Polish Journal of Occupational Medicine and Environmental Health, Vol. 5, No 1, 27-33, 1992

TOXIC EFFECTS OF COMBINED EXPOSURE TO TOLUENE AND M-XYLENE IN ANIMALS. III. SUBCHRONIC INHALATION STUDY

ZOFIA KORSAK, JERZY A. SOKAL and ROMAN GÓRNY

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

Key words: Toluene, m-Xylene, Combined exposure, Nervous system

Abstract. Effects of combined exposure to toluene and m-xylene in the conditions of subchronic inhalation experiments in rats were examined. Rats were exposed to vapours of individual solvents and their 1:1 mixture at concentrations of 1000 ppm or 100 ppm, 6 h/day, 5 days/week, for 3 or 6 months, respectively. In rats exposed for 3 or 6 months to toluene, m-xylene and their mixtures (1:1) at concentrations of 1000 ppm and 100 ppm, respectively, the observed disturbances in rotarod performance test and decrease in spontaneous motor activity were statistically significant in comparison to control. In animals exposed to mixtures (1:1) of toluene and m-xylene, changes were not significantly different but more pronounced when compared to single solvent groups. The decrease of red blood cells count and increase of rod neutrophil cell counts were observed only in rats exposed for 3 months to mixture of solvents. Results obtained in condition of acute and subchronic inhalation exposure and toxicokinetics data interpreted jointly indicate the more than additive toxic effects of combined exposure to toluene and m-xylene.

INTRODUCTION

It is well known that both toluene and xylene primarily depress the central nervous system and irritate respiratory tract. 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 (1). However, this assumption is not sufficiently validated by scientific data (2).

Our previous observations indicate the more than additive toxic effects of combined exposure to toluene and xylene (1:1) on central nervous system and respiratory tract in condition of acute inhalation study (3). The objective of the

Address reprint requests to: Z. Korsak, Department of Toxicity Evaluation, The Nofer Institute of Occupational Medicine, P.O. Box 199, 90-950 Lodz, Poland.

present study was to evaluate the neurotoxic effects of combined exposure to toluene and m-xylene in the condition of subchronic inhalation exposure.

MATERIALS AND METHODS

Chemicals

Toluene and m-xylene reagent grade were supplied by Reachim and Polish Chemical Company.

Animals

Male Wistar rats of Imp: DAK stock outbred, body weight 300–350 g 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 toluene, m-xylene or their mixture consisting of 50 Vol-% toluene and 50 Vol-% m-xylene in a dynamic inhalation chamber (1.3 m^3 volume). Vapours of toluene and m-xylene 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.

Forty eight rats were divided into 4 groups. Each group consisted of 12 rats. Control group was sham-exposed, the others were exposed to vapours of toluene; 1000 ppm (3750 mg/m^3), m-xylene; 1000 ppm (4350 mg/m^3) and their 1:1 mixture; 1000 ppm (1875 mg/m^3 of toluene + 2175 mg/m^3 of m-xylene) 6 h/day, 5 days/week, 3 months.

Other forty eight rats were divided into 4 gropus each consisting of 12 rats. Control group was sham-exposed and the others were exposed to toluene; 100 ppm (375 mg/m^3), m-xylene; 100 ppm (435 mg/m^3) and their 1:1 mixture; 100 ppm (187,5 mg/m^3 of toluene + 217,5 mg/m^3 of m-xylene) 6 h/day, 5 days/week, 6 months. All rats were deprived of food and water during the 6 h exposure period.

Clinical pathology determinations

Clinical laboratory studies were conducted for all animals 24 hours after termination of 3 or 6 months inhalation exposure. Animals were deprived of food 24 hours prior then were anasthetized in light ether anesthesia, exanguined from the abdominal aorta and subjected to gross necropsy. Blood samples were collected for serum chemistry determinations. These were made for alanine aminotransferase,

aspartate aminotransferase, sorbitol dehydrogenase, alkaline phosphatase, total protein and albumin and glucose. Determinations were done by using the biochemical autoanalyser – Backman Clinical System 700; the Beckman test kits were applied.

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 leucocyte count were conducted on the control and exposure groups.

Rotarod performance

Rotarod performance was tested according to the principle described by Kaplan and Murphy (4). 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 the experiment.

Rotarod performance test was conducted before the experiment and at each month during 3 and 6 months of inhalation exposure.

Spontaneous motor activity

One hour measurements were done 24 hour after termination of 3 or 6 month exposure by using of the UMA-2-10 actometer for small laboratory animals.

