than additive toxic effect of combined exposure to m-xylene and n-butyl alcohol.

INTRODUCTION

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 (7). However, this assumption is not significantly validated by scientific data (5, 12, 19). It

is well known that both m-xylene and n-butyl alcohol primarily depress the central nervous system and irritate the respiratory tract. Our previous observations indicate the less than additive toxic effects of combined exposure to m-xylene and n-butyl alcohol (1:1) on the central nervous system and respiratory tract in condition of acute inhalation study (10).

The objective of the present study was to evaluate the toxic effects of combined exposure to m-xylene and n-butyl alcohol in the condition of subchronic inhalation exposure.

Biological and health effects of exposure to mixture of m-xylene and n-butyl alcohol may result from their toxicokinetic interaction, since similar enzymatic pathway may be involved in their metabolism (16), the evaluation of metabolic (cytochrome P-450 system) and peroxidative effects of exposure to m-xylene, n-butyl alcohol and their mixture were carried out.

MATERIALS AND METHODS

Chemicals

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

Conversion factors (20°C)

m-xylene 1 ppm = 4.35 mg/m³

n-butyl alcohol 1 ppm = 3.078 mg/m³

Animals

Male Wistar rats of Imp: DAK stock outbred, were used. They were housed in plastic cages with wire-mesh covers and maintained under 12 h light/12 h dark cycle, lighting on from 6:00 to 18:00 hours. Food and water were available *ad libitum* in their home cages. Body weights for all animals were recorded prior to the start of the study and weekly during the experiment.

Inhalation exposure

Animals 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 m³ 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 chromasorb WHP (80–100 mesh) as a stationary phase at column temperature of 100°C.

Ninety six rats were divided into 7 groups each consisted of 12 animals except of control group of 24 animals. Control group was sham-exposed, the others were exposed to m-xylene 50 ppm (217 mg/m³) and 100 ppm (435 mg/m³); n-butyl alcohol 50 ppm (154 mg/m³) and 100 ppm (308 mg/m³) and their 1:1 mixture; 100 ppm (217 mg/m³ m-xylene + 154 mg/m³ of n-butyl alcohol) and 200 ppm (435 mg/m³



m-xylene + 308 mg/m³ of n-butyl alcohol) 6 hours/day, 5 days/week for 3 months.

Hematology parameters in the tail blood were evaluated prior to the beginning of the study and 1 week before termination of the experiment. Erythrocyte count, hemoglobin concentration, hematocrit, leucocyte count and differential leukocyte count were conducted in the control and exposure groups.

Biochemical assays

Clinical biochemistry studies were conducted for all animals 24 hours after termination of inhalation exposure. Animals were deprived of food 24 hours prior, then were anesthetized in light ether anesthesia, exsanguinated from the abdominal aorta and subjected to gross necropsy. Blood samples were collected for serum biochemical diagnostic tests. These were made for alanine aminotransferase (ALAT, E.C. 2.6.1.8.), aspartate aminotransferase (AspAT, E.C. 2.6.1.1.), sorbitol dehydrogenase (SDH, E.C. 1.1.1.14.), alkaline phosphatase (AP, E.C. 3.1.3.1.), total protein, albumin and glucose, and electrolytes – sodium, potassium, calcium, chloride. Beckman Clinical System 700 autoanalyser and standard test combination kits were used for determinations.

For assay of microsomal monooxygenases and lipid peroxidation, livers were homogenized in 3 vol (w/v) of cold 150 mmol KCl 20 mmol Tris-HCl buffer, pH 7.4 in a glass Potter-Elvehjem homogenizer with a teflon pestle to yield a 25% homogenate. The activity of aniline p-hydroxylase (EC 1.14.1.1) was assayed in 9000 g postmitochondrial supernatants of the liver according to Holtzman and Gillette (6) as adopted by Wiśniewska-Knypl and Jabłońska (21). Liver microsomes were prepared by CaCl₂ – aggregation method of Kamath et al. (8). Microsomal protein was determined by the method of Lowry et al. (11). Cytochrome P-450 in microsomes was determined according to Omura and Sato (14). Carbon monoxide difference spectra of dithionite-reduced microsomes were charted between 490 and 450 nm using Beckman ACTA CIII spectrophotometer and extinction coefficient of 91 mmol⁻¹ cm⁻¹ was employed for quantifying cytochrome P-450.