Statistics

For statistical evaluation analysis of variance (ANOVA) (5), Dunnett's test (5) and the Fisher exact test (6) were used.

RESULTS

All rats exposed for 3 or 6 months to single solvents and their mixture (1:1) at concentrations of 1000 ppm and 100 ppm, respectively survived experiments. Clinical observations were unremarkable. No significant differences in final body weights, absolute and relative organ weights were observed in rats exposed to the single solvents and their mixtures when compared with the controls (Table 1).

There were statistically significant differences in red blood cell counts between rats exposed to mixture (1:1) of solvents for 3 months and control groups (Table 2) and changes in differential white blood cell counts in animals exposed to the single solvents and their mixtures when compared wirth the controls.

No significant differences in clinical chemistry parameters were observed in animals exposed for 3 or 6 months to single solvents or their mixtures (1:1) at concentrations of 1000 ppm or 100 ppm (Table 3).

Table 1. Effect of 3 months exposure to toluene, m-xylene and their mixture on organ and final body weight.

Parameters	Control	Toluene	m-xylene	Mixture (1:1)
Final body weight (g)	439.5 ± 57.5	421.4 ± 55.5	436.2 ± 41.1	412.9 ± 36.7
Absolute organ weight (g)				
Heart	1.29 ± 0.16	1.17 ± 0.15	1.23 ± 0.16	1.19 ± 0.13
Lungs	1.86 ± 0.24	1.79 ± 0.33	1.82 ± 0.31	1.79 ± 0.24
Liver	12.25 ± 2.89	11.05 ± 2.79	11.30 ± 1.37	10.98 ± 1.93
Spleen	0.68 ± 0.12	0.70 ± 0.08	0.63 ± 0.07	0.64 ± 0.08
Kidneys	2.57 ± 0.36	2.48 ± 0.32	2.73 ± 0.29	2.35 ± 0.20
Adrenals	0.06 ± 0.03	0.06 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
Testes	3.41 ± 0.29	3.41 ± 0.34	3.26 ± 0.34	3.30 ± 0.30
Relative organ weight (g/100g b.w.)				
Heart	0.295 ± 0.028	0.279 ± 0.017	0.282 ± 0.022	0.289 ± 0.024
Lungs	0.425 ± 0.036	0.425 ± 0.043	0.420 ± 0.084	0.434 ± 0.047
Liver	2.75 ± 0.39	2.60 ± 0.39	2.60 ± 0.31	2.64 ± 0.32
Spleen	0.153 ± 0.011	0.168 ± 0.020	0.145 ± 0.014	0.155 ± 0.016
Kidneys	0.585 ± 0.045	0.591 ± 0.051	0.628 ± 0.061	0.570 ± 0.043
Adrenals	0.0133 ± 0.0059	0.0136 ± 0.0029	0.0125 ± 0.0027	0.0120 ± 0.0015
Testes	0.781 ± 0.054	0.818 ± 0.106	0.751 ± 0.077	0.802 ± 0.075

Rats were esxposed to toluene at concentration of 1000 ppm (3750 mg/m^3), m-xylene at concentration of 1000 ppm (4350 mg/m^3) and their mixture (1:1) at concentration of 500 + 500 (1875 mg/m^3 + 2175 mg/m^3).
Data are expressed as mean values ± SD for 12 rats. Changes not statistically significant.

Table 2. Effect of 3 months exposure to toluene, m-xylene and their mixture on hematological parameter.

Parameters	Control	Toluene	m-xylene	Mixture (1:1)
Hematocrit (%)	37.9 ± 3.5	42.3 ± 3.3	37.9 ± 5.3	34.7 ± 5.2
Hemoglobin (g/dl)	16.1 ± 1.2	15.7 ± 0.6	15.4 ± 0.9	15.2 ± 0.7
Red blood cells (x10^6/mm^3)	10.45 ± 0.66	10.19 ± 0.93	10.09 ± 0.89	9.22 ± 0.83*
White blood cells (x10^3/mm^3)	22.2 ± 4.7	30.5 ± 10.7	29.7 ± 10.0	28.4 ± 9.0
Differential variable				
rod neutrophil	0.5 ± 0.7	1.6 ± 1.4	1.4 ± 1.0	3.0 ± 1,9*
segmented neutrophil	21.7 ± 5.1	22.7 ± 5.9	26.3 ± 11.5	23.3 ± 6.9
eosinophil	8.7 ± 3.8	10.5 ± 3.1	10.5 ± 4.4	9.8 ± 5.0
lymphocyte	60.8 ± 6.4	50.6 ± 6.9*	45.5 ± 9.5*	47.5 ± 8.0*
monocyte	8.3 ± 4.2	14.6 ± 5.3*	16.3 ± 8.9*	16.4 ± 5.1*

Rats were exposed to toluene at concentration of 1000 ppm (3750 mg/m^3), m-xylene at concentration of 1000 ppm (4350 mg/m^3) and their mixture (1:1) at concentration of 500 + 500 (1875 mg/m^3 − 2175 mg/m^3); mean values ± SD for 12 rats).
* Statistically significant difference as compared to controls ($p \leq 0,05$).