Lipid peroxidation in fresh microsomal membranes was evaluated on the basis of detection of thiobarbituric acidreactive substance according to Mihara et al. (13). An extinction coefficient of 1.56×10^{-5} mmol⁻¹ according to Wills (20) was used for malondialdehyde formation. For assay of triglycerides, hepatic lipids were extracted by the method of Folch et al. (4): liver slices were homogenized with 20 volumes of chloroform-methanol (2:1, v/v) at 45°C and the extract washed with 0.1 mol NaCl, evaporated under vacuum and the residues dissolved in chloroform. Triglycerides were determined with a standard enzymatic kit of Boehringer-Mannheim "Test Combination-Triglycerides (neutral fat)" taking for analysis a lipid extract equivalent to 10–20 mg of fresh liver, and the decrease of NADH was measured at 340 nm in a Perkin-Elmer Lambda 15 spectrophotometer. Concentration of triglycerides in the liver were adapted for nmol per g tissue using a factor 1.14 (mol wt. of glycerol trioleate = 885.4).

Rotarod performance

Rotarod performance was tested according to the principle described by Kaplan and Murphy (9). 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 10 consecutive days were used in experiment.

Rotarod performance test was conducted before the experiment and at each month during 3 months of inhalation exposure.

Hot plate behaviour was tested after termination of a three-month exposure. The hot-plate 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.

Statistics

For statistical evaluation analysis of variance (ANOVA) (22), Dunnett's test (22) and the Fisher exact test (23) were used.

RESULTS

All rats exposed for 3 months to single solvents and their mixtures (1:1) survived experiments without any observable signs of toxicity. Changes in body weight gain

Table 1. Effect of a three-month exposure to m-xylene, n-butyl alcohol and their mixture on body weight (g) in rats

	Before exposure	Exposure		
		1 month	2 months	3 months
Control	327.9 ± 26.0	372.5 ± 24.4	395.0 ± 27.5	399.2 ± 27.9
n-butyl alcohol				
50 ppm	328.7 ± 23.7	390.8 ± 26.7**	420.4 ± 29.8**	426.7 ± 28.1
m-xylene 50 ppm	317.1 ± 18.0	375.4 ± 24.2*	400.0 ± 27.6	405.8 ± 26.3
n-butyl alcohol + m-xylene				
50 + 50 ppm	340.8 ± 28.2	402.1 ± 29.3**	428.3 ± 31.1*	423.3 ± 35.6
n-butyl alcohol				
100 ppm	322.5 ± 23.2	392.1 ± 26.0**	418.3 ± 34.3**	423.3 ± 29.2
m-xylene 100 ppm	322.5 ± 35.7	390.0 ± 41.6**	423.3 ± 56.9**	417.9 ± 40.9
n-butyl alcohol + m-xylene				
100 + 100 ppm	344.2 ± 28.2	402.5 ± 30.3*	432.1 ± 32.1*	439.2 ± 40.9**

Data are expressed as mean values ± SD for 24 rats in the control group and 12 rats in exposed groups

*statistically significant difference as compared to controls ($p \leq 0.05$)

**statistically significant difference as compared to controls ($p \leq 0.01$)

Table 2. Effect of a three-month exposure to n-butyl alcohol, m-xylene and their mixture on the organ and final body weight