Table 3. Effect of 3 months expsure to toluene, m-xylene and their mixture (1:1) on chemical chemistry values.

Parameters		Control	Toluene	m-Xylene	Mixture (1:1)
Aspartate aminotransferase	(U/l)	76.14 ± 20.22	83.09 ± 13.67	88.09 ± 19.01	82.02 ± 24.03
Alanine aminotransferase	(U/l)	42.63 ± 6.02	39.02 ± 10.08	42.64 ± 11.97	43.81 ± 15.56
Alkaline	(U/l)	46.1 ± 13.1	44.0 ± 6.0	38.0 ± 9.5	42.8 ± 5.9
Sorbitol dehydrogenase	(U/l)	2.017 ± 0.271	2.071 ± 0.337	2.591 ± 0.648	2.483 ± 0.851
Total protein	(g/dl)	7.32 ± 0.29	7.17 ± 0.31	7.09 ± 0.34	6.99 ± 0.30
Albumin	(d/dl)	3.31 ± 0.12	3.27 ± 0.14	3.30 ± 0.09	3.28 ± 0.12
Glucose	(mg/dl)	106.2 ± 23.5	111.8 ± 28.7	113.2 ± 20.4	107.7 ± 29.2
Trigliceride		6.99 ± 1.81	8.16 ± 2.08	9.20 ± 2.70	7.10 ± 1.70

Rats were expsed to toluene at concentration of 1000 ppm (3750 mg/m^3), m-xylene at concentration of 1000 ppm (4350 mg/m^3) and their mixture (1:1) at concentration of 500 + 500 (1875 mg/m^3 + 2175 mg/m^3).
Data are expressed as mean values ± SD for 12 rats. Changes not statistically significant.

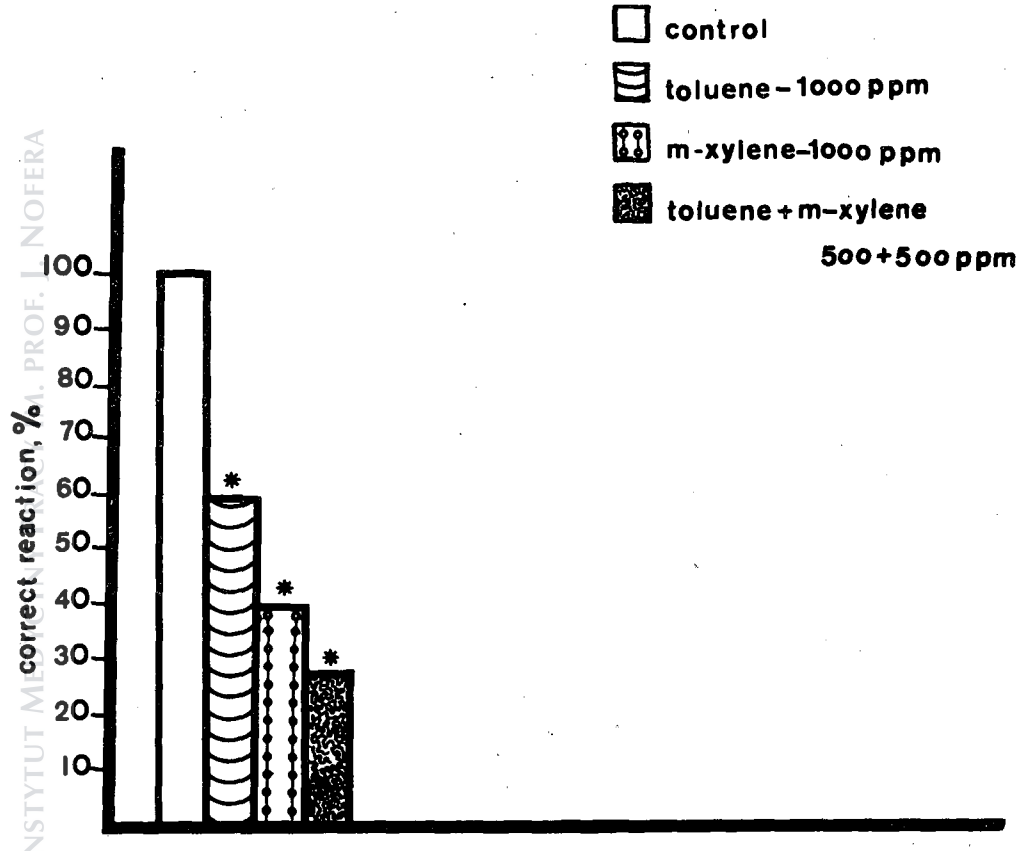

Fig. 1. Rotarod performance of rats exposed to toluene, m-xylene and their mixture containing 50 Vol-% toluene and 50 Vol-% m-xylene at concentrations of 1000 ppm. Rats were exposed to vapours of solvents for 6h/day, 5 days/week, 3 months. Rotarod performance was tested 24 hours after termination of 3 months exposure. Statistical significance marked by asterisks for $p \leqslant 0.01$.

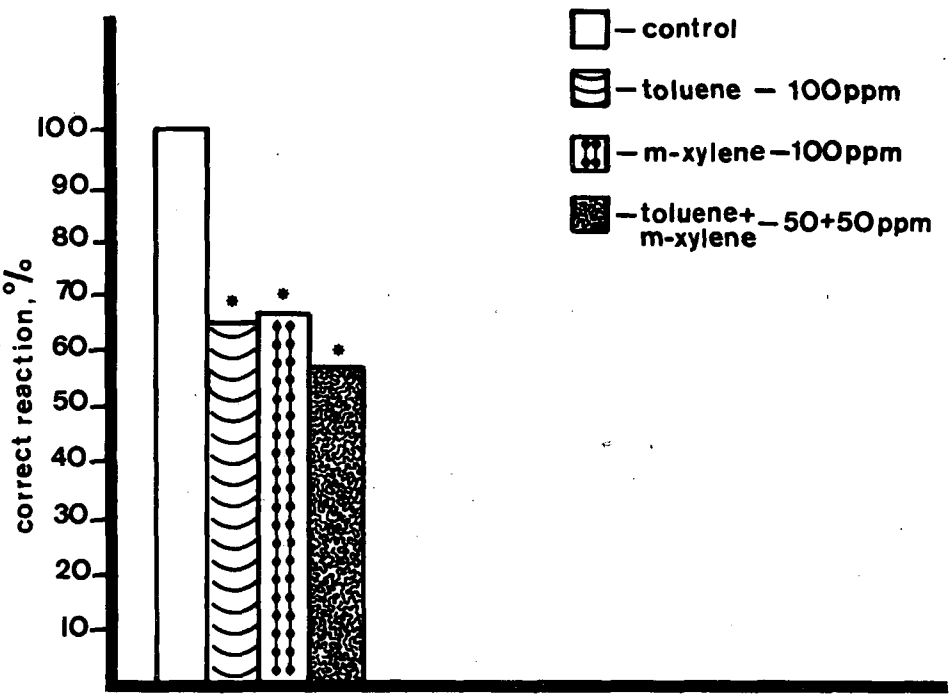

Fig. 2. Rotarod performance of rats exposed to toluene, m-xylene and their mixture containing 50 Vol-% toluene and 50 Vol-% m-xylene at concentrations of 100 ppm. Rats were exposed to vapours of solvents for 6 h/day, 5 days/week, 6 months. Rotarod peformance was tested 24 hours after termination of 6 months exposure. Statistical significance marked by asterisks for $p \leqslant 0.05$.