Parameters	Control	n-butyl alcohol 50 ppm	m-xylene 50 ppm	mixture (1:1) 50 + 50 ppm	n-butyl alcohol 100 ppm	m-xylene 100 ppm	mixture (1:1) 100 + 100 ppm
Final body weight (g)	399.1 ± 27.9	426.6 ± 28.0	405.8 ± 26.3	423.3 ± 35.5	423.3 ± 29.1	417.9 ± 42.5	439 ± 40.1
Absolute organ weight (g)							
Heart	1.13 ± 0.09	1.19 ± 0.12	1.15 ± 0.07	1.16 ± 0.10	1.16 ± 0.09	1.16 ± 0.09	1.22 ± 0.11
Lungs	1.59 ± 0.15	1.66 ± 0.15	1.61 ± 0.14	1.60 ± 0.12	1.68 ± 0.22	1.71 ± 0.19	1.87 ± 0.49
Liver	9.27 ± 1.21	9.69 ± 1.17	8.91 ± 0.75	9.87 ± 1.33	10.00 ± 1.14	9.36 ± 1.02	10.75 ± 2.32
Spleen	0.63 ± 0.11	0.72 ± 0.09	0.67 ± 0.12	0.76 ± 0.12	0.75 ± 0.10	0.71 ± 0.09	0.71 ± 0.11
Kidneys	2.47 ± 0.16	2.69 ± 0.25	2.47 ± 0.22	2.69 ± 0.34	2.62 ± 0.37	2.62 ± 0.42	2.68 ± 0.37
Adrenals	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
Testes	3.20 ± 0.45	3.24 ± 0.28	3.17 ± 0.31	3.23 ± 0.67	3.16 ± 0.25	3.12 ± 0.24	3.17 ± 0.78
Relative organ weight (g/100 g b.w.)							
Heart	0.28 ± 0.02	0.28 ± 0.01	0.28 ± 0.01	0.27 ± 0.01	0.27 ± 0.01	0.27 ± 0.01	0.28 ± 0.02
Lungs	0.40 ± 0.03	0.39 ± 0.03	0.39 ± 0.04	0.38 ± 0.02	0.40 ± 0.04	0.41 ± 0.04	0.42 ± 0.07
Liver	2.32 ± 0.26	2.27 ± 0.21	2.20 ± 0.20	2.32 ± 0.17	2.36 ± 0.16	2.23 ± 0.09	2.42 ± 0.31
Spleen	0.15 ± 0.03	0.17 ± 0.02	0.16 ± 0.02	0.17 ± 0.02	0.17 ± 0.02	0.17 ± 0.01	0.16 ± 0.02
Kidneys	0.62 ± 0.04	0.63 ± 0.05	0.60 ± 0.05	0.63 ± 0.05	0.62 ± 0.08	0.62 ± 0.05	0.61 ± 0.04
Adrenals	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Testes	0.80 ± 0.12	0.76 ± 0.08	0.78 ± 0.08	0.77 ± 0.17	0.75 ± 0.08	0.75 ± 0.09	0.72 ± 0.17

Data are expressed as mean values ± SD for 12 rats. Changes not statistically significant

Table 3. Effect of a three-month exposure to m-xylene, n-butyl alcohol and their mixture on hematological parameters in rats

Parameters	Control		n-butyl alcohol		m-xylene		mixture (1:1)		n-butyl alcohol		m-xylene		mixture (1:1)	
			50 ppm	50 ppm	50 ppm	50 ppm	50 + 50 ppm	50 + 50 ppm	100 ppm	100 ppm	100 ppm	100 ppm	100 + 100 ppm	100 + 100 ppm
Hematocrit (%)	40.0 ± 1.5	38.6 ± 2.9	37.4 ± 2.3	38.5 ± 2.5	37.4 ± 2.3	38.5 ± 2.5	38.5 ± 2.5	38.5 ± 2.5	38.5 ± 2.5	38.9 ± 2.4	38.9 ± 2.4	38.9 ± 2.4	39.2 ± 2.4	39.2 ± 2.4
Hemoglobin (g/dl)	15.9 ± 0.4	14.2 ± 0.8**	14.7 ± 0.6**	15.1 ± 0.6*	14.7 ± 0.6**	15.1 ± 0.6*	15.1 ± 0.6*	15.1 ± 0.6*	14.1 ± 0.7**	14.7 ± 0.6**	14.7 ± 0.6**	14.7 ± 0.6**	14.3 ± 0.6**	14.3 ± 0.6**
Red blood cells (x10 ⁶ /mm ³)	9.97 ± 0.02	9.45 ± 0.05	9.29 ± 0.05*	9.01 ± 0.05**	9.29 ± 0.05*	9.01 ± 0.05**	9.01 ± 0.05**	9.01 ± 0.05**	8.35 ± 0.06**	8.02 ± 0.05**	8.02 ± 0.05**	8.02 ± 0.05**	8.42 ± 0.05**	8.42 ± 0.05**
White blood cells (x10 ³ /mm ³)	10.5 ± 0.13	13.1 ± 0.26	13.3 ± 0.26	12.1 ± 0.27	13.3 ± 0.26	12.1 ± 0.27	12.1 ± 0.27	12.1 ± 0.27	16.5 ± 0.27**	14.2 ± 0.27**	14.2 ± 0.27**	14.2 ± 0.27**	15.0 ± 0.26**	15.0 ± 0.26**
Differential count														
red neutrophils (%)	0.33 ± 0.03	1.08 ± 0.07	0.33 ± 0.07	0.66 ± 0.07	0.33 ± 0.07	0.66 ± 0.07	0.66 ± 0.07	0.66 ± 0.07	0.33 ± 0.07	0.33 ± 0.07	0.33 ± 0.07	0.33 ± 0.07	0.16 ± 0.07	0.16 ± 0.07
segmented neutrophils (%)	38.8 ± 3.9	38.6 ± 6.9	33.1 ± 6.8	34.2 ± 6.8	33.1 ± 6.8	34.2 ± 6.8	34.2 ± 6.8	34.2 ± 6.8	32.7 ± 7.0	31.9 ± 7.1	31.9 ± 7.1	31.9 ± 7.1	29.6 ± 6.9*	29.6 ± 6.9*
eosinophils (%)	7.8 ± 2.3	11.5 ± 4.6	9.7 ± 4.6	10.2 ± 4.6	9.7 ± 4.6	10.2 ± 4.6	10.2 ± 4.6	10.2 ± 4.6	13.8 ± 4.6*	10.4 ± 4.6	10.4 ± 4.6	10.4 ± 4.6	14.0 ± 4.6*	14.0 ± 4.6*
lymphocytes (%)	48.3 ± 4.8	45.3 ± 8.5	51.6 ± 8.4	51.7 ± 8.5	51.6 ± 8.4	51.7 ± 8.5	51.7 ± 8.5	51.7 ± 8.5	50.5 ± 8.7	54.6 ± 8.7	54.6 ± 8.7	54.6 ± 8.7	53.0 ± 8.6	53.0 ± 8.6
monocytes (%)	3.2 ± 0.10	4.1 ± 0.22	5.0 ± 0.21	2.9 ± 0.21	5.0 ± 0.21	2.9 ± 0.21	2.9 ± 0.21	2.9 ± 0.21	3.0 ± 0.21	3.7 ± 0.22	3.7 ± 0.22	3.7 ± 0.22	3.3 ± 0.21	3.3 ± 0.21

Statistically significant difference as compared to controls - * p ≤ 0.05, **p ≤ 0.01

during 3 months of exposure to the vapours of single solvents and their mixtures are shown in Table 1. At the end of exposure statistically significant increase in body weight of animals exposed to the mixture of m-xylene and n-butyl alcohol at concentration of 100 + 100 ppm was observed.

No significant differences in absolute and relative organ weights were observed in rats exposed to single solvents or their mixtures when compared to controls (Table 2).

Significant decrease in hemoglobin and red blood cell counts in animals exposed to applied concentrations of single solvents and their mixtures when compared with the controls were observed (Table 3). The significant increase in white blood cell counts was observed in animals exposed to single solvents at concentration of 100 ppm and their mixture at concentration of 100 + 100 ppm. There were no significant differences in hematological parameters between animal exposed to single solvents and their mixture.

No significant differences in biochemical tests values were observed in animals exposed to single solvents or their mixtures (1:1) at applied concentrations (AspAT 82.3 U/l, ALAT 28.6 U/l, SDH 2.52 U/l, AP 43.4 U/l, total protein 7.16 g/ml, albumin 3.40 g/100 ml, glucose 108.7 mg/100 ml, Na⁺ 142.5 mM/l, K⁺ 5.18 mM/l, Ca⁺⁺ 9.14 mg/100 ml, Cl⁻ 100.1 mM/l) n-Butyl alcohol and m-xylene, singly or combined, none of the two paired concentrations of 50 and 100 ppm applied, have influenced the hepatic microsomal monooxygenase system (Table 4); the value of

Table 4. Effect of a three-month exposure to n-butyl alcohol, m-xylene and their mixtures on microsomal monooxygenases and lipid peroxidation and on the level of triglycerides in the rat liver