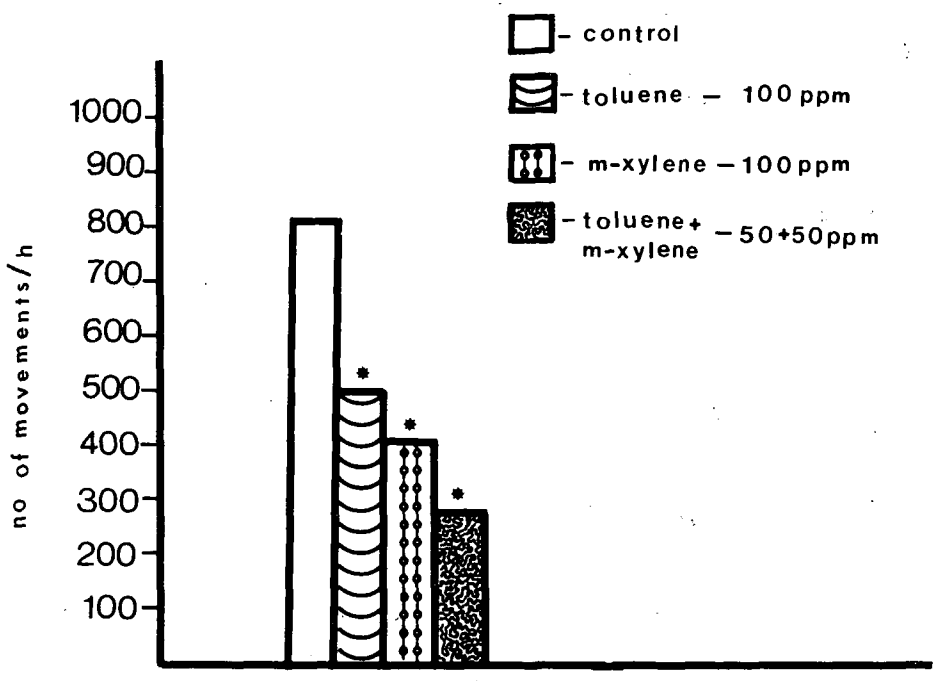

Fig. 3. Spontaneous motor activity in rats exposed to toluene, m-xylene and their mixture at concentrations of 100 ppm for 6 h/day, 5 days/week, 6 months. Spontaneous motor activity was measured 24 hours after termination of 6 month exposure. Statistical significance marked by asterisks for $p \leqslant 0.05$.

In animals exposed for both 3 or 6 months to single solvents and their mixtures (1 : 1) at concentrations of 1000 ppm and 100 ppm, respectively, the disturbances in rotarod performance were observed (Figs 1, 2). Effects were statistically significant in comparison to control group. The effect was more pronounced in groups exposed to mixtures of solvents but changes were not significantly different in comparison to groups exposed to single solvents. Also the decrease in spontaneous motor activity observed in all exposed groups was statistically significant when compared to controls (Fig. 3). Although the decrease in spontaneous motor activity was more pronounced in animals exposed to the mixture of solvents the differences were insignificant in comparison to animals exposed to the single solvents.

DISCUSSION

Clear more than additive neurotoxic and irritating effects of combined exposure to toluene and xylene in condition of acute inhalation (3) suggested that similar phenomenon might occur in conditions of subchronic combined inhalation exposure. In rats, exposed for 3 or 6 months to toluene, m-xylene or their mixtures at concentrations of 1000 ppm and 100 ppm, respectively, the observed disturbances in rotarod performance test and decrease in spontaneous motor activity were statistically significant in comparison to controls. In animals exposed to mixture of solvents, changes were more pronounced but not significantly different when compared to single solvents groups. The decrease in red blood cells count and increase in rod neutrophil cell count were observed only in rats exposed for 3 months to mixture of solvents at concentration of 1000 ppm. To define the type of combined toxic effect of toluene and m-xylene mixtures in condition of subchronic exosure is difficult because the observed toxic effects were not of great extent. They have not indicated clearly any synergistic toxic effects of combined subchronic exposure to toluene and m-xylene. However, the results obtained in condition of acute (3) and subchronic inhalation exposure and toxicokinetics data (7) interpreted jointly, idicate the more than additive toxic effects of combined exposure to toluene and m-xylene.

REFERENCES

1. Ikeda M. Interactions and health aspects of exposure to mixtures of organic solvents. In: Health effects of combined exposure to chemicals in work and community environments. WHO, Regional Office, Europe Copenhagen, Interim Document 11, 77–96, 1983.
2. Holmberg B, Lundberg P. Exposure limits for mixtures. Ann Am Ind Hyg 12: 111, 1985.
3. Korsak Z, Sokal J, Dedyk A, Tomas T, Jędrychowski R. Toxic effects of combined exposure to toluene and xylene in animals. I. Acute inhalation study. Pol J Occup Med 1: 45–50, 1988.
4. Kaplan M L, Murphy S D. Effect of acrylamide on rotarod performance and sciatic nerve beta-glucuromidase activity of rats. Toxicol Appl Pharmacol 22: 259–266, 1972.
5. Woolson R F. Statistical Methods for the Analysis of Biomedical Data. Wiley, New York, 1987.
6. Zar J H. Biostatistical Analysis. Prentice-Hall. New York, 1974.
7. Korsak Z Sokal J, Świercz R. The toxic effects of combined exposure to toluene and m-xylene. II. Blood toluene and m-xylene during single and combined exposure in rats. Pol J Occup Med Environ Health 4: 377–382, 1991.

Received for publication: February 26, 1992
Accepted for publication: March 31, 1992