Exposure chemical	Microsomal protein mg/g liver	Monooxygenases		Lipid peroxidation nmol MDA/mg microsomal protein equivalent to g liver	Triglycerides nmol/g liver
		Aniline p-hydroxylase nmol p-amino-phenol/15 min/g liver	Cytochrome P-450 nmol/g liver		
Control	23.79 ± 1.06	312.30 ± 13.81	20.92 ± 1.43	40.41 ± 2.10	8.09 ± 0.57
n-butyl (50 ppm) alcohol	22.55 ± 0.95	307.33 ± 10.80	19.44 ± 0.68	46.68 ± 2.16*	8.09 ± 0.47
m-xylene (50 ppm)	22.88 ± 0.87	297.82 ± 9.82	19.74 ± 0.69	43.50 ± 2.05	8.52 ± 0.93
n-butyl alcohol + m-xylene (50 + 50 ppm)	25.73 ± 1.29	377.71 ± 26.43	22.92 ± 1.80	39.93 ± 3.63	7.51 ± 0.51
n-butyl alcohol (100 ppm)	24.25 ± 0.95	314.84 ± 21.81	20.12 ± 1.70	52.45 ± 2.88*	9.15 ± 0.90
m-xylene (100 ppm)	25.80 ± 1.45	382.92 ± 28.63	21.73 ± 1.55	45.92 ± 2.58	8.34 ± 0.58
n-butyl alcohol + m-xylene (100 + 100 ppm)	23.09 ± 0.77	304.81 ± 15.44	20.34 ± 0.92	45.91 ± 2.23	7.83 ± 0.60

MDA - malondialdehyde

Test were performed 19 h after termination of the last inhalatory exposure

Results are the mean ± SD for 12 rats

* - significantly different from the control at p ≤ 0.05

the markers were comprised within a physiological range for the liver and brought about, on average, for microsomal protein 23 mg/g liver, for aniline p-hydroxylase 320 nmol p-aminophenol formed per mg microsomal protein per 15 min/g liver and for cytochrome P-450 20 nmol/g liver.

Nevertheless, one of the chemicals tested, namely n-butyl alcohol, exerted a prooxidative activity towards microsomal membranes at both concentrations, 50 and 100 ppm.

The effect of n-butyl alcohol at concentrations of 50 and 100 ppm was manifested by a significant stimulation of lipid peroxidation by 16 and 30 per cent, respectively, as compared with controls where the level of malondialdehyde was found to be 40.4 nmol per microsomal protein equivalent to g liver (Table 4).

The level of hepatic triglycerides, taken as a lipid index for feeding up status remained unchanged in each of the tested groups and brought about, on average, 8 nmol/g liver (former denotation 7 mg/g liver) (Table 4).

The disturbances in rotarod performance were observed in animals exposed for 3 months to single solvents and their mixtures (1:1) at applied concentrations (Fig. 1). At the end of a three-month exposure the effect was more pronounced and was statistically significant in groups exposed to single solvents and their mixture at higher concentrations (n-butyl alcohol 100 ppm, m-xylene 100 ppm and (1:1) mixture 100 + 100 ppm) (Fig. 1 ab).

Statistically significant motor coordination disturbances recorded after 1 month exposure to m-xylene at concentration of 100 ppm as well as disturbance tendency observed after 1 month exposure to m-xylene at concentration of 50 ppm remained at the same level until the end of a three-month exposure (Fig. 1 abc).

During exposure to n-butyl alcohol (at both concentrations) the motor coordination disturbances observed after 1 month became more intense and at the end of exposure (100 ppm) reached that of m-xylene level and were statistically significant (Fig. 1 abc).

In animals exposed to mixture (1:1) of n-butyl alcohol and m-xylene at concentrations of 50 + 50 ppm and 100 + 100 ppm the motor coordination disturbances were also observed (Fig. 1 bc). At higher concentration the disturbances progressively increased and after three month exposure they reached the level caused by single solvents at concentration of 100 ppm. Time course of disturbances in rotarod performance caused by mixture of m-xylene and n-butyl alcohol at concentration of 100 + 100 ppm was identical as that observed during three-month exposure to n-butyl alcohol at concentration of 100 ppm (Fig. 1b).

After a three-month exposure to m-xylene, n-butyl alcohol and their mixture (1:1) at applied concentrations, the pain sensitivity, measured as latency of the paw-lick response, was changed in animals exposed to m-xylene at both concentrations applied (Table 5). Statistically significant increase in sensitivity to pain (decrease in latency of the paw lick response) was observed.

No changes in sensitivity to pain were observed in animals exposed to n-butyl alcohol or to the mixture of solvents at both concentrations applied (Table 5).



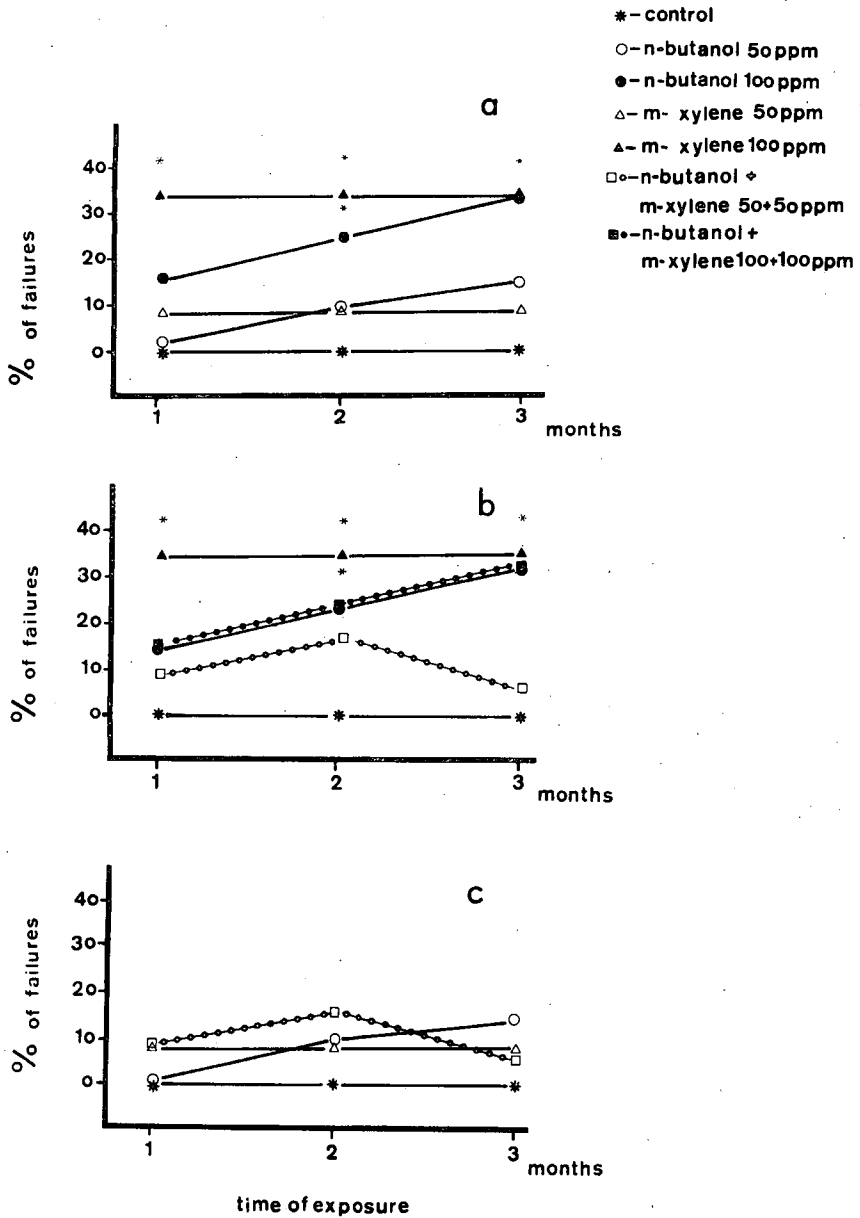


Fig. 1. Rotarod performance of rats exposed to m-xylene and n-butyl alcohol at concentrations of 50 and 100 and their mixture (1:1) at concentrations of 50 + 50 and 100 + 100 ppm.

a) effect of m-xylene and n-butyl alcohol at concentrations of 50 and 100 ppm;

b) effect of m-xylene and n-butyl alcohol at concentration of 100 ppm and their (1:1) mixture at concentrations of 50 + 50 ppm and 100 + 100 ppm;

c) effect of m-xylene and n-butyl alcohol at concentration of 50 ppm and their (1:1) mixture at concentration of 50 + 50 ppm;

Statistical significance marked by asterisks for $p < 0.05$.

Table 5. Effect of a three-month exposure to m-xylene, n-butyl alcohol and their mixture (1:1) on the latency of the paw-lick response (hot-plate behaviour) in rats

Group		Latency of the paw-lick response, sec.
Control	(n = 24)	12.2 ± 3.1
n-butyl alcohol 50 ppm	(n = 12)	9.7 ± 3.6
m-xylene 50 ppm	(n = 12)	8.7 ± 3.8*
n-butyl alcohol + m-xylene 50 + 50 ppm	(n = 12)	11.5 ± 4.2
n-butyl alcohol 100 ppm	(n = 12)	9.4 ± 4.7
m-xylene 100 ppm	(n = 12)	8.6 ± 2.7*
n-butyl alcohol + m-xylene 100 + 100 ppm	(n = 12)	9.6 ± 2.1

Mean values ± SD

*statistically significant difference as compared to controls ($p \leq 0.05$)

DISCUSSION

Positively less than additive neurotoxic and irritating effects of combined exposure to m-xylene and n-butyl alcohol in condition of acute inhalation (10) suggested that a similar phenomenon might occur in conditions of subchronic combined inhalation exposure. In rats exposed for 3 months to single solvents at concentration of 50 or 100 ppm and to their (1:1) mixture at concentration of 50 + 50 ppm or 100 + 100 ppm, the disturbances in rotarod performance test were observed.

In animals exposed to the mixture of solvents at concentration of 50 + 50 ppm the changes were similar to that observed with single solvents at concentration of 50 ppm and less pronounced than that recorded with single solvents at concentration of 100 ppm (Fig. 1). The time course of disturbances in rotarod performance caused by the mixture of m-xylene and n-butyl alcohol at concentration of 100 + 100 ppm which was identical to that observed during a three-month exposure to n-butyl alcohol at concentration of 100 ppm clearly indicated 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 rotarod performance test suggest less than additive effect of the combined exposure to n-butyl alcohol and m-xylene. Also, the statistically significant increase in sensitivity to pain in animals exposed to m-xylene at both applied concentrations (50 and 100 ppm) and parallel lack of changes in the pain sensitivity in animals exposed to n-butyl alcohol (50 ppm and 100 ppm) and to solvent mixtures (50 + 50 ppm and 100 + 100 ppm) indicate the less than additive effect of combined exposure to m-xylene and n-butyl alcohol.

It is difficult to define, on the basis of recorded changes in hematological parameters, the type of combined toxic effect of m-xylene and n-butyl alcohol in the condition of subchronic exposure because of its limited extent.

The standard clinical chemistry data have not shown any toxic effect of exposure to single solvents and their mixture at applied concentrations. However, the



present study demonstrated clearly that inhalatory exposure to low concentration of solvents up to 50 – 100 ppm, did exert biological effect: n-butyl alcohol provoked the increase of lipid peroxidation in hepatic microsomes without any induction of cytochrome P-450 monooxygenases (Table 4). Exposure to m-xylene at 50 and 100 ppm affects neither the activity of monooxygenase nor the lipid peroxidation rate in hepatic microsomes. Moreover, in the case of combined exposure to n-butyl alcohol the abolishing effect on n-butyl alcohol driven microsomal lipid peroxidation was noted (Table 4). The mechanism involved in the abolishing/inhibitory effect of m-xylene and n-butyl alcohol mediated lipid peroxidation may be only speculated on the basis of presumable competition of the solvents for enzymatic system involved in their biotransformation, namely cytochrome P-450 monooxygenase. Cytochrome P-450 is the main pathway for oxidation of m-xylene (15) and a minor one for n-butyl alcohol, supplementary to alcohol dehydrogenase (18). Cytochrome P-450 may act in catalysing the formation of superoxide anion and other free radicals, and in propagating the peroxidative chain through its peroxidase type of activity (21), the process may be stimulated by Fe^{2+} (2). In the case of n-butyl alcohol metabolism via cytochrome P-450, in opposition to xylene, very reactive hydroxyl radicals may be formed and its interaction with Fe^{2+} derived from heme moiety of cytochrome P-450 may stimulate lipid peroxidation (1). The above process may be overcome by m-xylene resulting in abolishing the stimulatory effect of n-butyl alcohol on lipid peroxidation.

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